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Implant Dentistry

The Most Promising Discipline of Dentistry

Edited by Ilser Turkyilmaz

IMPLANT DENTISTRY – THE MOST PROMISING DISCIPLINE OF DENTISTRY

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Meet the editor

Dr. Ilser Turkyilmaz obtained his dental degree from Hacettepe University, Ankara, Turkey in 1998. Immediately after graduation, he started his PhD program in the Department of Prosthodontics, Hacettepe University. He completed that program in 2004 and kept working as an instructor in the same department. Dr. Turkyilmaz then was invited by Goteborg University, Goteborg, Sweden for research collaborations. He worked in the Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, Goteborg University, Goteborg, Sweden in 2005. He returned to Hacettepe University in the end of 2005 and then worked in private practice in Ankara from February 2006 to May 2007. He was accepted for an implant prosthodontic fellowship program in the Department of Restorative and Prosthetic Dentistry, The Ohio State University, Columbus, Ohio, and worked in that university as an implant prosthodontic fellow from June 2007 to October 2008. He took up a full-time position as an assistant professor in the Department of Prosthodontics at the University of Texas Health Science Center in San Antonio, Texas, USA on November 1, 2008. Dr. Turkyilmaz maintains a private practice in the school's faculty practice. He treats patients with esthetic and reconstructive needs using implants, veneers, crowns, fixed partial dentures, complete dentures, and partial dentures. Dr. Turkyilmaz is particularly interested in dental implant studies regarding early/immediate loading protocols, implant stability measurements using resonance frequency analysis, bone density evaluations using computerized tomography (CT), flapless implant surgeries using CT-generated surgical guides, and the biomechanical aspects of implants. He has currently 50 scientific articles published in well-known international journals. He has also given lectures including dental implants at local, national and international meetings. He is currently serving as an editorial board member or reviewer for several international dental journals. Dr. Turkyilmaz earned Diplomate status within the International Congress of Oral Implantologists in 2011, which is the highest honor showing efforts in education, research and actual clinical experience with dental implants.

Contents

Preface

Since Dr. Branemark presented the osseointegration concept with dental implants, implant dentistry has changed and improved dramatically. The use of dental implants has skyrocketed in the past thirty years. As the benefits of therapy became apparent, implant treatment earned a widespread acceptance. The need for dental implants has resulted in a rapid expansion of the market worldwide. To date, general dentists and a variety of specialists offer implants as a solution to partial and complete edentulism. Implant dentistry continues to advance with the development of new surgical and prosthodontic techniques.

The purpose of *Implant Dentistry - The Most Promising Discipline of Dentistry* is to present a comtemporary resource for dentists who want to replace missing teeth with dental implants. It is a text that integrates common threads among basic science, clinical experience and future concepts. This book consists of twenty-one chapters divided into four sections.

The first section of the book, *Bone Grafting Procedures in Implant Dentistry*, includes seven chapters and provides basic information regarding bone physiology and bone grafting procedures.

The second section of the book, *Bone-Implant Interface: Biomechanics and Surrounding Tissues*, consists of four chapters, and focuses on stress distribution in the jawbone, load transfer along the bone-implant interface and response of the bone/soft tissue around implants.

The third section of this book with six chapters, *Implant Surfaces and Clinical Practice*, concentrates on bone response to various implant surfaces, implant placement techniques and complications that might be encountered.

The fourth section of the book, *Computer-Aided Implant Dentistry and Imaging*, consists of four chapters and emphasizes state-of-the-art computer softwares for treatment planning and the value of computer softwares in more predictable implant treatment outcomes. Chapters in this section describe computer-guided implant surgeries, and the importance of computerized tomography in implant dentistry.

Our goal in writing this text is to help elevate and advance the discipline of implant dentistry, and we think that, *Implant Dentistry – The Most Promising Discipline of Dentistry,* will be a valuable source for dental students, post-graduate residents and clinicians who want to know more about dental implants.

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Part 1

Bone Grafting Procedures in Implant Dentistry

1

Various Ways to Enhance the Results of Maxillary Sinus Augmentation Procedures

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1. Introduction

The placement of dental implants in the distal edentulous maxillary region is challenging due to the significant resorption following tooth extraction and the pneumatization of the maxillary sinus (Tadjoedin et al., 2003). Maxillary sinus augmentation is a well-established procedure for functional rehabilitation of partially or completely edentulous patients, as demonstrated by Boyne and James (1980) (Boyne and James 1980) (Figures 1-4). The survival rate for implants placed in graft sinuses showed comparable results to those generally reported for implants placed in pristine bone in the non-grafted posterior maxilla (Wallace and Froum 2003).

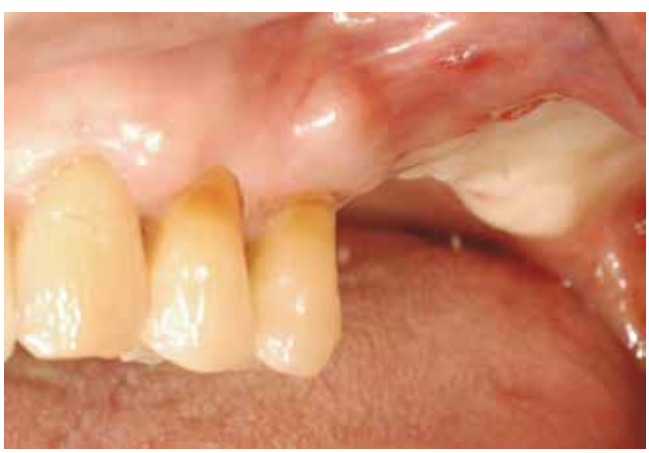

Fig. 1. Preoperative clinical view.

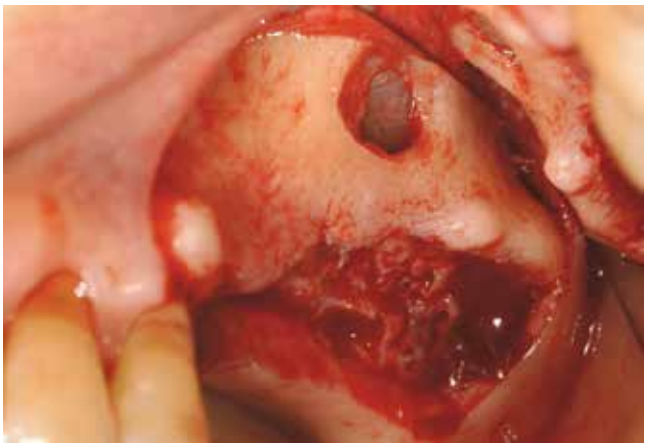

Fig. 2. Buccal view after elevation of sinus membrane.

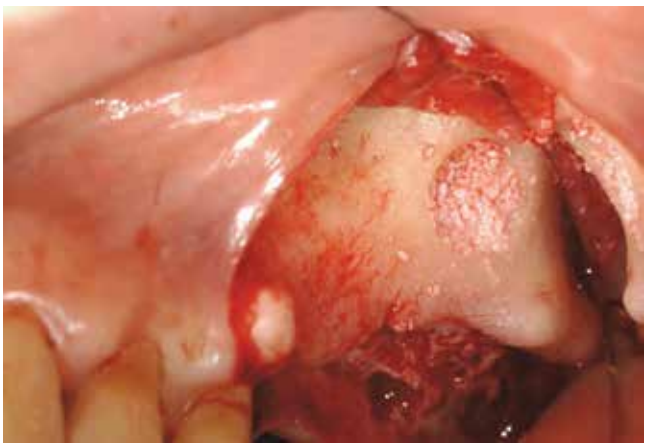

Fig. 3. Clinical view after application of graft material.

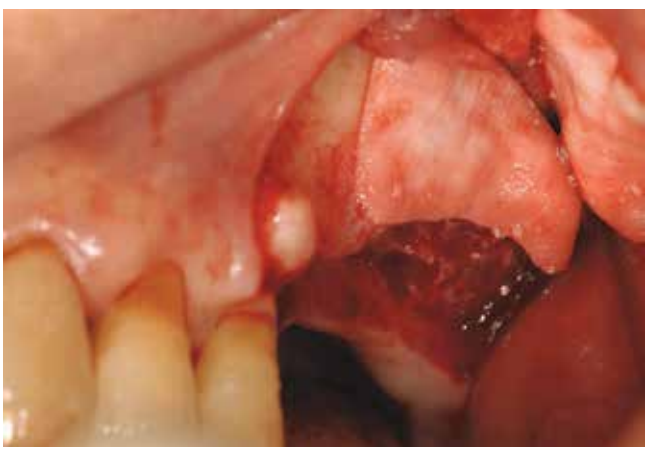

Fig. 4. The lateral wall is covered with a membrane.

Autogenous bone obtained from various donor sites from the patients performed with good results in bone regeneration and served as the gold standard (Tadjoedin et al., 2003). Various bone-grafting materials are being used in sinus augmentation including freeze-dried bone allograft (Avila et al., 2010), bovine xenograft (Chaushu et al., 2009), and alloplastic material (Bae et al., 2010) as an alternative or supplement to autogenous bone. Sinus augmentation procedures are highly predictable, with many studies reporting over 95% success (Del Fabbro et al., 2008). However, sinus augmentation may be influenced by the choice of graft material and implant surface micromorphology (Del Fabbro et al., 2008), and controversies still exist related to the most suitable way to perform sinus augmentation.

2. Type of graft

2.1 Autogenous graft

Although autogenous bone grafting is still the gold standard, according to the clinical results (Nkenke and Stelzle 2009), it is considered to have osteoinductive and osteoconductive properties, and it is known to contain growth factors (Turhani et al., 2005). Autogenous corticocancellous bone cores can be obtained intraorally or extraorally (Galindo-Moreno et al., 2007), however, this requires an additional second surgical donor site and additional morbidity (Park 2010).

A bone trap may be used to harvest bone debris during implant preparation, with additional bone collected by further drilling adjacent to the implant sites or by using a bone scraper to harvest cortical bone chips from the zygomatic buttress and from the lateral sinus wall before opening a bony window (Johansson et al., 2010). By using this approach, the autogenous bone can be achieved adjacent to the surgical site without the need for a remote second surgical donor site and additional morbidity (Park 2010). Furthermore, the amount of autogenous bone graft available for harvesting is limited and may be insufficient to fill large osseous defects (Mirzayan et al., 2001). Due to the complications associated with harvesting autogenous bone and its limited supply, many surgeons have sought bone-graft-substitute materials (Mirzayan et al., 2001).

2.2 Allograft

Allograft has been used for grafting in multiple intraoral applications such as periodontics (infrabony defects), oral surgery (extraction sites), and implant dentistry (ridge augmentation) (Froum et al., 2006). Allografts are bony tissue from a donor of the same species and they are known to contain no viable cells (Hallman and Thor 2008). This allograft may be prepared by demineralization of the bone in hydrochloric acid to expose bone morphogenetic protein and this demineralized freeze-dried bone allograft may be considered to have osteoconductivity and osteoinductivitiy (Kalish et al., 2008).

Atrophic maxillary floor augmentations were performed by mineralized human bone allograft in sinuses of different size, and the results showed that this material produced satisfactory bone formation (Maria Soardi et al., 2010). Histologic evaluation using mineralized freeze-dried bone allografts for sinus augmentation revealed a mean of 29.1% newly formed bone and graft particles were mainly in close contact with newly formed bone, primarily with features of mature bone with numerous osteocytes (Kolerman et al., 2008). Similar results were achieved with other reports that most of the specimens presented newly formed bone, and the interface areas between new and old bone were not discernible (Stacchi et al., 2008). There was no evidence of an acute inflammatory infiltrate in both studies (Kolerman et al., 2008; Stacchi et al., 2008).

However, histological evaluation revealed a chronic inflammatory reaction when demineralized freeze-dried bone was used in sinus augmentation, and the authors suggested that demineralized freeze-dried bone homografts may not be recommended to be used alone (Haas et al., 2002).

2.3 Xenograft

Deproteinized bovine bone is one of the most widely researched grafting materials and is used in the maxillofacial region because of its similarity to human (Hallman and Thor 2008). The complete absence of protein has been demonstrated, and its safety with respect to transmission of disease has been confirmed (Norton et al., 2003). Deproteinized cancellous bovine bone was placed as a grafting material for sinus floor elevation and histologic evaluation was performed after 6 months of healing (Valentini et al., 1998). Histologic evaluation showed that, in the grafted area and the previously existing area of the sinus floor, the bone was primarily of lamellar structure and intimate contact between newly formed bone and the particles of the graft was present (Valentini et al., 1998).

Dental implants installed in sinuses augmented with xenograft showed bone-to-implant contact of 27% to 63%, and xenograft was shown to be very slowly restored and seemed to behave as a semipermanent grafting material (Wallace and Froum 2003). If xenograft is used with autogenous bone, this may give additional advantages such as an increase in the volume of the graft and longer space-maintaining effects due to prolonged resorption (Galindo-Moreno et al., 2007).

2.4 Alloplastic materials

Alloplastic materials consist of a large group of chemically diverse synthetic calcium-based biomaterials, including calcium sulfate, calcium phosphate, bioactive glasses, and polymers (Hallman and Thor 2008), and these bone substitutes possess osteoconductive properties (Moore et al., 2001).

A study by Zijderveld, Zerbo, et al. showed that sinus augmentation using a limited volume of beta-tricalcium phosphate appeared to be a clinically reliable procedure, although autogenous bone grafting is still the gold standard (2005) (Zijderveld et al., 2005). This material is reported to act as an osteoconductive material and it allows osteoprogenitor cells to grow on its surface or into its porosity and differentiate into osteoblasts, thus bringing about bone deposition (Zerbo et al., 2005).

Calcium sulfate hemihydrate has been proposed as a grafting material for sinus augmentation, and this resulted in good, new tissue formation within the sinuses when clinically and radiographically evaluated. The histomorphometiric analysis revealed bone density of 34.3% to 55.54% (De Leonardis and Pecora 2000). Similar results were achieved by other investigators (Pecora et al., 1998), and the study done at two years revealed new irregular trabecular design was seen radiographically and revealed normal, vital trabecular bone with woven and lamellar structure in all the histollogically examined sections (Guarnieri et al., 2006).

2.5 Titanium granule

Resorption of grafting material may lead to unpredictable long-term results when rehabilitating the resorbed posterior maxilla. Nonresorbable, osteoconductive bone substitutes may therefore be an advantage over autogenous bone grafts. Titanium granules were used as

bone substitute in patients scheduled to receive augmentation of the sinus floor prior to or in conjunction with placement of dental implants (Bystedt and Rasmusson 2009).

3. Growth factors and the cell-based approach

3.1 Platelet-rich plasma

The use of platelet-rich plasma to enhance bone regeneration has been documented in periodontal defects, in extraction sockets, during implant placement, and in guided bone regeneration procedures around implants, including sinus augmentation (Kim et al., 2002). The use of platelet-rich plasma in conjunction with autogenous bone graft materials has been advocated for use in sinus augmentation surgery as a means of enhancing both quantity and quality of newly forming bone (Danesh-Meyer et al., 2001). The use of platelet-rich plasma is based on the premise that autogenous plasma, rich in platelets, contributes large quantities of mitogenic polypeptides such as platelet-derived growth factor, transforming growth factor-beta and insulin-like growth factor-I, thereby enhancing osteogenesis, improving soft tissue healing, and reducing postoperative morbidity (Danesh-Meyer et al., 2001; Boyapati and Wang 2006; Browaeys et al., 2007). The handling of the particulate bone grafts is reported to be improved due to the adhesive capacity of platelet-rich plasma via its hemostatic capacity of fibrin (Galindo-Moreno et al., 2007; Arora et al., 2010).

In some reports, sinus augmentations were performed successfully with greater bone maturation when using a composite graft of cortical autogenous bone, bovine bone, and platelet-rich plasma (Galindo-Moreno et al., 2007). A randomized clinical trial was performed to test the efficacy of platelet-rich plasma in sinus augmentation procedures (Badr et al., 2010). No appreciable clinical effect could be observed when using platelet-rich plasma with autologous iliac crest bone graft in the maxilla. No statistically significant differences were observed for soft tissue healing indices ($P=0.4$) and mean graft resorption ($P=0.5$) between groups (autogenous bone only vs. bone + platelet-rich plasma). The findings suggest that the addition of platelet-rich plasma to bone derivative/substitute materials may not significantly enhance bone formation in the maxillary sinus area (Danesh-Meyer et al., 2001).

The results from the other study showed that the marginal bone level measurements showed no significant differences, although there was a tendency toward less resorption on platelet-rich plasma sides. Resonance frequency analysis measurements showed statistically significantly higher implant stability quotient values for platelet-rich plasma sites at abutment connectios in the anterior but not in the posterior regions (Thor et al., 2005).

Theoretically, it seems to have significant beneficial effects on the soft and hard tissue healing; however, the disparity in study design, surgical techniques, and different outcome assessment variables used makes it difficult to assess the practical benefit of its clinical use (Arora et al., 2010). Although platelet-rich plasma has been extensively studied for over a decade, there are no definitive reports proving the benefit of using platelet-rich plasma in sinus augmentation procedures (Arora et al., 2010). Therefore, the use of platelet-rich plasma may not be recommended as a standard method to support bone regeneration for maxillary sinus augmentation (Schaaf et al., 2008).

3.2 Platelet rich fibrin

Platelet-rich fibrin, a second-generation platelet concentrate, is a leucocyte- and platelet-rich fibrin biomaterial (Dohan Ehrenfest et al., 2009). Platelet-rich fibrin belongs to a new

generation of platelet concentrates characterized by simplified processing and without biochemical blood handling (Dohan et al., 2006). Platelets are activated during platelet-rich fibrin processing by centrifugation, and massive degranulation allows the release of cytokine (Mazor et al., 2009). Platelet-rich fibrin is reported to release transforming growth factor-beta1, platelet-derived growth factor-AB and vascular endothelial growth factor up to the whole experimental time (Dohan Ehrenfest et al., 2009). Slow fibrin polymerization during platelet-rich fibrin processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes (Mazor et al., 2009). Thus, results showed that platelet-rich fibrin, unlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling, leading enhanced healing properties in experimental and clinical situations (Dohan et al., 2006).

Platelet-rich fibrin was used in sinus augmentation procedures both in lateral and crestal approaches. In the lateral approach, platelet-rich fibrin was applied with or without the graft material, and the use of autogenous bone in combination with platelet-rich fibrin glue as a grafting material for maxillary sinus augmentation with simultaneous implant placement was tested in dogs (Lee et al., 2007). The results showed that the use of autogenous bone mixed with platelet-enriched fibrin glue can achieve results superior to those for grafts of autogenous bone alone in terms of enhanced osseointegration of dental implants (40.5±14.4% vs. 32.3±12.0%) and increased height of new bone (12.0±1.0% vs. 10.7±1.0%) (Lee et al., 2007).

Other studies used no bone substitutes, and the biopsies were taken in the center of the regenerated osteotomy window of the sinus lift. Therefore, all of the observed bone must be considered new bone built starting from the sole platelet-rich fibrin matrix (Mazor et al., 2009). All biopsies showed well-organized and vital bone, often with more than 30% bone matrix (33% ± 5%).

Osteotome-mediated sinus floor elevation was performed using only platelet-rich fibrin, and of the 138 implants that had been placed, 97 have been restored function for an average loading time of 5.2 months with a survival rate of 97.8% (Toffler et al., 2010). Early review of the osteotome-mediated sinus floor elevation with platelet-rich fibrin technique presented for localized sinus floor elevation and implant placement demonstrates a high degree of safety and success at sites with 5- to 8-mm residual subantral bone height (Toffler et al., 2010).

3.3 Bone morphogenetic protein

Growth factors are present at low concentrations in bone matrix and plasma, but they execute important biologic functions (Hallman and Thor 2008). The growth factors are believed to have an osseous regenerative effect on the mesenchymal stem cells and contribute to cellular proliferation, matrix formation, collagen synthesis, and osteoid production (Yamada et al., 2008). Bone morphogenetic proteins are multi-functional growth factors belonging to the transforming growth factor-beta superfamily, and bone morphogenetic proteins are reported to have a variety of functions (Wang et al., 1990; Xiao et al., 2007). Bone morphogenetic protein molecules not only induce the formation of both cartilage and bone but also play a role in a number of non-osteogenic developmental processes (Xiao et al., 2007), and they are capable of inducing ectopic cartilage and bone formation, a process that mimics embryonic endochondral bone formation (Xiao et al., 2007). Bone morphogenetic protein 2, 4 and 7, in particular, are reported to be the most effective growth factors in terms of osteogenesis and osseous defect repair (Schilephake 2002), and three bone morphogenetic proteins— bone morphogenetic protein 2, 7, and 14—have been

applied in sinus augmentation procedures (Park 2009). The efficacy of Bone morphogenetic proteins for defect repair is strongly dependent on the type of carrier and has been subject to unknown factors in clinical feasibility trials resulting in ambiguous results.

Autogenous bone graft demonstrated greater increases in mineralization and probably angiogenesis of bone than the other bioimplants in rabbit sinus augmentation models when bone morphogenetic protein was delivered at the same time (Hu et al., 2010).

The effectiveness of recombinant human bone morphogenetic protein-2 on an absorbable collagen sponge was compared with an autogenous bone graft when used for two-stage maxillary sinus floor augmentation (Triplett et al., 2009). No marked differences were found in the histologic parameters evaluated between the two groups, but the induced bone in the recombinant human bone morphogenetic protein-2/absorbable collagen sponge group was significantly denser than that in the bone graft group (Triplett et al., 2009). Additionally, collagen sponge was shown to induce new bone equally as well as the other composite material when loaded with bone morphogenetic protein (Pekkarinen et al., 2005). The effects of mineralized bone replacement grafts (e.g., xenografts and allografts) on recombinant human bone morphogenetic protein-2/absorbable collagen sponge were tested, and bone graft densities tended to increase more with the xenograft than with the allograft (Tarnow et al., 2010). The increased density in allograft cases was likely the result of both compression of the mineralized bone replacement graft and vital bone formation, seen histologically (Tarnow, Wallace, et al., 2010). Loss of volume was greater with the four-sponge dose than the two-sponge dose because of compression, and resorption of the sponges and vital bone formation in the allograft cases ranged from 36% to 53% (Tarnow et al., 2010). Hydroxyapatite, biphasic tri-calcium phosphate-hydroxyapatite, and natural coral are reported to be equally good as framing material for bone morphogenetic protein (Pekkarinen et al., 2005).

Deproteinized bovine bone mineral is known to show excellent osteoconductive properties, and it has been used for sinus augmentation clinically (Terheyden et al., 1999). The bovine xenograft composite promotes formation of new bone in a similar fashion to autogenous bone when bone morphogenetic proteins were bound to the graft material, and could therefore be considered a biomaterial with potential applications as a bone substitute in maxillary sinus floor augmentation (Sicca et al., 2008).

It has been shown that beta-tricalcium phosphate is suitable as a biodegradable, highly biocompatible, and osteoconductive carrier for bone morphogenetic proteins in sinus augmentation procedures (Gruber et al., 2008). Both experimentally and clinically, recombinant human bone morphogenetic protein-14/beta-tricalcium phosphate was found to be effective and safe as the control treatment with autologous bone mixed with beta-tricalcium phosphate in sinus floor augmentation (Koch et al., 2010). Self-setting alpha-tricalcium phosphate was used in maxillary sinus augmentation, and it was shown to have bone-conductive activity and shown to maintain the rigidity of implanted bone screws (Marukawa et al., 2010).

Composite material has been used for bone morphogenetic protein-2 release in mandibular defect areas (Wen, Karl, et al., 2011). While in-vitro bone morphogenetic protein-2 release was highest for the polyethylene glycol hydrogel, the scaffold most successful in vivo was the collagen-hydroxyapatite composite infused with polyethylene glycol-hydrogel scaffold because it had the necessary mechanical strength to perform well in the mandibular bone environment (Wen et al., 2011). The in vitro release studies suggested a threshold dose of 5

μg that was confirmed by the in vivo dose response studies (Wen, Karl, et al., 2011). Many studies have demonstrated the potential for the bone morphogenetic proteins to increase bone formation in sinus augmentation procedures and further studies are warranted to find an appropriate carrier scaffold for the optimal release of bone morphogenetic proteins (Park 2009).
Bone morphogenetic protein-7 was loaded onto a poloxamer carrier or demineralized bone matrix in a poloxamer carrier (Ho et al., 2010). These bioimplants had more rapid initial bone production than all other materials, including autogenous bone, and these bone morphogenetic protein-containing bioimplants demonstrated promise as alternatives to autogenous bone grafts for sinus-augmentation procedures (Ho et al., 2010).
In the future, such biomaterials may enable earlier placement of dental implants into augmented maxillary sinuses.

3.4 Platelet-derived growth factor

Platelet-derived growth factor plays an important role in inducing proliferation of undifferentiated mesenchymal cells (Schilephake 2002); it is a stimulator of the proliferation and recruitment of both bone cells and periodontal ligaments (Nevins et al., 2003). It is an important mediator for bone healing and remodeling during trauma and infection, and it is reported to enhance bone regeneration in conjunction with other growth factors (Schilephake 2002).
Recombinant human platelet-derived growth factor-BB was used in simultaneous vertical guided bone regeneration and guided tissue regeneration in the posterior maxilla (Urban et al., 2009). The results demonstrated successful use of recombinant human platelet-derived growth factor-BB in conjunction with autogenous bone, anorganic bone mineral, and barrier membranes to reconstruct severe alveolar bone defects (Urban et al., 2009). A significant amount of periodontal bone gain was achieved in close apposition to a previously denuded root surface, giving the possibility of vertical periodontal regeneration (Urban et al., 2009).
The use of purified recombinant human platelet-derived growth factor-BB mixed with bone allograft in humans is reported to result in periodontal regeneration in both Class II furcations and interproximal intrabony defects in human Class II furcation defects (Nevins et al., 2003).
The regenerative outcomes in maxillary sinus augmentation procedures were tested when recombinant human platelet-derived growth factor-BB was combined with particulate anorganic bovine bone mineral (Nevins et al., 2009). The surgical outcomes in all treated sites were uneventful at 6 to 8 months, with sufficient regenerated bone present. Large areas of dense, well-formed lamellar bone were seen throughout the intact core specimens, and a number of cores demonstrated efficient replacement of the normally slowly resorbing anorganic bovine bone mineral matrix particles with newly formed bone.

3.5 Mesenchymal stem cell

Long-term success of dental implants has been demonstrated when placed simultaneously with or after a sinus augmentation procedure (Gonshor et al., 2011). However, optimal bone formation can be from 6 to 9 months or longer with grafting materials other than autogenous bone (Pieri et al., 2008). Various osteoconductive materials have been applied to augment the sinus floor, but these materials are cell-free and may require more time for bone healing (Pieri et al., 2008).

Bone marrow contains a population of rare progenitor cells capable of differentiating into bone, cartilage, tendon, and other connective tissues, and these mesenchymal stem cells can be purified and culture-expanded from animals and humans and have been shown to regenerate functional tissue when delivered to the site of musculoskeletal defects in experimental animals (Bruder et al., 1998). Cells can be also be achieved intraorally from the maxillary tuberosity and the periosteum of the mandible (Turhani et al., 2005; Springer et al., 2006) The study was done to assess whether differences occur in bone formation after maxillary sinus floor elevation surgery with bovine bone mineral mixed with autogenous bone or autogenous stem cells (Rickert et al., 2010). Mesenchymal stem cellseeded on xenograft particles can induce the formation of a sufficient volume of new bone to enable the reliable placement of implants within a time frame comparable to that of applying either solely autogenous bone or a mixture of autogenous bone and xenograft; this technique could be an alternative to using autografts (Rickert et al., 2010).
Sinus augmentation procedures using either an allograft cellular bone matrix, containing native mesenchymal stem cells and osteoprogenitors, or conventional allograft showed similar results (Gonshor et al., 2011). Histomorphometric analysis of the allograft cellular bone matrix grafts revealed average vital bone content of 32.5% ± 6.8%, but results for the conventional allograft showed an average vital bone content of 18.3% ± 10.6% at an average healing period of 3.7 ± 0.6 months (Gonshor et al., 2011).
An ovine split-mouth study was applied to compare bovine bone mineral alone and in combination with mesenchymal stem cells regarding their potential in sinus augmentation (Sauerbier et al., 2010). The authors concluded that the high percentage of vital bone content, after a relatively short healing phase, may encourage a more rapid initiation of implant placement or restoration when a cellular grafting approach is considered (McAllister et al., 2009; Sauerbier et al., 2010).

4. Conclusion

Sinus augmentation procedures have been used clinically with very high success rates. Bone-substitute materials are reported to be as effective as autogenous bone when used alone or in combination with autogenous bone. It may be concluded that bone substitutes can be successfully used for sinus augmentation, reducing donor-site morbidity. Attempts have been made to accelerate bone formation with different scaffolds, growth factors, and mesencymal stem cells. Further studies are needed to find an optimal approach that can enhance bone formation in sinus augmentation procedures.

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2

Biomaterials Applicable for Alveolar Sockets Preservation: In Vivo and In Vitro Studies

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1. Introduction

Bone density and quantity are primary conditions for the insertion and stability of dental implants. In cases of a lack of adequate maxillary or mandibulary bone, e.g. in terms of front to back depth or thickness, bone augmentation will be necessary. Lack of bone or bone defects can be caused by inflammation, congenital malformation, trauma or oncological surgery. Several procedures and materials for augmenting bone height have been developed to overcome the problem of a reduced amount of bone. In dentistry, bone substitution materials were used for the following applications: (1) socket preservation; (2) periodontal defects; (3) third molar extraction sites to support 2nd molars; (4) ridge augmentation; (5) defects following cyst removal / apicoectomies; (6) sinus lifts; (7) distraction osteogenesis; and (8) implant dentistry. The treatment of bone-defects and socket preservation include autografting (from one location to another within the same individual), xenografting (from a donor of another species) and allografting (from a genetically dissimilar member of the same species) cancellous bone. After blood, bone is the most commonly transplanted tissue. Worldwide, an estimated 2.2 million grafting procedures are performed annually to repair bone defects in orthopaedics, neurosurgery, and dentistry (Giannoudis et al. 2005). The increasing number of grafting procedures and the disadvantages of current autograft and allograft treatments (e.g. limited graft quantity, risk of disease transmission) drive the quest for alternative methods to treat bone defects.

The use of synthetic bioactive bone substitute materials is of increasing importance in modern dentistry as alternatives to autogenous bone grafts. Various alloplastic bone substitution materials of different origin, chemical composition, and structural properties have been investigated in the last years. The materials commonly used in all approaches are ceramics, polymers or composites (Burg et al. 2000). These alloplastic materials are either absorbable or non-absorbable, as well as naturally derived or synthetically manufactured (Figure 1).

Various types of biomaterials (minerals and non-mineral based materials as well as natural and artificial polymers) with different characteristics have been used for studying ossification and bone formation. For example, calcium phosphate ceramics include a variety of ceramics such as hydroxyapatite, tricalcium phosphate, calcium phosphate cement, etc. These mentioned ceramics have excellent biocompatibility and bone bonding or bone regeneration properties. Recently non-biodegradable and degradable membranes have been

tested for their appliance in bone defects (Zhao et al. 2000). Cell-biomaterial interactions depend on surface characteristics, e.g. chemistry or topography. Surface characteristics determine the ionic exchange dynamics and the protein attachment as well as cell attachment, cell proliferation and cell differentiation.

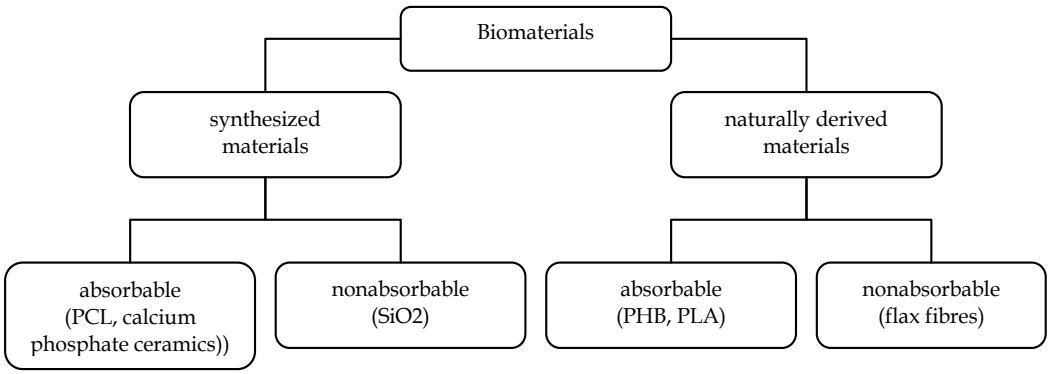

Fig. 1. The classification of used biomaterials for bone tissue engineering modified according to Burg (Burg et al. 2000)

Xenogenic grafting has been shown to be one of the most effective methods of creating bone in areas where there is none, whereas alloplastic graft material constitutes the second of the most popular forms of bone grafting material in dentistry.

2. Bone regeneration

Bone is a dynamic tissue that constantly undergoes remodelling. The signal that initiates bone remodelling has not been identified, but there is evidence that mechanical stress can alter local bone architecture. This can be sensed by osteocytes followed by secretion of paracrine factors such as insulin-like growth factor (IGF-1) in response to mechanical forces (Lean et al. 1996). Bone formations result from a complex cascade of events that involves proliferation of primitive mesenchymal cells (osteoinduction), differentiation into osteoblast precursor cells, maturation of osteoblasts, formation of matrix, and finally mineralization. Osteoblasts converge at the bottom of the resorption cavity and form osteoid which begins to mineralize after 13 days. The osteoblasts continue to form and mineralize osteoid until the cavity is filled (Hill 1998). Some of the osteoblasts differentiate into osteocytes and become embedded in the matrix.

Even if pre-existing osteoblasts may help to form new bone, it is generally agreed that such osteoblasts only contribute a minor portion of the new bone needed in a fracture-healing situation. The initial event must be the chemotactic attraction of osteoblasts or their precursors to sites of the resorption defect. This is likely to be mediated by the release of local, biochemical and biophysical messengers. The second event involved in the formation phase of the coupling phenomenon is proliferation of osteoblast precursors. This is likely to be mediated by osteoblast-derived growth factors and those growth factors released from bone during the resorption process. The third event of the formation phase is the differentiation of the osteoblast precursor into the mature cell. The differentiating osteoprogenitor cells express

several bone matrix macromolecules, namely alkaline phosphatase, type I collagen, bone sialoprotein, osteopontin, Cbfa1 and osteocalcin (Hill 1998).

It is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblast (Frost 1964). Bone remodelling occurs in small packets of cells called basic multicellular units (BMU), which turn bone over in multiple bone surfaces (Frost 1991). BMU consist of osteoblasts, other bone-forming cells such as osteocytes and bone-lining cells, bone-resorbing cells - osteoclasts, the precursor cells of both, and their associated cells like endothelial cells and nerve cells (Papachroni et al. 2009). Osteoblasts are key components of the bone multicellular unit and have a seminal role in bone remodelling, which is an essential function for the maintenance of the structural integrity and metabolic capacity of the skeleton. Osteoblasts originate from the non-hematopoietic part of bone marrow, which contains a group of fibroblast-like stem cells with osteogenic differentiation potential, known as the mesenchymal stem cells (MSCs) and also referred as skeletal stem cells (SSCs), bone marrow stromal cells (BMSCs) and multipotent mesenchymal stromal cells (MMSCs) (Abdallah and Kassem 2008; Heino and Hentunen 2008). MSCs are capable of multi-lineage differentiation into mesoderm-type cells such as osteoblasts, adipocytes and chondrocytes (Dezawa et al. 2004; Luk et al. 2005). Osteoblast growth and differentiation is determined by a complex array of growth factors and signalling pathways. The following three families of growth factors influence the main aspects of osteoblast activity and induce, mediate or modulate the effects of other bone growth regulators:

- the transforming growth factor-β (TGF-β) family
- the insulin-like growth factors (IGFs)
- bone morphogenetic proteins (BMPs) (Zhou et al. 1993; Mundy 1994; Bikle 2008).

Furthermore, other growth factors, such as the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) as well as platelet derived growth factor (PDGF) are involved in bone formation.

Many growth factors involved in the natural process of bone healing have been identified and tested as potential therapeutic candidates to enhance the regeneration process. *In vitro*, BMPs can differentiate mesenchymal stem cells into the osteoblastic phenotype (Cheng et al. 2003; Luu et al. 2007). Furthermore, the osteoinductive BMPs (e.g. BMP-2/-4/ and BMP-5 to -8) can initiate the complete cascade of bone formation when implanted ectopically (Wozney 2002). In general, TGF-β stimulates migration of skeletal stem cells and regulates cell proliferation, cell differentiation, and extra cellular matrix synthesis (Bostrom and Asnis 1998; Janssens et al. 2005). Stimulatory effects on bone healing and bone formation have been evaluated in various experimental settings (Janssens et al. 2005). FGFs are considered potent regulators of cell growth and wound healing and have mitogenic effects. The best studied member of FGFs is FGF-2. Although FGF-2 alone is not capable of inducing ectopic bone formation, it plays an important role in the regulation of normal bone healing (Marie 2003). In various animal studies exogenous recombinant FGF-2 enhances callus formation and stimulates bone healing (Kato et al. 1998; Nakamura et al. 1998; Radomsky et al. 1998). IGFs are the most abundant growth factors produced by bone cells and are stored at the highest concentration of all growth factors in the bone matrix. It was shown that IGF has an anti-apoptotic effect on pre-osteoblast cells and enhances bone matrix synthesis (Niu and Rosen 2005). In animal models and clinical trials of osteoporosis, systemic IGF infusion showed an increase in bone formation, bone volume, and / or bone turnover (Rosen 2004). VEGF is considered one of the key regulators of angiogenesis during bone formation

(Gerstenfeld et al. 2003). In various experimental models the stimulation of the bone regeneration process has been shown in response to VEGF administration (Street et al. 2002; Eckardt et al. 2005). In addition, PDGF regulates general tissue repair as well as enhances proliferation of various bone cell types and angiogenesis (Hollinger et al. 2008).

Mechanisms that promote skeletal tissue specificity are necessary, because none of these growth factors are specific for cells in the osteoblastic lineage and these involve interactions with other circulating hormones in addition to the action of specific intracellular mediators on bone-specific transcription factors. It is certain that bone remodelling is regulated by systemic hormones and by local factors (Canalis 1983), which affect cells of both the osteoclast and osteoblast lineages and exert their effects on the replication of undifferentiated cells, the recruitment of cells, and the differentiated function of cells (Hill 1998). The following hormones are involved in bone formation:

- Calcitonin (CT) – decreases the reabsorption of calcium from bones thereby lowering blood calcium levels. It can inhibit osteoclastic bone resorption and probably osteocytic osteolysis (Weiss et al. 1981; Wallach et al. 1993).
- Estrogens and androgens - slow the rate of bone remodelling and protect against bone loss. They help retain calcium in bones, thereby maintaining a strong bone matrix. Sex steroids not only influence the accrual of bone mass and bone mineral density but also bone growth (Manolagas et al. 2002; Bertelloni et al. 2010).
- Growth hormone (GH) - is one of the most important regulator substances for both bone growth and bone remodelling. It directly increases longitudinal bone growth by stimulating prechondrocites. GH is a polypeptide complex that regulates the processes of bone physiology, increases the rate of mitosis of chondrocytes and osteoblasts, and increases the rate of protein synthesis (collagen, cartilage matrix, and enzymes for cartilage and bone formation) (Ohlsson et al. 1998; Calvo-Guirado et al. 2011).
- Insulin – increases energy production from glucose. It has been established as an osteoblast regulator. In bone organ culture, insulin stimulates collagen synthesis at periphysiological hormone concentrations (Wettenhall et al. 1969; Cornish et al. 1996).
- Parathyroid hormone (PTH) - a major systemic regulator of bone metabolism, increases the reabsorption of calcium from bones to the blood, thereby raising blood calcium levels and increases the absorption of calcium by the small intestine and kidneys. The anabolic effect of intermittent PTH on bone has made it an effective treatment for osteoporosis in humans, where it was shown to increase bone mass and reduce fracture rate (Neer et al. 2001).
- Thyroxine – increases the rate of protein synthesis and increases energy production from all food types. It directly stimulates the metabolic activity of bone cells and profoundly affects bone turnover. Thyroxine influences the responsiveness of bone cells to 1,25-dihydroxycholecalciferol, parathyroid hormone, and calcitonin (High et al. 1981).

Besides growth factors and regulating hormones, expression of transcription factors is necessary and sufficient for mesenchymal cell differentiation. Runx2 is an essential bone-specific transcription factor. It was recently shown, that the complete Runx2 gene inactivation in transgenic mice leads to complete lack of intramembraneous and endochondral ossification owing to lack of mature osteoblasts (Komori et al. 1997). In addition, heterozygous Runx2 mice demonstrate specific skeletal abnormalities that are characteristic of the human heritable skeletal disorder cleidocranial dysplasia (Otto et al. 1997).

3. Pre-conditions of bone surrogates

Selection of graft materials is based on operator preference, type and size of the bone defect, resorbability of graft material as well as cost and patient acceptance. Furthermore, bone graft materials are generally evaluated based on their osteogenic, osteoinductive, or osteoconductive potential. Osteoinduction is a basic biological mechanism that occurs regularly, e.g. in fracture healing but also in implant incorporation (Albrektsson and Johansson 2001). Osteoconduction means that bone grows on a surface and is defined as the ability to stimulate the attachment, migration, and distribution of vascular and osteogenic cells within the graft material (Albrektsson and Johansson 2001). Several physical characteristics can affect the graft osteoconductivity, including porosity, pore size, and three-dimensional architecture. In addition, direct interactions between matrix proteins and their appropriate cell surface receptors play a major role in the host response to the graft material. An osteoconductive material guides repair in a location where normal healing will occur if left untreated (Kulkarni et al. 1971; Helm and Gazit 2005).

The ability of a graft material to independently produce bone is termed its direct osteogenic potential. The main critical considerations in bone tissue-engineering scaffold design are:

- Promotion of bone in-growth
- Average pore sizes approximately 200-400 μm
- Sterilization without loss of properties
- Absorbation with biocompatible components
- Good bony apposition
- Correct mechanical and physical properties for application
- Maximal bone growth through osteoinduction and/or osteoconduction
- Adaptation to irregular wound site (malleableness)
- Absorbation in predictable manner in concert with bone growth
- Availability to surgeon on short notice (Peter et al. 1998; Burg et al. 2000)

To have direct osteogenic activity, the graft must contain cellular components that directly induce bone formation. Polymers have been shown to be an excellent substrate for cellular or bioactive molecule delivery. They can differ in their molecular weight, polydispersity, crystallinity, and thermal transitions, allowing different absorption rates. Their relative hydrophobicity and percent crystallinity can affect cellular phenotype (Hollinger and Schmitz 1997). Various types of biomaterials (minerals and non-mineral based materials as well as natural and artificial polymers) with different characteristics have been used for studying ossification and bone formation. For example, calcium phosphate ceramics include a variety of ceramics such as hydroxyapatite, tricalcium phosphate, calcium phosphate cement, etc. Local tissue responses to polymers *in vivo* depend on the biocompatibility of the polymer as well as its degradative by-products (Hollinger and Battistone 1986). The mentioned ceramics have excellent biocompatibility and bone bonding or bone regeneration properties. They have been widely used in no or low load-bearing applications (Milosevski et al. 1999). Furthermore, natural polymers like collagen have been used for bone tissue engineering purposes (Hutmacher et al. 2001a; Lauer et al. 2001). Recently non-biodegradable and degradable membranes have been tested for their appliance in bone defects (Zhao et al. 2003). Scores of artificial polymers of diverse character are already in use for bone supply. One of them, poly(3)hydroxybutyrate (PHB) due to its form stability combined with little inflammatory response after implantation, may serve as a scaffold for tissue engineering (Gogolewski et al. 1993; Schmack et al. 2000).

4. Alloplastic materials in dentistry

Today, the above-mentioned clinical problems are solved by bone grafting. Autogeneic bone graft from the iliac crest is the main source of trabecular bone. It has a good osteoinductive capacity, but the sources are limited and the harvest procedure causes postoperative discomfort to the patient. Allogeneic bone is also widely used but provides mainly osteoconductive properties. Moreover, despite of extensive testing, there are still potential risks for transmitting diseases. Synthetic or alloplastic materials are alternative materials to avoid the drawbacks of autografts or allografts, because alloplastic materials show very little risk of morbidity and mortality. Alloplastic materials are divided into two main groups: (1) non-resorbable materials that acts as replacement bone and (2) resorbable frameworks or scaffolds for bone to grow into at its normal rate. A large percentage of alloplastic biomaterials are based on one raw material, such as hydroxyapatite (HA) and tri-calcium phosphate (TCP) or bioactive silicates (SiO2). Recent developments have focused on composites with different chemical phases, such as HA+TCP (Straumann® BoneCeramic, Straumann, Freiburg, Germany), SiO2+HA (Nanobone®,Artoss, Rostock, Germany) or SiO2+Ha+TCP (BonitMatrix®, DOT, Rostock, Germany).

4.1 Homogenous chemical composition

Until now HA-based biomaterials are the most abundant materials used in modern bone substitution. At the moment calcium phosphate cements play a secondary role in dentistry, although they often have an excellent biocompatibility (Noetzel and Kielbassa 2005). For HA scaffolds both pore size and porosity have effects on mineralisation and bone formation (Harris and Cooper 2004; Kruyt et al. 2004).

In vitro testing is used primarily as a first stage test for acute toxicity and cytocompatibility. A precondition for osteoconductivity and osteoinductivity are proliferating cells. Reichert and coworkers could show that neither Cerasorb® (TCP; Curason AG, Sonneberg, Germany), Geistlich Bio-Oss® (HA; Geistlich Pharma, Wolhusen, Germany) nor Perioglas® (SiO2; Novabone, Jacksonville, U.S.A.) has a negative influence on cellular proliferation, as compared to the control (Reichert et al. 2009). In fact, the tested bone substitution materials led to an increased AlamarBlue reduction over the observation period of 7d. Only PerioGlas® showed slight, but not significant decrease in AlamarBlue reduction, compared to controls (Reichert et al. 2009). The effect on the proliferation of osteoblast *in vitro* was investigated using phytogene HA (Algipore®), TCP (Bio-Base®) and bovine HA (Bio-Oss®). Kübler et al. have found that human osteoblast cells seeded with Bio-Oss® showed the lowest proliferation and differentiation rate after comparison with the other tested bone graft materials (Kubler et al. 2004). Furthermore, there were no obvious differences in cell migration and growth behaviour between BioOss® and Vitoss® scaffolds, but significantly higher osteocalcin expression in cells seeded on BioOss® scaffolds (Payer et al. 2010). In contrast, DNA content, LDH (lactate dehydrogenase) and alkaline phosphatase activity as well as expression of bone-related genes, such as alkaline phosphatase, osteonectin, osteopontin and bone sialoprotein II revealed proliferation and osteogenic differentiation of osteoblasts on Cerasorb®, but not on BioOss® during cultivation over 28 days (Bernhardt et al. 2010).

Bioactive glass ceramic was seeded with human primary osteoblasts and evaluated after 2, 6 and 12 days. Incubation with Bioglass 45S5 increased human osteoblast proliferation to 155% as shown by flow cytometric analysis (Xynos et al. 2000b). Analysis of osteoblast specific markers, such as alkaline phosphatase and osteocalcin indicate that Bioglass

advanced osteoblast augmentation. Furthermore, expression of IGF2 was 2,9fold increased compared to control cells (Xynos et al. 2000a).

However, *in vitro* characterization is not able to demonstrate the tissue response to materials. Animal models are essential for evaluating biocompatibility. Bone substitution materials, such as hydroxyapatite and β-tricalcium phosphate were shown to be osteoinductive (Okumura et al. 1997; Habibovic et al. 2008; Sun et al. 2008). The *in vivo* bone-regenerative capacity of calcium silicate was investigated in rabbits and results were compared with TCP. Micro-CT and histomorphometric analysis showed resorption and newly formed bone with both materials, but resorption as well as bone formation of calcium silicate was higher than that of TCP (Xu et al. 2008). Wistar rats were used to histologically evaluate the healing of surgically created defects on the tibia after implantation of bioactive glass. Bioactive glass promoted comparable bone formation independently of the size of glass granules (Macedo et al. 2005). Also new bone formation was found after implantation of NovaBone 45S5 bioglass particulate (NovaBone, Jacksonville, USA) in sheep. In addition, acute as well as chronic inflammatory reactions were associated with the use of these glass granules (Kobayashi et al. 2010). Furthermore, cylindrical porous hydroxyapatite implants were implanted in rabbit femurs. Histological analyses of bone sections with toluidine blue showed new bone formation (Damien et al. 2003). Various publications discussed the use of porous hydroxyapatite in maxillary sinuses. Histologically, the grafted sinuses exhibited a significant amount of new bone formation. The porous hydroxyapatite granules appeared integrated with the new formed bone (Browaeys et al. 2007).

To date, predominantly histological staining and immunohistochemical analyses were used to study the behaviour of bone substitute materials on bone graft healing. Only a few numbers of studies present the molecular mechanisms of bone formations associated with bone substitutes. Recently it was shown, that bovine hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) up-regulate the expression of Runx2, Alpl and osteocalcin in biopsies from human jaws (Gotz et al. 2008).

Calcium phosphate cements and sintered ceramics as well as calcium sulphate cements and bioglass have been used as BMP carriers (del Real et al. 2002; den Boer et al. 2003; Kroese-Deutman et al. 2005). Synthetic porous hydroxyapatite might be providing a delivery system for bioactive agents. It was recently shown that the supplementation of a synthetic porous hydroxyapatite with IGF-1 significantly increased new bone formation and bone mineral apposition rate compared with hydroxyapatite alone (Damien et al. 2003). In contrast, the resorption of IGF1, TGFbeta1 or bFGF onto a carrier of TCP does not enhance new bone formation (Clarke et al. 2004). A multi-centre, randomized clinical trial using recombinant human platelet-derived growth factor with β-TCP for the treatment of intra-osseous periodontal defects showed significantly higher extent of linear bone growth and per cent bone fill compared to β-TCP alone after six months (Jayakumar et al. 2011).

An example for commercially available products containing growth factors is Gem 21S™ (Osteohealth, Shirley, USA), β-tricalciumphosphate and recombinant human PDGF.

4.2 Composites with different chemical phases

Advances in biomaterial fabrication have introduced numerous innovations in designing scaffolds for tissue engineering. The combination of different bone substitution materials is an important research topic. Biomaterials on the basis of calcium phosphates are most widely used in craniofacial bone surgery and considered to be biocompatible, non-

immunogenic and osteoconductive (Ruhé et al. ; Abukawa et al. 2006; Weinand et al. 2006). It is well know that the surface topography has an influence on cell attachment. Figure 2 showed surface topographies of three different commercially available bone substitution materials, especially Cerasorb®, Straumann® BoneCeramic and NanoBone®.

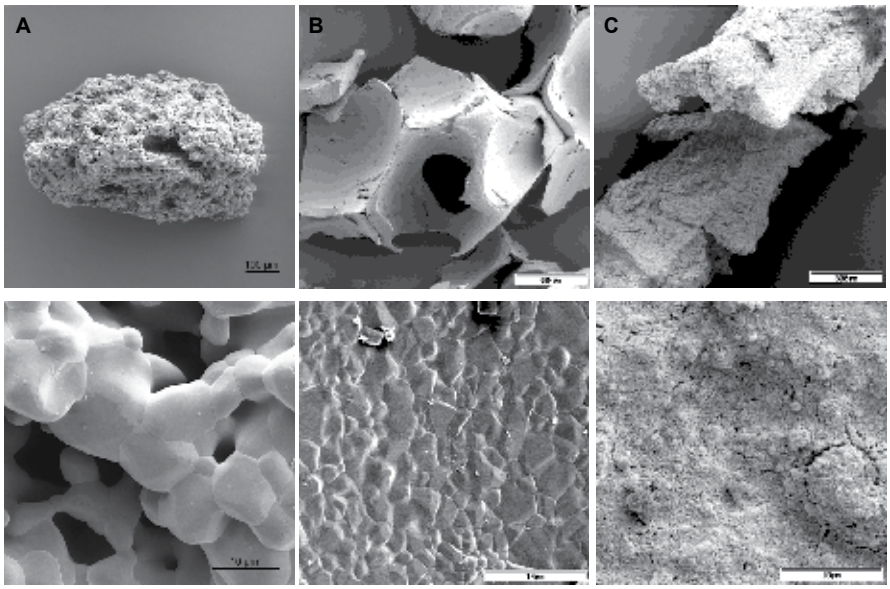

Fig. 2. Scanning electron micrographs of different biomaterials on the basis of calcium phosphates at different magnifications. (A) Cerasorb® (Curason AG, Germany), (B) Straumann® BoneCeramic (Straumann, Freiburg, Germany), (C) NanoBone® (Artoss, Rostock, Germany)

The composition of bioceramics influences the cell attachment and proliferation. It was shown that incorporation of zinc or silicate in calcium phosphate ceramics is followed by an increase of osteoblast attachment and proliferation (Ishikawa et al. 2002; Thian et al. 2005), whereas carbonate had contradictory effects on these cells (Redey et al. 2000).
Viability, proliferation and growth characteristics of fibroblasts cultured together with NanoBone® and Straumann® BoneCeramic were analysed over a period of 28 days. Fibroblast viability does not show any differences between cells incubated with the different bone graft materials and the control cells. Electron microscopy showed that fibroblast cells grew and proliferated at the surface of both bone graft materials (Kauschke et al. 2006). The same results could be observed by Reichert and coworkers (Reichert et al. 2009). They show no differences in the cellular proliferation between control cells and cells incubated with Straumann® BoneCeramic, NanoBone® and BonitMatrix® (Reichert et al. 2009). Furthermore, endothelial cells predominantly spread on BonitMatrix® and cells expressed endothelium-specific surface marker proteins (Thimm et al. 2008).
The biocompatibility of Straumann® BoneCeramic, NanoBone® and BonitMatrix® were tested using different animal models. A rapid vascularization and good integration within the peri-implant tissue was shown for BonitMatrix® after subcutaneously implantation in Wistar rats (Ghanaati et al. 2010).
In vivo studies using the mouse dorsal skinfold chamber model revealed a high biocompatibility comparable to that of cancellous bone for NanoBone®. Both NanoBone

granules and plates do not show any venular leucocyte activation after implantation. Furthermore, signs of angiogenesis could be observed (Abshagen et al. 2009). Vascularization as well as osteoneogenesis after implantation of NanoBone® was observed using guinea pigs (Punke et al. 2008). Our own group could establish a FE model of remodelling processes occurring in a bone area provided with bone graft substitutes (Gedrange et al. 2008). MicroCT images revealed specimen changes in the spongious and compact areas provided with the bone substitution material (Figure 3). The use of NanoBone® as bone substitution material seems to decrease the bone atrophy after teeth extraction in pigs as compared to untreated alveolars, but the reduction is not significant (Figure 4).

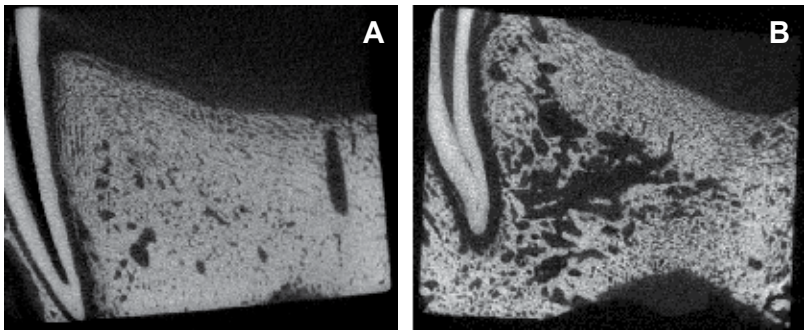

Fig. 3. MicroCT images of embedded bone specimens showing the remodelling of the area provided with NanoBone®. (A) NanoBone treated animal; (B) control animal (Gedrange et al., 2008).

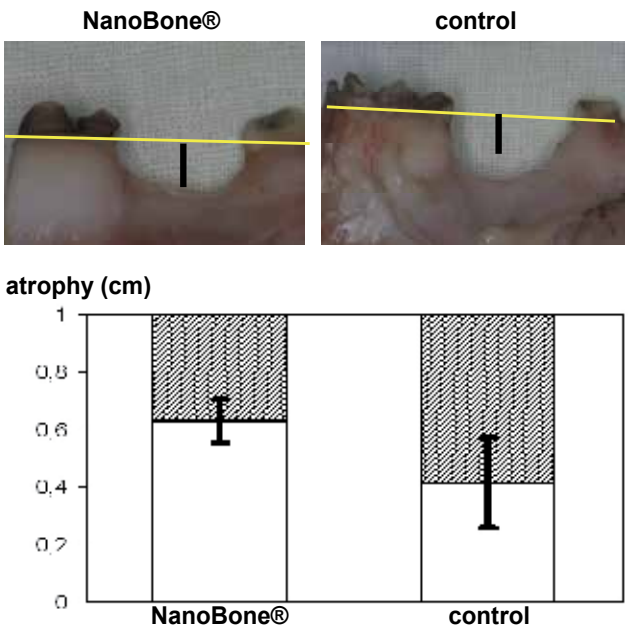

Fig. 4. Measurement of alveolar bone atrophy 70 days after teeth extraction and animal treatment with or without NanoBone®

The ideal substrate for the synthesis of bone should be able to promote the expression of the osteoblastic phenotype as well as provide a template for bone deposition (Kaufmann et al. 2000). An osteoblastic phenotype expression on the surface of hydroxyapatite ceramics, with subsequent gene expression of osteocalcin was found (Okumura et al. 1997). Recently it has been shown that OSSA NOVA and BONITmatrix® could stimulate bone regeneration of surgically created cranial defects in rats, but OSSA NOVA leads, in comparison with granular BONITmatrix®, to an accelerated more comprehensive bone regeneration (Kunert-Keil et al. 2009). Studies from our lab demonstrated an increased expression of the IGF1 mRNA in cranial defects treated with BONITmatrix® and OSSA NOVA (Gredes et al. 2010a). The increase in the IGF1 expression was followed by an accelerated, more comprehensive bone regeneration using both synthetic biomaterials consisting of calcium phosphates embedded in a silica matrix (Gredes et al. 2010a). Furthermore, Real-time RT-PCR analyses showed that the amount of the osteocalcin and Alpl mRNA was significantly increased after treatment with BONITmatrix®. It was recently shown that mesenchymal stem cells differentiated to osteoblasts within 14 days on hydroxyapatite and BONITmatrix® in expansion medium with and without osteogenic differentiation additives (Muller et al. 2008). Cells revealed a higher Alpl activity and increased mRNA expression of osteocalcin and collagen 1 (Muller et al. 2008). Another bone graft material consisting of nanocrystalline hydroxyapatite embedded in a porous silica gel matrix, Nanobone® was implanted in the mandible of minipigs. It was shown that Nanobone® is able to stimulate the differentiation of bone cells into osteoblasts and osteoclasts, because osteocalcin, alkaline phosphatase, osteopontin and BMP-2 were located in newly formed bone and focally in particles (Gerber et al. 2006). Furthermore, the matrix of all Nanobone® granules showed very strong immunoreactivity of osteocalcin and alkaline phosphatase in human jaw bone augmented with Nanobone® (Gotz et al. 2008). In addition, two other common markers for osteogenesis, mineralization and bone remodelling, namely Runx2 and Phex were analysed. Similar to the outcomes of other bone substitution materials like NanoBone® and their influence on bone healing process (Gotz et al. 2008), the expression of Runx2 gene was unaltered after application of BONITmatrix® and OSSA NOVA. Otherwise BONITmatrix® slightly increased the expression of the osteogenic transcription factor Runx2 in mesenchymal stem cells (Muller et al. 2008). In addition, it was suggested that diverse forms of hydroxyapatite nanoparticles may differentially affect the osteoblast cell function as shown on gene and protein levels (Xu et al. 2009). That NanoBone® could up-regulate the expression of Runx2, Alpl and osteocalcin was also shown in sinus lift procedures in rabbits (De Souza Nunes et al. 2010).

The degree of osteoclast activity on the most hydroxyapatite containing scaffolds depends on material qualities such as crystal size and surface roughness (Rumpel et al. 2006). Therefore the silica gel matrix granules of NanoBone® were replaced by an organic matrix by accompanying osteoblastic and osteoclastic relationships. Bio-Oss®, another type of hydroxyapatite material derived from bovine bone, is also degraded by osteoclasts with ruffled borders and acid phosphatase activity (Rumpel et al. 2006). The osteoclast-like cells are not only localized along the surface of the newly formed bone but also directly on the biomaterial. In the case of Bio-Oss® signs of osteoclastic resorption were evident in the formerly vascular osteon channels not surrounded by bone lamellae. Contrastingly, osteoclast attached to NanoBone® granules also occurred in defect regions without visible bone formation (Rumpel et al. 2006). Similar findings showed osteoclast-like cells on the surface of the NanoBone® bodies as well as on newly formed bone in human jaw bone augmented with Nanobone®. An osteoclastic as well as osteoblastic compartment could be observed after TRAP staining (Gotz et al. 2008).

Results of clinical studies using bone graft materials have recently shown that the implant success rate was 100% after three years when NanoBone® was used as grafting material. Furthermore the periotest values indicated a solid osseointegration (Heinemann et al. 2009).

4.3 Polymers

Tissue repair and regeneration by tissue engineering is dependent on the use of biodegradable polymer scaffolds which serve as a carrier matrix for bioactive substances or for incorporated cells. The adhesion of cells to bioresorbable scaffolds and the proliferation of cells on these scaffolds are important components for tissue engineering projects and play a fundamental role in regulating cell differentiation, growth and survival. Biodegradable polymers have displayed several properties of a suitable implant scaffold for the growth of osteoblast precursor cells. The ideal polymer would satisfy the following criteria: i) fill defects of various sizes and shapes, ii) mechanical and physical properties for a particular application, iii) a long shelf life, and iv) biocompatible degradation products.

Polymers are large organic macromolecules composed by many monomers in a regular pattern (structural units). Cellulose, collagen, agarose, chitin or hyaluronan are members of natural polymeric materials or so-called biological polymers. Natural polymers like collagen have been used for bone tissue engineering purposes (Hutmacher et al. 2001a). The most widely used bioresorbable materials are polymers of monocarboxylic acid derivates. A number of natural and synthetic polymers, such as poly-lactid acid (PLA), poly-glycolic acid (PGA), polyurethane (PU), and polycaprolactone (PCL), are in use as tissue scaffolds (Kiremitci and Piskin 1990; Vunjak-Novakovic et al. 1998; Hayashi et al. 2008). Recently these materials have been joined by linear polyesters of microbiological origin – polyhydroxykanoates (PHA). Local tissue responses to polymers *in vivo* depend on the biocompatibility of the polymer as well as its degradative by-products (Hollinger and Battistone 1986).

At the moment, two polymers are commercially available as a bone substitution material, first a PLA granulate (TruGraft™, Osteobiologics, San Antonio, U.S.A.) and second NovoSorb™ (PolyNovo Biomaterials, Port Melbourne, Australia).

The poly(α-hydroxy acid) polymers such as PLA, polygylcolide (PLG) and their copolymers PLGA are the most commonly used synthetic polymers to deliver BMPs (Isobe et al. 1999). Additional polymers used as BMP delivery systems include polyanhydrides, polypropylene fumurate, polyethylene glycol-PLA as well as polyphosphate (Lucas et al. 1990; Miyamoto et al. 1992; Renier and Kohn 1997; Behravesh et al. 1999). Incorpoartion of BMP-2 and BMP-7 in polycaprolactone suppressed bone marrow mesenchymal stem cell proliferation and increased the alkaline phosphatase activity (osteogenic differentiation) (Yilgor et al. 2010). An *in vivo* study determined the effects on osseointegration when polycaprolactone with BMP-2 coating was applied to bone screws. BMP-2 within the polycaprolactone coating did not stimulate osteogenesis (Niehaus et al. 2009). In contrast, using PCL scaffold, platelet-rich plasma and recombinant human BMP-2, a critical-size defect in the anterior mandible of a 71-years-old female patient was regenerated using de novo-grown bone (Schuckert et al. 2009).

4.3.1 Poly-3-hydroxybutyrate (PHB)

The most widespread and best studied polyhydroxyalkanoat (PHA) is the lipidic polymer, poly-3-hydroxybutyrate (PHB), which is found in the plasma membranes of Escherichia coli

complexted to calcium polyphosphorate (Reusch and Sadoff 1983). Different bacterial types of microorganisms produce PHB from renewable sources, e.g. sugar and molasses, as intracellular storage materials. PHB is an ideal biomaterial, because it is a biodegradable polyester and completely degrades in D,L-b-hydroxybutyrate (HB), a normal component of blood and tissue (Miller and Williams 1987; Suwantong et al. 2007). PHB is perfectly isotactic viz. the monomers have all branch groups on the same side of the polymeric chain and are oriented in the same way (Figure 5).

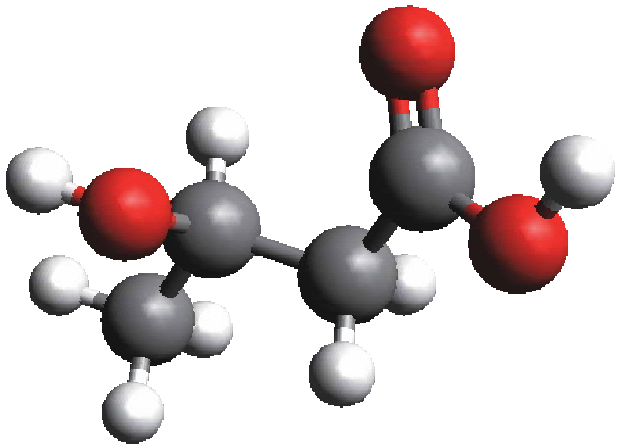

Fig. 5. Schematically illustration of a PHB monomer. The PHB monomer consists of hydroxybutyric acid ($C_4H_6O_2$ units) and a methyl group as side chain. Grey = carbon molecule; white = hydrogen molecule, red = oxygen molecule.

The biocompatibility of PHB has been confirmed *in vitro*, in cell cultures of various origins. It was found that mouse fibroblast cells and chondrocytes do not grow very well on microbial PHB films (Deng et al. 2002; Yang et al. 2002). PHB showed a high degree of crystallization and this rapid crystallization generates pores and protrusions on the PHB surface film. It was speculated that this surface could prohibit the attachment and growth of mammalian cells (Zhao et al. 2003). It is well known that biocompatibility of bio materials depends not only on the chemical structure but, to a large extent, on the size. Suwantong and coworker could show using indirect cytotoxicity assessment that PHB fibre mats were acceptable to both mouse L929 fibroblast cells and mouse Schwann cells (RT4-D6P2T) (Suwantong et al. 2007). Mouse fibroblast cells cultured with polymeric PHB microspheres revealed no cytotoxic effects which were indicated by the absence of changes in the cell morphology, cell viability and proliferative activity (Shishatskaya and Volova 2004). In addition, human osteoblast cells grow very well on PHB embroidery (Mai et al. 2006).
Several *in vivo* studies have shown that PHB may serve as a scaffold for tissue engineering due to its excellent biocompatibility as evidenced by lack of toxicity and compatibility with tissue and blood (Saito et al. 1991; Clarotti et al. 1992; Gogolewski et al. 1993; Schmack et al. 2000; Mai et al. 2006; Mack et al. 2008; Gredes et al. 2009). PHB sheets did not caused any inflammation in the chorioallantoic membrane of the developing egg (Saito et al. 1991). In mouse tissues no acute inflammation, abscess formation, or tissue necrosis was observed after subcutaneously implantation. In contrast, mononuclear macrophages, proliferating fibroblasts, and mature vascularized fibrous capsules were typically found (Gogolewski et

al. 1993). Furthermore, PHB sutures implanted intramuscularly did not cause any acute vascular reaction or inflammation, necrosis calcification of the fibrous capsule or malignant tumour formation in rats (Shishatskaya et al. 2004). On the other hand, implantation of PHB implants significantly increased the mRNA expression of VEGF (vascular endothelial growth factor), IGF1 and IGF2 (insulin-like growth factor) and decreased the GDF8 (growth differentiation factor 8; myostatin) mRNA amount in Musculus latissimus dorsi of rats (Table 1) (Gredes et al. 2009).

Tested gene	**mRNA expression (gene/β-actin)**		
	controls	**6 weeks of treatment**	**12 weeks of treatment**
VEGF	3.9 ± 1.0	6.2 ± 1.6 *	
IGF1	14.2 ± 3.0	29.0 ± 7.0 *	19.2 ± 3.0 *
IGF2	5.5 ± 1.8	8.2 ± 1.8 *	
GDF8	4.8 ± 1.6	2.9 ± 1.0 *	3.3 ± 0.8 *

Table 1. Relative expression of the VEGF, IGF1, IGF2 and GDF8 mRNA in M. latissimus dorsi of control rats and in M. latissimus dorsi of rats 6 and 12 weeks after PHB scaffold implantation. Mean ± S.E.M. are given. Students t-test * = $p < 0.05$.

In addition, PHB scaffolds showed synergistic effects with the surrounding muscle, because of an increase in the slow myosin isoform after implantation (Mack et al. 2008). Another study showed ectopic bone formation in nude rats after intramuscular implantation of PHB embroidery seeded with human osteoblast cells (Mai et al. 2006). The osteogenic potential of different PHB scaffolds was also shown in nude rats (Rentsch et al. 2009). In mini pigs PHB was used for covering defined bone defects in the anterior skull base including a Dura mater lesion. The anterior skull base bone defect was completely closed after 9 months. Furthermore no reaction or adhesions between brain and PHB or Dura mater and PHB was observed (Bernd et al. 2009).
PHB patches were used to close experimentally induced atrial septal defects in calves. Twelve months postoperatively no shunt or sign of infection was found. This experimental model thus prompted formation of regenerated endothelial tissue and complete degradation of the PHB patches (Malm et al. 1992). Recently it was shown that PHB can be used as an alternative to epineural suturing in the treatment of peripheral nerve injuries at the wrist/forearm level of the arm (Aberg et al. 2009).

4.3.2 Polylactid (PLA)

Among the family of biodegradable polyesters, polylactides have come to the fore because, they are produced from renewable resources, they are biodegradable, have high mechanical performance and very low or no toxicity. The degradable material PLA is applied in dentistry as Guided Tissue/Bone Regeneration membranes even though these membranes do not have any significant biological function such as the facilitation of cell adhesion. Polylactic acid or polylactide (PLA) is the most commonly used biodegradable, thermoplastic, aliphatic polyester derived from renewable resources in surgery especially in osteosynthesis (Ashammakhi et al. 2003). PLA can be used for biomedical purposes because

of its biodegradability in contact with biological tissues (Rimondini et al. 2005). Polylactide acid is degraded by hydrolysis or specific cleavage of oligopeptides (Drury and Mooney 2003). It is known that the degradation product of PLA, lactic acid, is normally present in the body, can enter the tricarbonxylic acid cycle and is excreted as H_2O and CO_2 (Gunatillake and Adhikari 2003). On the other hand degradation products of PLA are partially cytotoxic (Nesic et al. 2006).

In our own experiments we could show that cultivation of L929 mouse fibroblast cells with PLA membranes significantly decreased cell proliferation and increased the amount of death cells compared to control cells. Our results are in agreement with findings from Yang et al (Yang et al. 2002). They show that growth of L929 cells was poor on PLA films. The numbers of adhered cells cultured on PLA were significantly lower than those on control disks in early periods of incubation but became comparable after 21 days (Iwamatsu-Kobayashi et al. 2005). Human keratinocytes delayed growth has been shown on a PLA film with respect to culture on standard tissue culture polystyrene, even though the same plateau level was observed after 2 weeks (Garric et al. 2005). Former *in vitro* studies have shown that osteoblast cells are able to attach well to PLA or poly-glycolic acid (PGA) (Ishaug et al. 1994). We could demonstrate that primary osteoblast cells growth on PLA membranes (Figure 6).

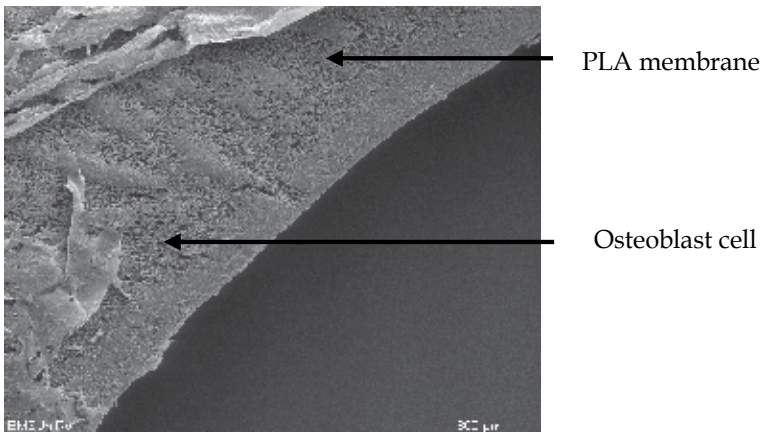

Fig. 6. Scanning electron micrograph of osteoblast cells (PO62) growing on a PLA membranes. Heterogenous morphology of these cells was observed after 10 days of cell culture. Bars = 300 μm

Collagen coating and NaOH treatments of the PLA film improved skin cell adhesion and proliferation but their extent depends usually on the culture time (Garric et al. 2005). Furthermore, an enhanced 3-dimensional porous structure of PLA coated with collagen showed a significantly higher amount of initial cell attachment than PLA porous scaffold without collagen immobilization (Fuse et al. 2009). Other PLA surface modifications with chloric acid mixture solution increased the surface wettability and with it the cell adhesion on the surface (Lee et al. 2002). In contrast, human RT-112 cells did not show any differences in the proliferation rate after incubation with PLA alone or PLA-copolymers (Holzl et al. 2000).

In vivo biocompatibility of poly(L-lactide) resorbable mesh was shown in rats. It was found that PLA induces important immunological reaction. Amongst others PLA caused mild

inflammatory response by infiltrating mononuclear and polynuclear inflammatory cells in animals (de Tayrac et al. 2008). Furthermore, stereocomplexed PLA nanofibers induced milder inflammatory reaction than poly(L-lactide) nanofibers after subcutaneous implantation in rats (Ishii et al. 2009).
To date, predominantly histological staining and immunohistochemical analyses were used to study the behaviour of bone substitute materials on bone graft healing. Only a few numbers of studies present the molecular mechanisms of bone formations associated with bone substitutes. Recently it was shown that the expression of type I and type II collagen was increased in human mesenchymal progenitor cells as well as adipose stem cells seeded on PLA (Heckmann et al. 2007; Maenpaa et al. 2010). PLA nanofibres possessed a growth inhibitory effect on human tendon derived fibroblasts and no meaningful influence on the gene expression of collagen I was observed (Theisen et al. 2010). Furthermore, PLA nanofibres tend to result in a down-regulation in bone morphogenetic protein 2 (BMP-2) and VEGF expression during the period of human mesenchymal stem cell differentiation towards osteoblasts (Schofer et al. 2009). On the other hand PLA membranes do not have any influence on the gene expression of different growth factors, collagen I and II as well as myostatin after subcutaneously implantation in rats (Gredes et al. 2010b).

4.3.3 Polycaprolactone (PCL)

Polycaprolactone (PCL), a thermoplastic polymer derived from the chemical synthesis of crude oil, has a very low melting point of about 60°C and degrades by hydrolysis of its ester linkage under physiological conditions. Polycaprolactone has also been regarded as a tissue-compatible biodegradable polymer with good mechanical properties (Khor et al. 2003). PCL has been proven to be a tissue-compatible biodegradable polymer with satisfying mechanical properties although it is not very favourable for cell growth because of its intrinsic hydrophobicity and lack of bioactive functional groups (Zhu et al. 2002). *In vitro* studies showed that human calvarial periosteal cells attached and proliferated on PCL membranes with the formation of extracellular matrix (Schantz et al. 2002). Further studies were conducted with primary human fibroblast cells. Light, environmental scanning electron, and confocal laser microscopy as well as immunohistochemistry showed cell proliferation and extracellular matrix production on the polycaprolactone surface in the first culturing week. Over a period of 3-4 weeks in a culture, the fully interconnected scaffold architecture was completely 3D-filled by cellular tissue (Hutmacher et al. 2001b). Human primary craniofacial cells proliferated on PCL as shown by DNA-assay and collagen-I staining. Short- and long-term attachment studies demonstrated the expression of osteoblast cell markers on the PCL (Gough et al. 2003). Figure 7 illustrates a natural cover of a PCL membrane with primary osteoblast cells after 10 d of cell culture.
On the other hand, decreased fibroblast cell density was present by Vance et al. after 5 days of treatment with NaOH-modified PCL (Vance et al. 2004). These findings are in agreement with our own observations that mouse fibroblast cells incubated with PCL membranes showed a significantly decreased proliferation rate and a higher amount of death cells as compared to control cells.
The ability of PCL to repair bone defects were tested in white rabbits. Bone defects of 4.5 x 12 mm in the bilateral femoral condyle were prepared and PCL cylinders implanted into the defects. After 3 to 12 months of implantation, it was shown that bone defects were filled with new bone on the PCL-surface, and no inflammatory reaction appeared. Furthermore, the bone mineral density was greater in the PCL treated animals compared to untreated control group (Aahmat et al. 2005). PCL has a good biocompatibility and high osteoinductivity.

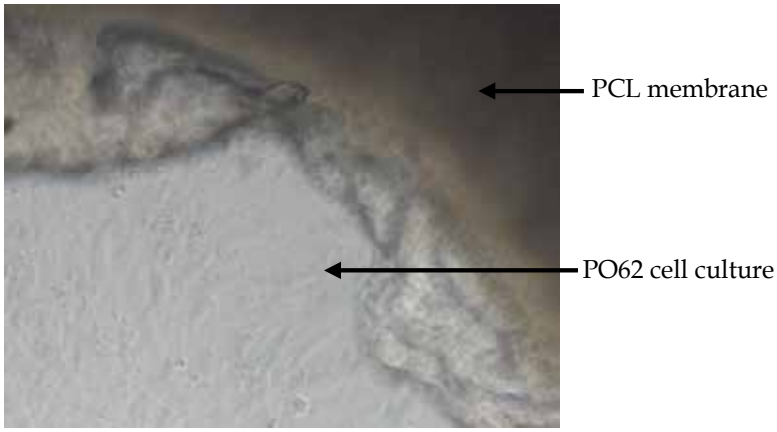

Fig. 7. Transmission microscopy of osteoblast cells (PO62) growing on a PCL membrane. Normal morphology of these cells was observed after 10 days of cell culture. Magnification: x 200.

To increase the osteoinductivity and osteoconductivity of PCL scaffolds, the PCL surface was modified. Previously, PCL-tricalcium phosphate scaffolds were developed and implanted into rat femoral defects. Histological evaluation illustrated infiltration of vascularized connective tissue and bone in treated rats, whereas the bone in-growth of untreated defects is minimal (Rai et al. 2007a). Polycaprolactone-20% tricalcium phosphate (PCL-TCP) scaffolds were assessed for the treatment of critical-sized defects of the mandible. Micro-CT measurements showed an increase in the bone volume fraction in defects grafted with scaffolds. The increase in the bone volume fraction was more pronounced after combination of the PCL-TCP scaffolds with platelet-rich plasma (Rai et al. 2007b). Another modification is the incorporation of hydroxyapatite (HA) particles in PCL scaffolds. These PCL/HA scaffolds increase the expression of osteogenic differentiation markers, such as type I collagen and osteocalcin in an in vitro model. Furthermore, the use of the PCL/HA scaffolds in a mouse calvarial model showed significantly greater amounts of new bone compared to pure PCL scaffolds (Chuenjitkuntaworn et al. 2010).

4.3.4 Collagen

Natural polymers used in bone tissue engineering include collagen, fibrin, alginate, silk, hyaluronic acid, and chitosan (Hutmacher et al. 2001a). Many of the naturally occuring animal-derived polymers are components of the extracellular matrix. They are biocompatible, bioresorbable and can be formulated into many configurations with variable residence time using enzymatic treatment and chemical crosslinking. Collagen is the most abundant extracellular matrix protein and component of connective tissue. Collagen, in the form of elongated fibers, is mostly found in tendon, ligament and skin, as well as in cartilage and bone. The collagen used in dental procedures is readily isolated and purified from various animal species by enzyme treatment. Collagen type I is the main organic component that is originally secreted by osteoblasts, which then becomes mineralized at a later stage of bone development. Collagen has been actively investigated as a favourable artificial environment for bone ingrowth. It was shown that endothelial cells adherent, spread and proliferated on a collagen membrane (Lycoll®, Resorba, Nuernberg, Germany) (Breithaupt-

Faloppa et al. 2006). Twardowski and co-workers found that type I collagen potently stimulates angiogenesis *in vitro* and *in vivo* (Twardowski et al. 2007). Furthermore, mesenchymal stem cell osteogenic differentiation was demonstrated on collagen scaffolds (Donzelli et al. 2007; Schneider et al. 2010; Jurgens et al. 2011). Alveolar ridge augmentation was found utilizing collagen wound dressing, so called COLLACOTE® (Zimmer Dental, Carlsbad, USA) (Ceravolo et al. 1987).

Type I collagen gel matrix was used for nasal reconstruction in rats. Histological sections of the collagen implant revealed restoration of the nasal anatomy with a thin plate of immature bone (Lindsey et al. 1996; Toung et al. 1999). Recently it was shown that resorbable collagen membranes can be used for guided bone regeneration after apicectomy or around dental implants. Both studies demonstrate that quantity and quality of new bone formation were much better in bone defects covered with collagen compared to untreated bone defects (Fei et al. 2008; Dominiak et al. 2009). At the moment two collagen-based bone augmentation materials are commercially available: Foundation™ (J. Morita USA, Inc.) and COLLAPLUG® (Zimmer Dental, Carlsbad, USA). Foundation™, an absorbable atelo-collagen sponge consists of 85 to 95% bovine collagen type I and 5 to 15% bovine collagen type III and has been used with great success since 1998 in Japan. Using rats it was shown that TERUPLUG® (different brand name for Foundation™) is able to stimulate endochondral ossification within two weeks after implantation into the calf muscle. Alkaline phosphatase activity and the calcium content had increased markedly in treated rats (Shinji et al. 2003). In addition, four weeks after implantation of TERUPLUG® in rabbit cranial defects a lot of newly formed collagen fibers around the inserted material were observed. Newly formed and matured bone was found eight weeks after implantation of TERUPLUG® compared to untreated animals (Kim et al. 2008). TERUPLUG® was used for maxillary sinus augmentation in rabbits. Gradually increased new bone formation was observed after 4, 12 and 24 weeks (Marukawa et al. 2011). In contrast, little or no new bone formation was observed on the maxillary sinus floor at six months following sinus membrane elevation and support with COLLAPLUG® in humans (Ahn et al. 2011).

Despite the good *in vitro* and *in vivo* biocompatibility, collagen undergoes rapid degradation upon implantation within 4-5 weeks (Donzelli et al. 2007). Furthermore, commercial collagen sponge (COLLAPLUG®) and membrane (BioGuide®, Geistlich Pharma, Wolhusen, Germany) induced considerable cell death, impaired initial function, and generated extraordinary intracellular reactive oxygen species in attached osteoblast cells. These effects substantially ameliorated after N-acetyl cysteine pre-treatment of the collagen sponge and membrane (Yamada et al. 2010).

Collagen is very often used as matrix for application of growth factors, e.g. TGF-β or BMP-2. Intramuscular application of TGF-β-loaded collagenous matrix is resulted in bone induction in primates (Ripamonti et al. 1997; Duneas et al. 1998). TERUPLUG®, as a carrier for Escherichia coli-derived recombinant human bone morphogenetic protein-2 (ErhBMP-2), was implanted into the calf muscle of Wistar rats. After 21 days, mature bone was formed and on days 14 to 21 after implantation, alkaline phosphatase activity and calcium content increased (Shinji et al. 2003).

Examples for a commercially available product containing growth factors is INFUSE® Bone Graft (Medtronic GmbH, Meerbusch, Germany), recombinant human bone morphogenetic protein 2 in an absorbable collagen sponge.

4.4 Biocomposites containing flax fibers in a polyester matrix

The usage of barrier membranes for osteogenesis has increased in the last years. Small bony defects seem not to depend on the application of membranes to regenerate bone, but large bone defects benefit from membranes, because they reduce the indispensable resorption of bone grafts when used alone. The application of barrier membranes to promote bone regeneration was described in orthopedic research. However, the clinical potential of these membranes was recognized some years ago for periodontal regeneration (Hermann and Buser 1996; Sculean et al. 2008).

Fibre-reinforced plastics have successfully proven their value in various applications because of their excellent specific properties, such as high strength and stiffness. The use of natural fibres for fabrication of composites in combination with biodegradable polymers is an excellent approach for biomedical application. Natural fibres are of basic interest since they have the capability to replace glass, ceramics or carbon fibres (Bax and Müssig 2008). Natural fibres combined with biodegradable polymers like polyhydroxyalkanoate (PHA), polylactic acid (PLA), polycaprolactone (PCL) and starch polymers frequently used as a matrix resulted in composites called "green" because of their complete biodegradability (Mohanty et al. 2005). The influence of plant fibres like flax, jute, ramie, oil palm fibres or cellulose fibres on the mechanical properties of biodegradable polymers was tested several years ago. Wollendorfer and Bader found in increased tensile strength as well as a four times better wheat starch of the reinforced polymer compared to the polymers without fibres (Wollendorfer and Bader 1998) Composites reinforced with natural fibres have been used in several branches of industry (i.e. automobile) and medicine with special focus on tissue artificial scaffold, drug-release systems, cardiovascular patches and nerve cuffs (Williams and Martin 2002; Misra et al. 2006). Flax fibres are amongst the oldest fibre crops in the world. The use of flax for the production of linen goes back at least to ancient Egyptian times. It is known that flax fibres are stronger than cotton fibres but less elastic. Flax fibres provide natural raw materials for composites and pulp / paper (van Dam et al. 1996). Former studies have shown an increased biological reaction around linen threads compared to synthetic threads (Jarosz-Cichulska 1988). Recently, the cytotoxicity of flax fibres, oil emulsion, and seedcake extract from transgenic flax overproducing various antioxidative compounds was determined in a culture of mouse fibroblasts. No changes in the total number of fibroblasts and comparable numbers of dead cells were shown in the presence of each type of flax material (Skorkowska-Telichowska et al. 2010).

In order to improve the biochemical and mechanical properties of flax fibres, transgenic flax over-expressing the bacterial polyhydroxybutyrate (PHB) gene was produced (Wrobel et al. 2004; Wrobel-Kwiatkowska et al. 2007). Composites containing fibres from transgenic plants did not differ from the control plants in the level of major fibre constituents (i.e. cellulose, lignin and pectin content) but was significantly stronger and more elastic than those containing fibres from control flax plants (Szopa et al. 2009). Furthermore, genetically modified flax plants producing PHB showed significantly increased parameters such as strength, Young´s modulus, energy for failure of flax fibres and phenolic acid level, a reason for improved plant resistance to pathogen infection (Wrobel-Kwiatkowska et al. 2007; Wrobel-Kwiatkowska et al. 2009).

We found that composites containing flax fibres from transgenic flax plants producing polyhydroxybutyrate (M50) and control (wt-Nike) plants in a polylactid (PLA) or polycaprolactone (PCL) matrix showed a good *in vitro* biocompatibility despite the cell viability of mouse fibroblast cells treated with these composites being slightly reduced and

the amount of dead cells significantly increased compared to untreated cells after incubation for 12h to 48h. The biocompatibility of composites from transgenic flax plant fibres producing PHB did not differ from composites of non-transgenic flax plant fibres. Both linen membranes coated with PLA showed a significant increase in the proliferation rate of fibroblast cells compared to cells treated with membranes composed of PLA alone, whereas both flax membranes in the PCL matrix significantly decreased the proliferation rate of mouse fibroblast cells compared to cells treated with membranes composed of PCL alone. No differences were found between genetically modified and non-modified flax (Figure 8).

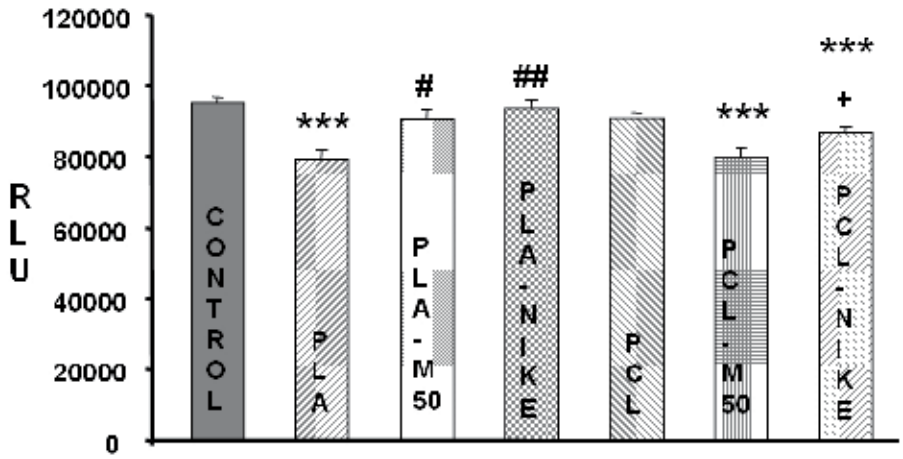

Fig. 8. Determination of cell viability by measurement of ATP. Viability of mice fibroblast cells (L929) cultured without and with membranes produced of polylactide (PLA), linen from PHB producing flax fibres coated with PLA (PLA-M50), linen from normal flax fibres coated with PLA (PLA-NIKE), polycaprolactone (PCL), linen from PHB producing flax fibres coated with PCL (PCL-M50) and linen from normal flax fibres coated with PCL (PCL-NIKE) for 24h was analysed using the CellTiter-Glo® assay. Means ± S.E.M. are given in all cases for n = 14-48 samples. Stars indicate significant differences: ***) $p < 0.005$, membrane-treated cells versus control; ##) $p < 0.01$, ###) $p < 0.005$, PLA versus linen membrane, +) $p < 0.05$, PCL versus linen membrane, unpaired t-test. RLU = relative luminescence units.

Beside macroscopically and histological examination the biocompatibility can be assessed by analyses of the biomaterial influence on the gene expression. Because of this we analysed the gene expression of growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) as well as myostatin also known as growth differentiation factor 8 (GDF8) after subcutaneous implantation. We found that subcutaneously insertion of the different biocomposites had no influence on the gene expression of the most tested genes. The constantly happening muscle contraction of the Musculus latissimus dorsi is not followed by stress-induced changes in muscle growth factor expression (Gredes et al. 2010b). The influence of the new biocomposites on bone regeneration must be checked using *in vitro* models. Cell culture experiments with primary osteoblast cells and these biocomposites showed that osteoblast cells are able to attach and proliferate on these membranes. The proliferation is much better on PLA-biocomposites compared to PCL-flax membranes (Figure 9).

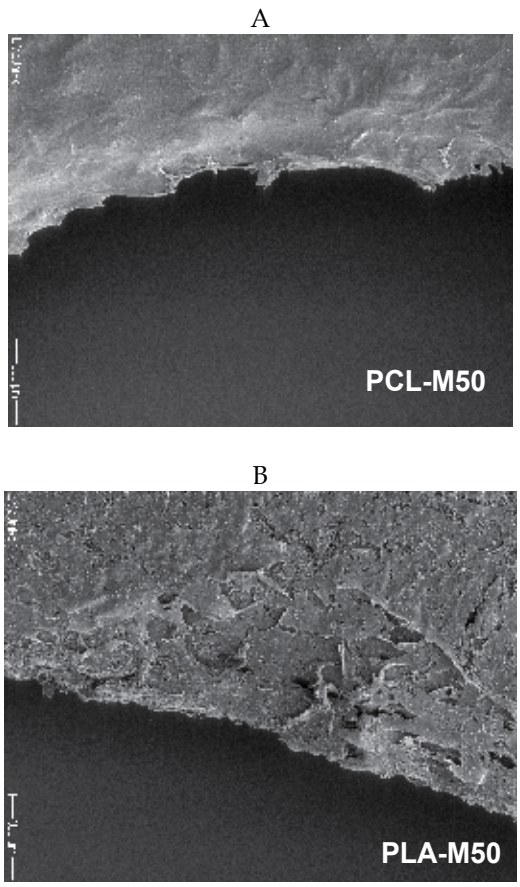

Fig. 9. Scanning electron micrographs of osteoblast cells (PO62) growing on a PCL-M50 (A) or a PLA-M50 membrane (B). After 10 days of cell culture on PCL-M50 membranes moderate natural cover with osteoblast cells could be observed, whereas culturing on PLA-M50 increased the amount of growing cells. Bars = 300 µm

4.5 Carrier for growth factors

As described earlier in this chapter bone formation is mediated by the release of local, biochemical and biophysical messengers, e.g. growth factors. In therapeutic use the bone growth factors need a carrier. Carrier requirements for most bone repairs are also simplified by the concurrent use of either internal or external fixation or coaptation, eleminating the need for mechanical strenght or resistance to motion. Furthermore, the carrier should assure local, sustained release of the growth factors which may otherwise be rapidly absorbed before instituting their effect. Bone is also highly vascular and carriers used for bone repair have to be capable of supporting vascular in-growth. Several biodegradable materials have been investigated as carriers for bone growth factors, including (1) organic materials such as inactive demineralized bone proteins, collagen, fibrin, squalene, and coral; (2) ceramics, including tricalcium phosphate, hydroxyapatite, calcium-sulphate composites, and bioactive glasses; and (3) synthetic polymers such as polylactide, polylactide-polyglycolide co-polymer, polyanhydride, and polyorthoester (Table 2).

Biodegradable materials as carriers for bone growth factors		Quality characteristics	References
organic materials	collagen	Collagen molecules assemble into heterotypic aggregates that define the biological and mechanical properties of most tissues and organs. Type I collagen, the major component of the organic bone matrix can bind osteoblasts via specific cell surface receptors, the integrins.	(Burgeson and Nimni 1992; Tzaphlidou 2005)
	fibrin	High adhesion to many biological surfaces, excellent seeding effects and good tissue development. It is not toxic, allergenic or inflammatory.	(Ye et al. 2000)
	coral	Natural coral is radiodense, it resorbs more rapidly than ceramic materials and the natural pore size of native coral facilitates osteoconduction and bone growth.	(Sciadini et al. 1997)
ceramics	tricalcium phosphate	Porous beta-tricalcium phosphate scaffolds are employed for local drug delivery in bone. It is used alone or in combination with resorbable polymer or autologous materials.	(Bansal et al. 2009; Kundu et al. 2010)
	hydroxyapatite	An identical composition to the bone tissue of living organisms and has similar physical, mechanical, and other properties. It shows high biocompatibility, does not give rise to inflammatory phenomena, and is non-toxic.	(Yarosh et al. 2001)
	calcium-sulphate composites	Calcium sulfate (plaster of Paris) is an absorbable, moldable material that has easy handling properties. It promotes osseous formation.	(Pecora et al. 1997)
	bioactive glass	This material has osteoconductive and osteo-promotive abilities in the biocompatible interface for osseous migration, and a bioactive surface colonized by osteogenic cells free in the surgical wound. It bonds to soft and osseous tissues.	(Wilson and Low 1992; Macedo et al. 2004)

synthetic polymers	polylactide (PLA)	Prosperities of PLA depend on the component isomers, processing temperature and molecular weight. In nature, polymer degradation is induced thermal activation, hydrolysis, biological activity, oxidation, photolysis, or radiolysis. In biomedical fields it is also used as bone fixation material and drug delivery microsphere.	(Zhao et al. 2004; Madhavan Nampoothiri et al. 2010)
	polylactide-polyglycolide copolymer	It promotes specific cell adhesion, differentiation and bone formation. Its degradation depends on the formulation, amorphous/crystalline structure, isomeric characteristics, molecular weight and amount of material used.	(Lu et al. 2003; Serino et al. 2003)
	polyanhydride	Polyanhydrides and their degradation products have not been found to cause significant harmful responses and are considered to be biocompatible. *In vivo*, polyanhydrides degrade into non-toxic diacid monomers that can be metabolized and eliminated from the body.	(Tamada and Langer 1992; Kumar et al. 2002)
	polyorthoester	This polymer degrades at its surface and becomes thinner with time rather than crumbling. It can cause minor moderate inflammation reaction, compared with PLA.	(Ekholm et al. 1997)

Table 2. Carrier materials for bone growth factors.

5. Conclusion

The literature has shown that early bone loss can be significantly reduced by socket grafting. The process of socket grafting requires an understanding of wound healing and an appreciation of the biological properties of the products available for socket grafting. Various types of alloplastic bone substitution materials have been created and showed ossification and new bone formation. The huge variety of biomaterials makes it difficult to decide which material is adequate for which indication. To date, tissue engineered bone is far from a routine clinical application. Clinical investigations using alloplastic bone substitution materials are very rare in the literature but, because of similar bone remodelling processes in pigs and humans, we found the same details about usage of alloplasts in dentistry. The most alloplastic materials are not popular in dentistry and, because of this, further studies are necessary.

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3

New Bone Formation in the Maxillary Sinus With/Without Bone Graft

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1. Introduction

Pneumatization of maxillary sinus causes insufficient vertical bone volume on posterior maxilla. So the restoration of edentulous posterior maxilla with dental implants is challenging due to a deficient posterior alveolar ridge, unfavorable bone quality and increased pneumatization of the maxillary sinus.[1] For maxillary sinus augmentation, the crestal approach and the lateral window approach have been used. In this chapter, sinus augmentation using crestal and lateral window approach with/without bone graft is described.

2. Indications and contraindications for sinus augmentation

Even sinus augmentation procedure is considered as highly predictable augmentation procedure, presurical evaluation on maxillary sinus and patient's medical and dental history should carefully be evaluated.
The following are indications for sinus augmentation.

- No history of sinus pathosis
- Insufficient residual bone height (less than 10mm of bone height)
- Severely atrophic maxilla
- Poor bone quality and quantity in the posterior maxilla

Sinus augmentation is not indicated when patient has history as below

- Recent radiation therapy in maxilla
- Uncontrolled systemic diseases such as Diabetes Mellitus
- Acute / chronic maxillary sinusitis (Fig. 1)
- Heavy smoker
- Alcohol abuse
- Psychosis
- Severe allergic rhinitis
- Tumor or large cyst in the maxillary sinus (Fig. 2)
- Oroantral fistula (Fig. 3)

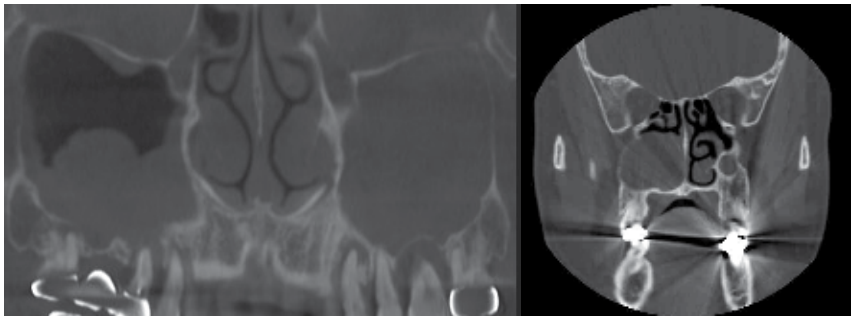


Fig. 1. (Left) demonstrates odontogenic sinusitis in the both sinus. Fig. 2. (right) shows mucocele in the right sinus. Sinus pathosis should be eliminated before sinus augmentation.

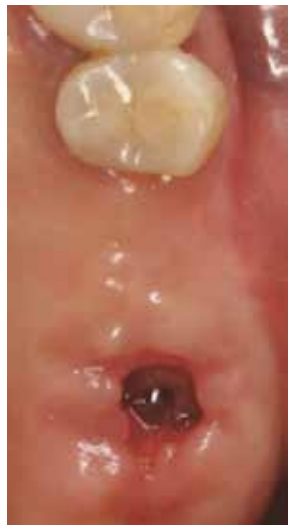


Fig. 3. Oroantral fistula should be corrected before sinus augmentation.

3. Sinus augmentation using crestal approach

Sinus augmentation using the lateral window procedure has been predictable for several decades. However, this procedure has some complications such as postoperative swelling, pain, and a long edentulous healing period. To reduce the complications from the lateral window approach in maxillary sinus augmentation, various crestal approaches such as osteotome mediated sinus floor elevation (OMSFE)[2], piezoelectric internal sinus elevation (PISE)[3-5], hydraulic sinus condensing (HSC) technique[6], internal sinus manipulation (ISM) procedure method[7], and crestal window technique (CWT)[8] and hydrodynamic piezoelectric internal sinus elevation (HPISE)[9-10] have been introduced as alternative to lateral window approach for sinus augmentation.

3.1 Osteotome Mediated Sinus Floor Elevation (OMSFE)

OMSFE technique utilizes osteotome and a surgical mallet to break sinus floor and to compact bone graft into the sinus cavity.

Surgical procedure of OMSFE (Fig. 4-16)

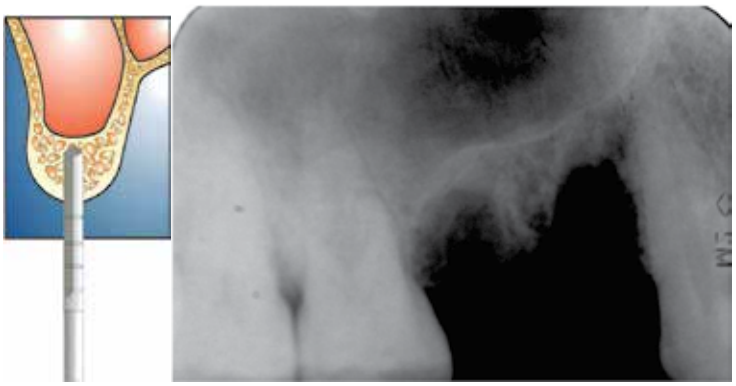


Fig. 4. and 5. A pilot drill is usually used to the depth 1-2mm short to the sinus floor to accommodate osteotome to sinus floor. However as shown on fig 5 , pilot drill is omitted when bone height is less than 2mm from sinus floor at the implant sites

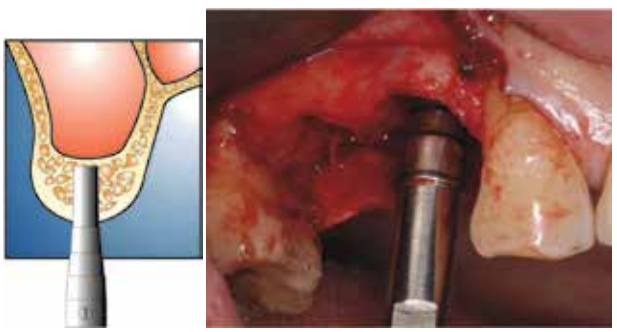


Fig. 6. and 7.

Small diameter osteotome is inserted into the prepared bone to compress sinus floor using a surgical mallet. Wider osteotome is inserted continuously into the prepared bone to accommodate implants. The insertion of osteotome would impose a light pressure on the sinus floor.

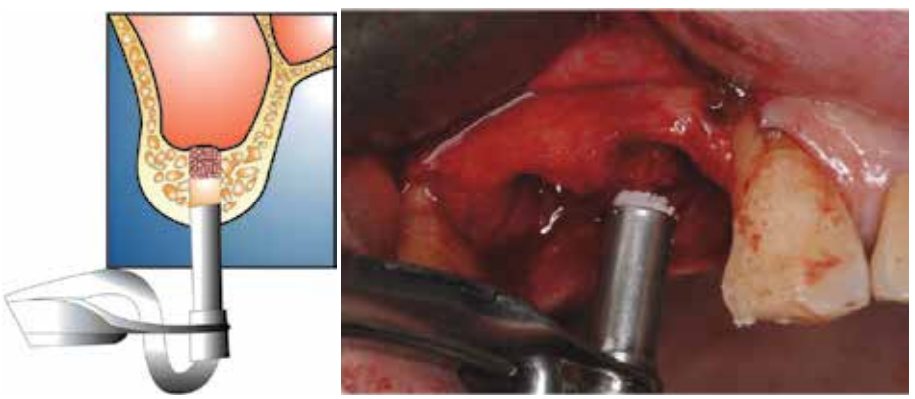
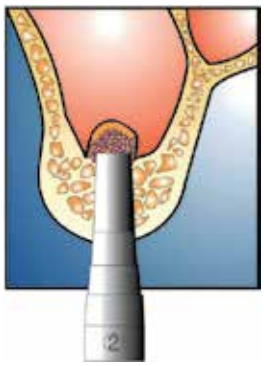


Fig. 8. and 9.

In order to elevate the sinus floor indirectly and provide buffering effect to sinus floor, bone graft material is added using an amalgam carrier. For the graft material, the radiopaque bone grafts, such as mineral allograft or xenograft, are preferred over radiolucent materials such as demineralized allograft.



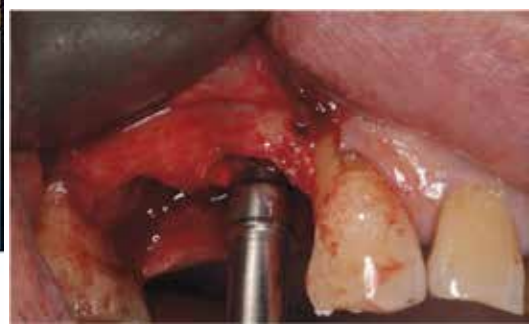
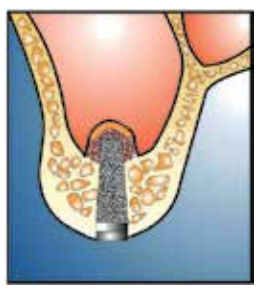


Fig. 10. and 11.

The sinus floor is elevated by repeated bone grafting and osteotome insertion. For sinus floor elevation of 3~4mm, 4~6 times of grafting and osteotome insertion and for the elevation of 5~7mm, 7~10 times of the repetition is required.



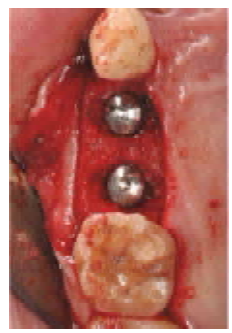


Fig. 12. and 13. The implants are placed.

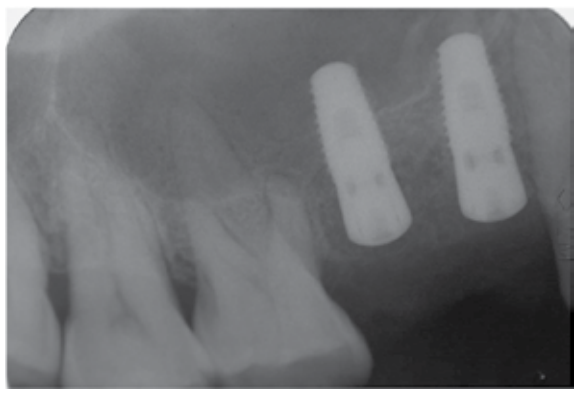


Fig. 14. Postoperative radiogram shows sinus elevation over the implant apex.

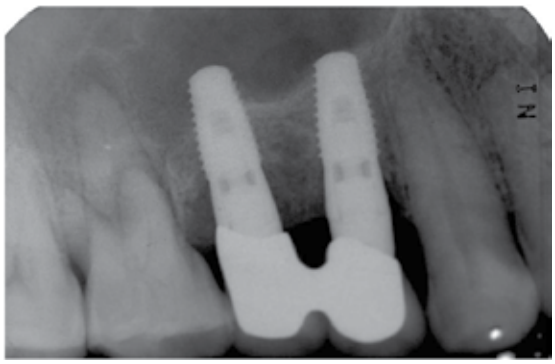


Fig. 15. Final prosthesis was cemented after 6 months healing. New sinus floor is seen in the radiogram.

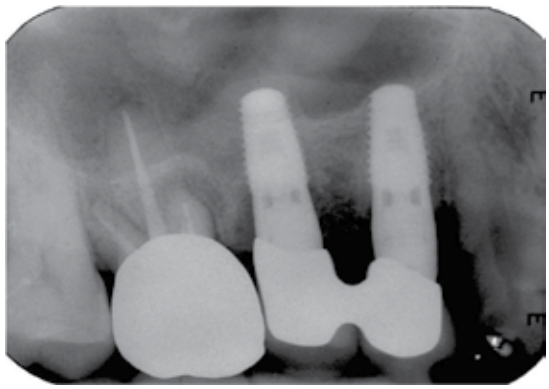


Fig. 16. 2 years after loading. Note newly formed sinus floor.

OMSFE has the advantage of surgical simplicity, resulting in minimal post-operative symptoms. But this technique also has the possibility of complications such as perforation of sinus membrane during bone drilling and bone compaction using osteotome. In addition, benign positional paroxysmal vertigo (BPPV) can be caused by the damage to the internal ear from striking osteotome and surgical mallet when sinus floor is broken.[11-13] It is difficult to break sinus floor in the area of slope of the sinus and in the site of septum with conventional crestal approach, and it could be time consuming and traumatic procedure to the internal ear (Fig 17 and Fig 18). Author experienced one case of BPPV caused from excessive striking of mallet in the anterior slope of sinus cavity in 2000. BPPV is not a common complication but surgeons should avoid excessive force to the surgical mallet when sinus floor is broken. In addition, OMSFE is a blind technique, so sinus augmentation is limited. The OMSFE technique has lower success rates when residual bone height is 4mm or less (when compared to cases with 5mm or more residual bone height).[14]

Sinus membrane integrity should be maintained when performing OMSFE because the membrane perforation causes cessation of sinus floor elevation. It is recommended that drilling should be stopped 1-2mm short to sinus floor in conventional OMSFE but this is not always easy because of magnification of plain radiogram.[6] Accidental sinus membrane perforation can be developed from improper drilling due to magnification of radiogram,

improper use of osteotome and excessive compaction of bone graft. Membrane perforation can cause the failure of osseointegration and sinus pathosis. (Fig 19 and Fig 20)

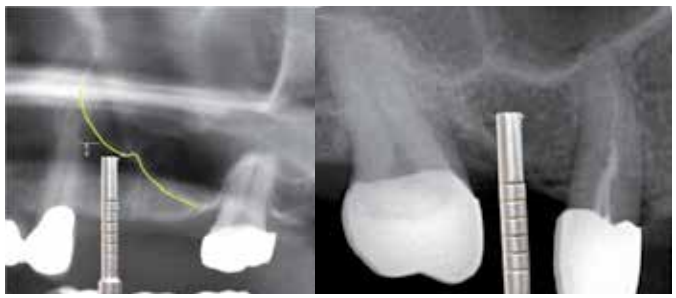


Fig. 17. and 18. Sinus elevation can be difficult at the anterior slope of sinus cavity due to too much remained bone height after initial osteotomy using pilot drill and at the sinus septum because it is very dense bone to break with OMSFE. Benign positional paroxysmal vertigo can be caused by the damage from the internal ear from striking osteotome and surgical mallet to sinus floor at these sites.

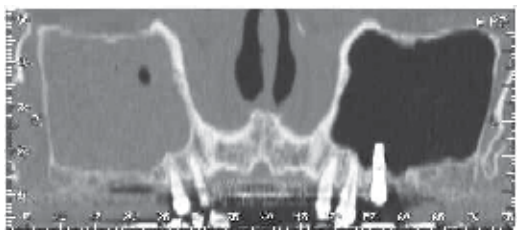


Fig. 19. OMSFE was performed at the site of # 26 and # 27 at private clinic. However membrane was perforated at the both sites. Two implants were placed at the same time. After 6 months healing, # 27 implant was removed because of mobility of implant during uncovering procedure and #26 implant showed the mobility too. The patient was referred to my department for the removal of implant and sinus graft. #26 implant was removed out, followed by sinus graft using lateral window approach. The integrity of sinus membrane should be maintained for successful sinus augmentation when performing OMSFE procedure. Note chronic sinusitis in the right sinus.

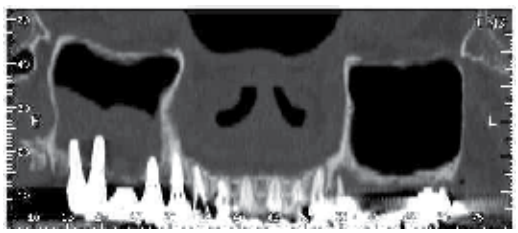


Fig. 20. OMSFE was performed at the both sinus by private clinician. Implants were placed at the same time. All implants placed in the left posterior maxilla were failed due to membrane perforation. She was referred to my department for sinus augmentation in the left sinus. Right sinus shows mucosal thickening due to intrusion of implant in to the sinus cavity. To maintain healthy sinus mucosa, the integrity of sinus membrane should be maintained.

3.2 Piezoelectric internal sinus elevation (PISE)

The PISE and HSC technique are innovative crestal methods which a surgical mallet is not required to break the sinus floor. PISE uses ultrasonic piezoelectric vibration and HSC technique uses high speed diamond bur to break sinus floor directly. These techniques are free from postoperative vertigo, but bone compaction is needed to elevate the sinus membrane. The CWT overcomes the blind nature of conventional OMSFE,[8] but the indication of this technique is limited because this technique is indicated when wide diameter implants (>5mm) are available.

Surgical procedure of PISE (Fig. 21- Fig. 33)

In PISE technique, PISE tip, connected with an ultrasonic piezoelectric device (Surgybone®, Silfradent srl, Sofia, Italy) is used to break the sinus floor. The working head of PISE tip (S028E® tip, Bukbu Dental Co, Daegu, Korea) is 2.8mm wide and 4mm high. A piezoelectric carbide-type tip is more powerful and effective for osteotomy than diamond coated tip. Thanks to selective cut of ultrasonic piezoelectric vibration, the perforation of sinus membrane is very rare when sinus floor is eroded. After braking sinus floor with piezoelectric tip directly, gel or putty conditioned bone graft was prepared to elevate sinus membrane with buffering function. To prepare gel or putty conditioned bone graft, author prefer to mix bovine bone powder (Bio-Cera™, Oscotec Co, Cheonan, Korea) and gel-onditioned allograft (OrthoBlast® II, Isotis Orthobiologics Inc, Irvine, USA). Bovine bone acts as radiopaque material and the gel conditioned allograft acts as buffer during membrane elevation.

The amalgam carrier is used when placing the graft into the narrow socket. A narrow diameter osteotome (usually 2mm in diameter) or PISE tip is inserted to compact the graft in the osteotomy site. After the membrane was elevated properly, the regular diameter implant is placed (3.7-4.0mm wide implant) without additional drilling procedure. To place wide body implant implant site should be widened with drill to accommodate the implant

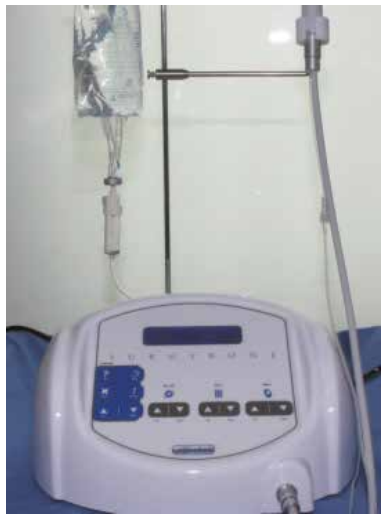


Fig. 21. Ultrasonic piezoelectrci device (Surgybone®, Silfradent srl, Sofia, Italy). This decice works in hard tissue, not in the soft tissue. Therefore membrane perforation is rare when breaking sinus floor.

Fig. 22. Carbide type PISE tip (S028E) with external irrigation.

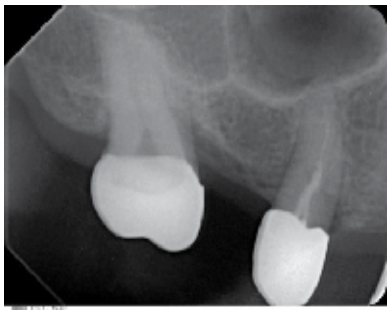


Fig. 23. A periapical radiogram shows high septum in the sinus. The bone around septum is dense, so the breaking of sinus floor using OMSFE technique is time consuming and could be traumatic to internal ear due to heavy striking of mallet. Therefore PISE is applied to break sinus floor directly.

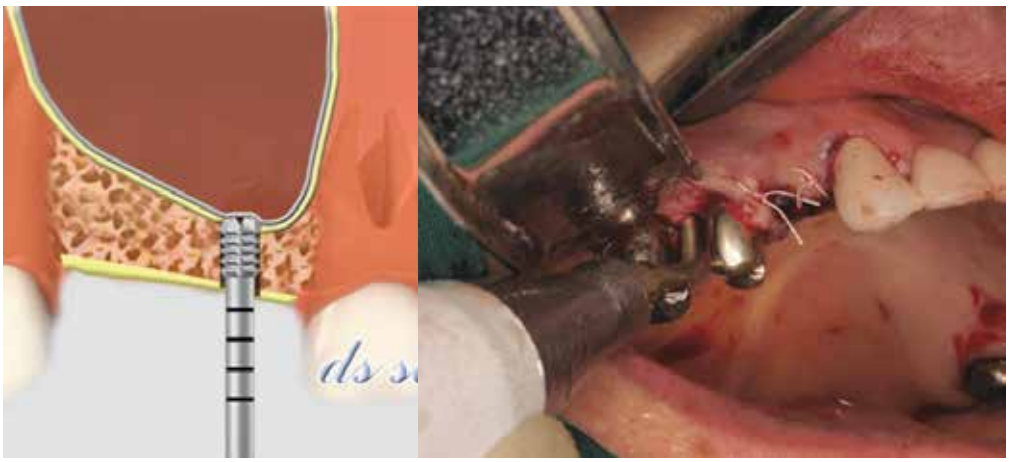


Fig. 24. (left) and Fig. 25. (right). Sinus floor is penetrated with PISE tip directly. A this stage, the exact bone height from alveolar crest to sinu floor is estimated.

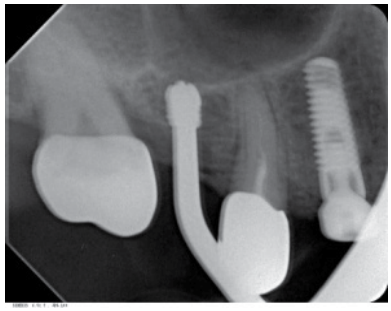


Fig. 26. The sinus floor is completely broken at the implant site by PISE tip. Note the broken sinus floor without membrane perforation.

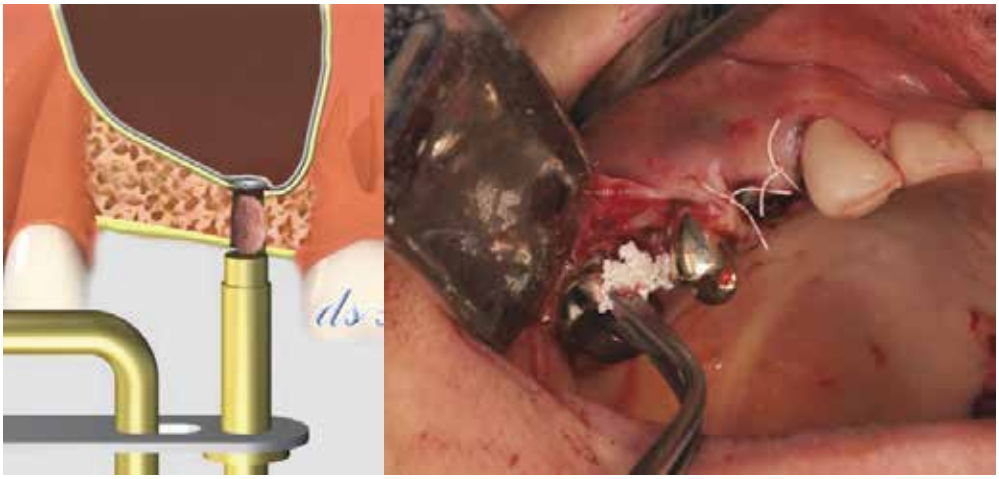


Fig. 27 (left) and Fig. 28. (right). Gel conditioned bone graft materials are carried into the osteotomy site.

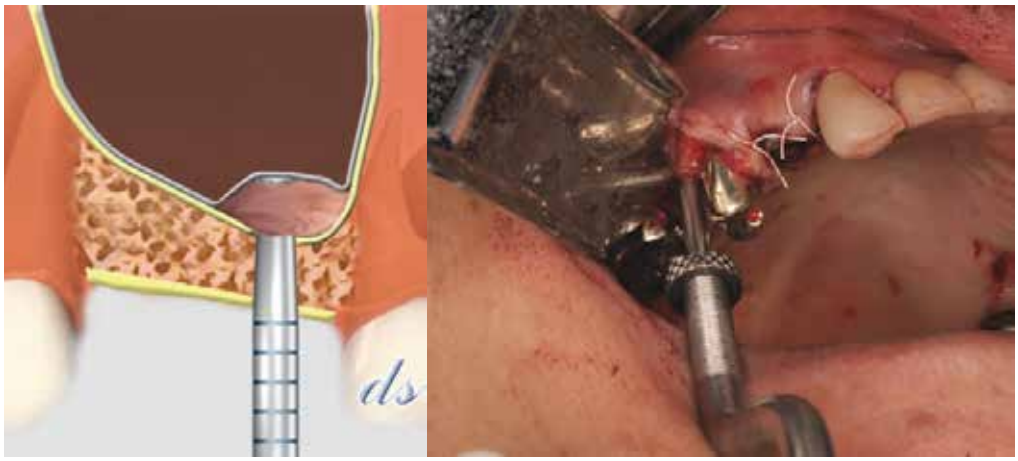


Fig. 26. (left) and Fig. 30. (right). Bone graft is compacted with the narrow osteotome or PISE tip using vibration to elevate sinus membrane.

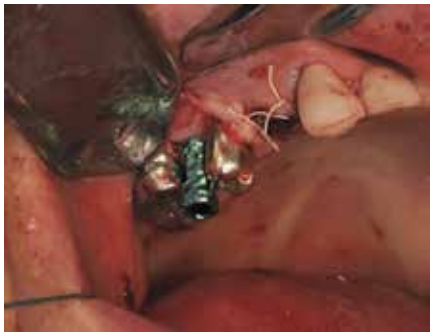
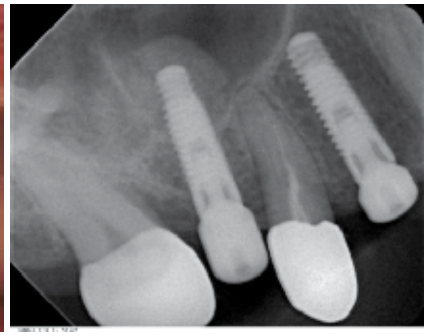



Fig. 31. (left) and Fig. 32. (right). A 3.7mm wide and 13mm high implant is placed without additional drilling procedure. Sinus elevation is seen at the radiogram.

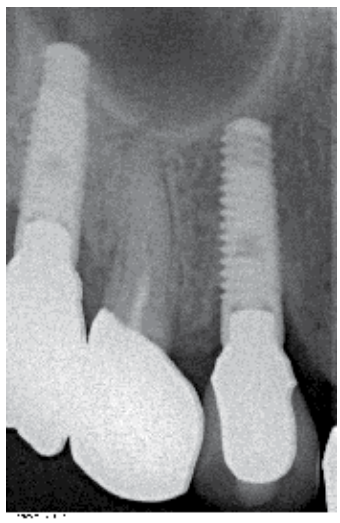


Fig. 33. Final prosthesis is cemented on after 6 months healing. A radiogram indicates sufficient augmentation of the sinus floor.

3.3 Hydrodynamic piezoelectric internal sinus elevation (HPISE)

HPISE is the updated crestal approach from PISE. This technique utilizes ultrasonic piezoelectric vibration to break sinus floor as same as PISE. However, unlike PISE and other conventional crestal approaches, HPISE usually doesn't rely on bone compaction to elevate sinus membrane. HPISE uses water pressure to elevate the sinus membrane. Hydraulic pressure from internal irrigation facilitates gentle and broad elevation of the sinus membrane before bone grafting. The HPISE uses a specially designed tip attached to a piezoelectric ultrasonic unit (Surgybone®, Silfradent srl, Sofia, Italy or compatible device). HPISE tip (S028I®, BukBu Dental Co, Daegu, Korea) allows internal irrigation to apply water pressure to the sinus membrane (Fig 34). Bone graft is dependent on surgeon's personal preference. Bone graft is indicated when heavy cyst is located in the sinus, more than 6mm high sinus elevation is required, implant is not placed simultaneously and postoperative radiopaque image is required to verify sinus elevation.

If the required sinus elevation is minimal (less than 5mm) and implant is placed at the same time, bone graft is not a prerequisite for sinus augmentation. New bone formation is achieved in the new compartment under the elevated sinus membrane thanks to tenting effect. As an alternative to bone graft, collagen sponge or fibrin rich block with concentrated growth factors (CGF®, Medifuge, Silfradent srl, S.Sofia, Italy) can be used. Fibrin rich block with concentrated growth factors can be used to accelerate new bone formation in the sinus

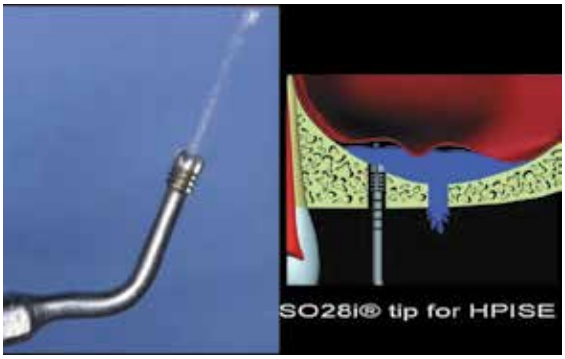


Fig. 34. HPISE (S028I®) tip with internal irrigation. Hydraulic pressure from internally irrigated saline elevates sinus membrane.

Surgical procedure of HPISE without bone graft (Fig. 35- Fig. 47)

As a first step, a 1.6mm wide carbide round insert (S016®, BukBu Dental Co, Daegu, Korea) with external irrigation is used to penetrate sinus floor directly. After breaking the sinus floor with the round tip, a 2.8mm wide HPISE tip is utilized to enlarge the osteotomy site and elevate the sinus membrane using hydraulic pressure. The HPISE tip has 4mm working tip height, and depth indicating lines are marked by 2mm intervals. Hydraulic pressure to the sinus membrane from internally irrigated sterile saline detaches sinus membrane from the sinus floor. Hydraulic pressure is applied for several ten seconds to detach sinus membrane from sinus floor. After the detachment of sinus membrane, surgeons can observe up and down movement of sinus membrane whenever patient takes a breath.
When the diameter of implant is less than 4mm wide, the HPISE insert is the last instrument to make the osteotomy prior to implant placement. Undersizing the osteotomy ensures favorable initial stability of the implant in the posterior edentulous ridge with poor bone quantity and quality. When a wider implant is placed, intermittent drilling is often required to accommodate the wide body implant.

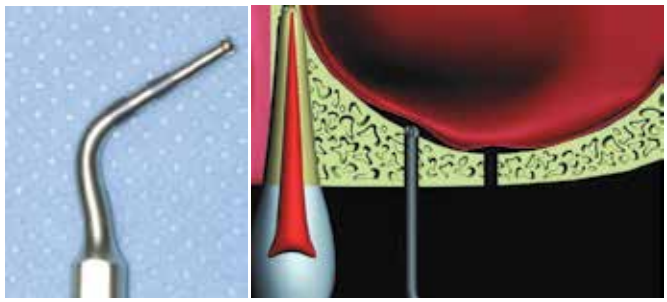


Fig. 35. (left) and Fig. 36. (right). Round carbide tip is used to break sinus floor directly. This tip provides surgeon tactile feeling of sinus membrane.

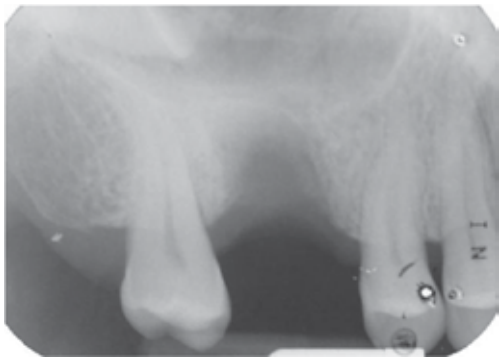
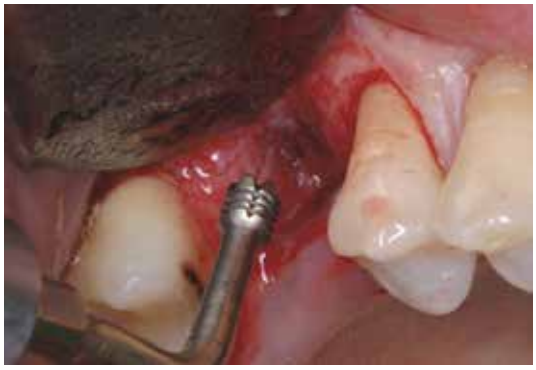


Fig. 37. When remaining bone height at the implant site is very low as shown on this radiogram, round tip can be omitted.



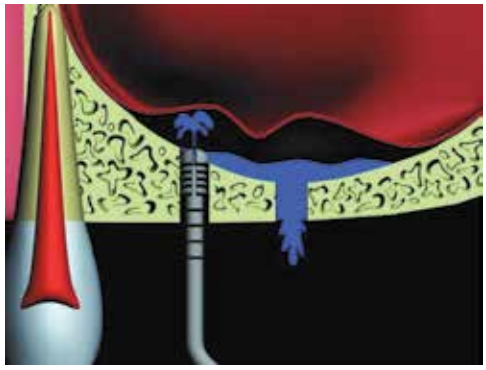


Fig. 38. (left) and Fig. 39. (right). HPISE is inserted to break sinus floor using ultrasonic vibration and elevate sinus membrane using water pressure.

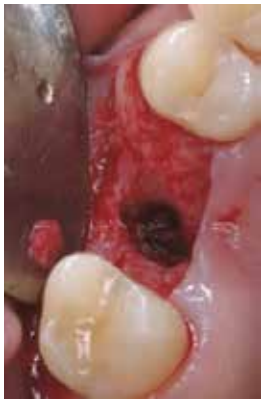


Fig. 40.

After applying water pressure, the detachment of membrane from sinus floor is seen. Surgeon can observe up and down movement of sinus membrane whenever patients take a breath.

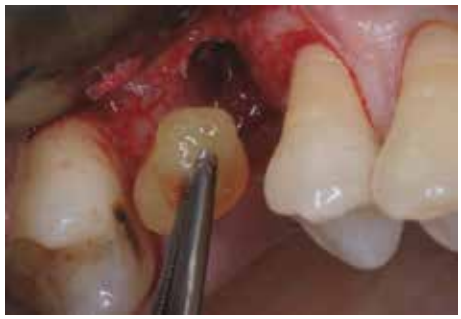


Fig. 41. As alternative to bone graft, CGF alone is inserted in order to accelerate bone reformation in the sinus,

Fig. 42. 4.7mm wide and 11.5mm long HA coated implant is placed at the same time.

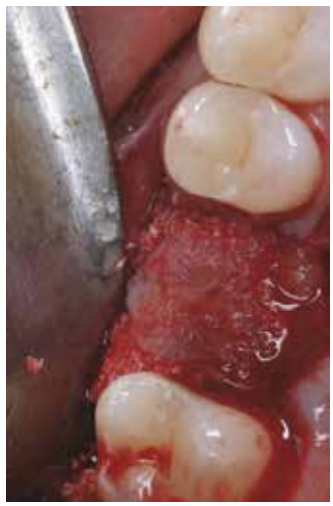


Fig. 43. Bovine bone is grafted in the defect around implant. CGF barrier is covered over the bone graft,

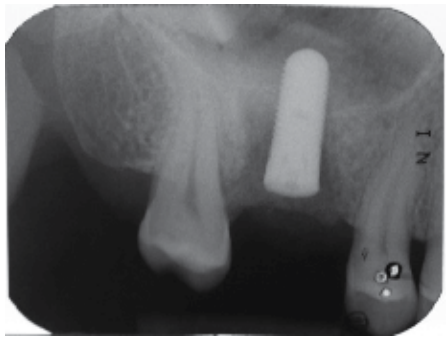
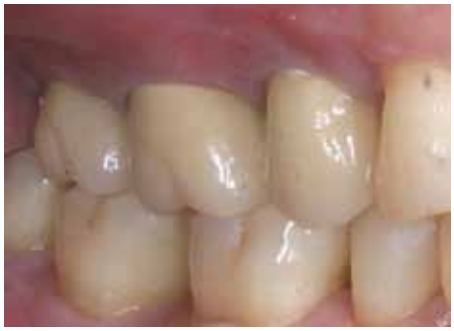


Fig. 44. A radiogram shows membrane elevation without bone graft.



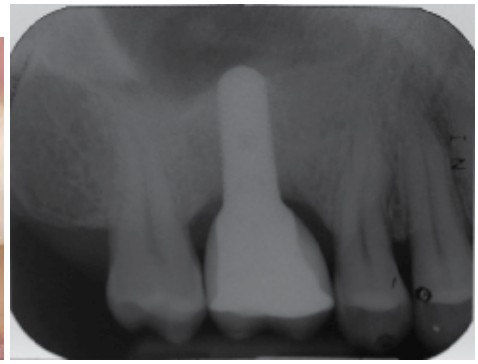


Fig. 45. (left) and Fig. 46. (right). Final prosthesis is cemented after 4 months healing. A radiogram shows sinus augmentation.

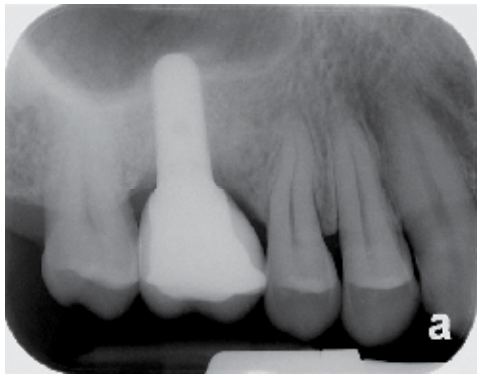


Fig. 47. A radiogram after 18 months loading. New bone formation is evident in the sinus without bone graft.

Surgical procedure of HPISE with bone graft (Fig. 48 – Fig. 60)

After elevating sinus membrane using water pressure, bone graft is optional. Bone graft in the new compartment under the elevated sinus membrane is indicated when heavy cyst is located in the sinus, more than 6mm high sinus elevation is required, implant is not placed simultaneously and postoperative radiopaque image is required to verify sinus elevation.

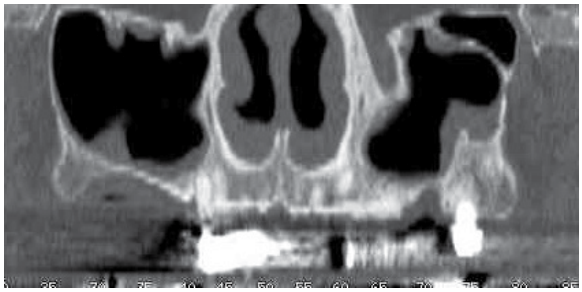


Fig. 48. 69 aged man was referred to my department for sinus and vertical ridge augmentation on the right posterior maxilla. He wants bone augmentation only. A radiogram shows very low bone height on the right posterior maxilla.

Fig. 49. Round carbide tip is used to break sinus floor at the implant sites.

Fig. 50. HPISE tip is inserted into the sinus to elevate sinus membrane with water pressure.

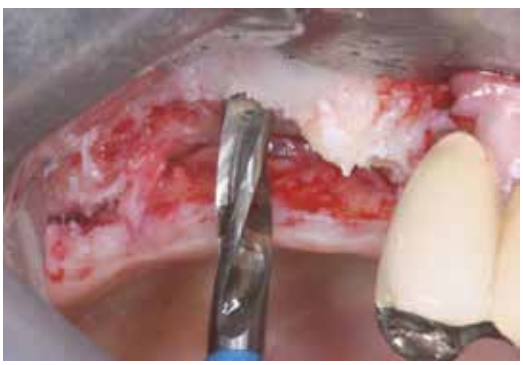


Fig. 51. In order to facilitate bone grafting, 4mm wide implant drill is used to expand the site of bone graft .

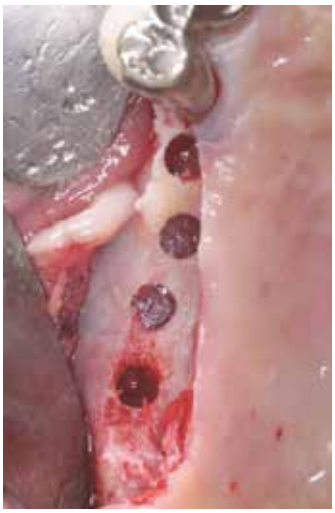


Fig. 52. Note detached sinus membrane from sinus floor. Whenever patient takes a breadth, up and down movement of the membrane is seen.

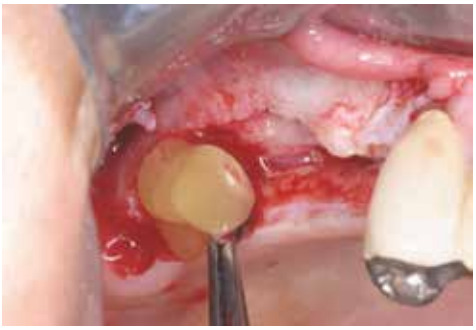


Fig. 53. CGF is inserted under the elevated sinus membrane. The CGF prevents membrane perforation as buffering effect during bone grafting and accelerates bone formation in the sinus.

Fig. 54. Gel conditioned allograft is injected into the sinus cavity

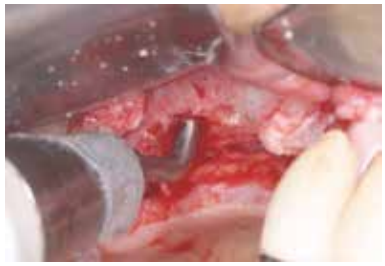


Fig. 55. Bone graft is compacted with HPISE tip

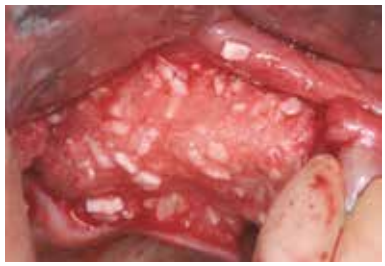


Fig. 56. Vertical bone defect is augmented with the mixture gel conditioned allograft and mineral allograft.

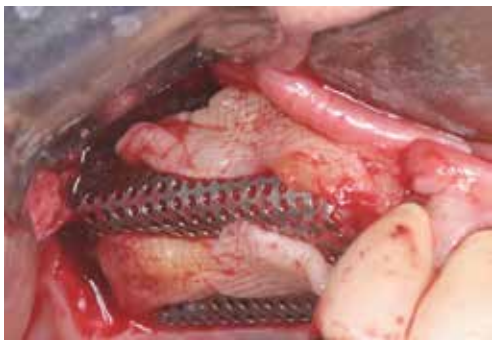


Fig. 57. Ti-mesh is stabilized with bone tack to stabilize bone graft. CGF is covered over the mesh to induce rapid soft tissue healing.

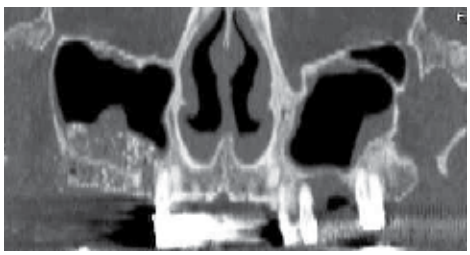


Fig. 58. Postoperative radiogram shows favorable sinus augmentation and ridge augmentation.

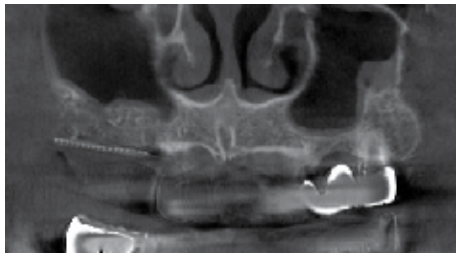


Fig. 59. A radiogram after 6 months healing. Sinus augmentation is evident.

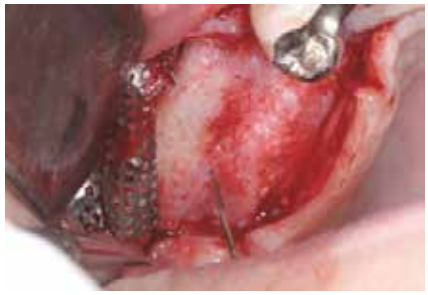


Fig. 60. Ti-mesh is removed after 6 months healing. Favorable idge augmentation is observed. The patient is referred to private clinic for implant placement.

231 implants were placed with HPISE technique at 3 centers from January 2008 to May 2010. 10 implants showed failure. Membrane perforation was developed in 14 implants (6.0% of perforation).CGF alone was inserted in the new compartment under the elevated sinus membrane in 127 implants (54.9%). Bone graft was used in 100 implants (43.2%). Collagen membrane was inserted in 3 implants (1.3%). Hyaloss matrix was used in 1 implant (0.4%). The success rate of implants was 96%. The success rate of HPISE is compatible to lateral window approach. However postoperative patient's discomfort is very minimal than lateral approached sinus augmentation.

4. Sinus augmentation using lateral window approach

Lateral window approach using various bone substitutes have been performed to overcome deficient bone volume on posterior maxillary ridge for decades.[15-17] However some studies reported new bone formation in animal and human's sinus with membrane elevation alone, resorbable gelatin sponge alone, venous blood alone and autologous fibrin gel with concentrated growth factors alone as alternatives to bone materials.[18-25] According to these studies, bone substitutes may not be a prerequisite for sinus augmentation. The function of elevated sinus membrane is controversial, but some studies reported that sinus membrane acted as periosteum and showed osteoinductive potetnial.[26, 27]

Sohn et al reported that significantly higher new bone formation was demonstrated in the sinus without bone graft than inorganic bovine bone grafted sinus.[23] According to this study, bone reformation in the new compartment under the elevated sinus membrane started from elevated sinus membrane and repositioned bony window. Osteoinductive function of sinus membrane seems to be similar to periosteum. Grafted bovine bone acted as

only space maker and scaffolding effect in this study. The key for bone reformation in the sinus is not grafting materials but space making under the elevated sinus membrane. Therefore simultaneous implant placement is required to maintain blood clot in the new compartment under the elevated sinus membrane when bone materials are not used.

4.1 New bone formation in the sinus with membrane elevation alone

Lundgren et al first reported radiographic finding of bone reformation in the sinus with membrane elevation alone in human. However the study didn't demonstrate histological evidence to verify new bone formation in the sinus. [18] Palma et al demonstrated histologic evidence to verify new bone formation in the monkey's sinus. According to this study, no differences on new bone formation, implant stability and bone-implant contacts was demonstrated between two groups with and without adjunctive autogenous bone graft.[19]

Sohn et al. first demonstrated histological evidence of new bone formation in human maxillary sinuses with sinus membrane elevation alone and simultaneous implant placement.[22]

Patient selection

Ten sinus surgeries were performed under local anesthesia. The subjects were divided into two groups according to the method of treating the lateral bony window of the sinus. Group A (five cases) used a non-resorbable membrane to seal the lateral window, but a replaceable bony window was used in Group B (five cases) to maintain blood clot in the sinus. The residual bone height of the edentulous site for implant placement varied from 1-9 mm (mean 5 mm). After average six months healing, implants were allowed to connect healing abutment and bone biopsy was performed through the site of previous bony window for histologic evaluation.

Surgical procedure of Group A (with barrier membrane)

A bony window was prepared with round carbide insert (S016®, BukBu Dental, Daegu, Korea) connected with ultrasonic piezoelectric device (Surgybone®, Silfradent srl, Sofia, Italy). After careful elevation of sinus membrane, two 4.2mm wide and 13mm high implants (Seven®, MIS implants Technologies Ltd, Shlomi, Israel) were inserted simultaneously (Fig. 61 – Fig. 62). The bony window was sealed with non-resorbable membrane (Gore-Tex®, W. L. Gore & Associates, INC. Flagstaff, Arizona, USA) and the membrane was stabilized with pin (TiTac™, IMTEC Co., Ardmore, USA) to retain the blood in the sinus (Fig. 63). The bony defect around dental implant was augmented with autogenous bone taken from bony portion of window and gel conditioned allograft (Orthoblast II®, IsoTis OrthoBiologics Inc, California, USA) and covered with a resorbable membrane (Tutoplast Pericardium®) over the bone graft (Fig. 64). Antibiotics (Levofloxacin, 100mg) and analgesics (Zaltoprofen, 80mg), three times a day for seven days, were give to all patients. Postoperative radiographic views were taken to confirm the implant position and to check coagulum formation after surgery (Fig. 65). After average six months healing, the CT scan was taken to evaluate new bone formation in the sinus (Fig. 66 and Fig. 67). Bone biopsy was performed through the site of previous bony window for histologic evaluation (Fig. 68). Follow up radiographic evaluation was continued after the placement of the final prosthetics and at follow-up after loading (Fig. 69 and Fig. 70).

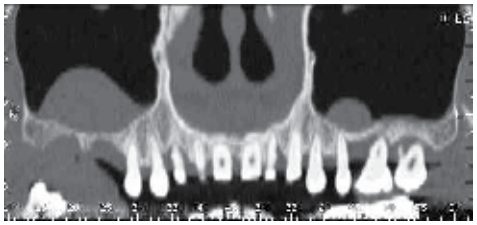


Fig. 61. Preoperative radiogram showing insufficient bone height in the right posterior maxilla.

Fig. 62. The preparation of bony window is performed with round carbide tip, connected with ultrasonic piezoelectric device. After careful elevation of sinus membrane, two 13mm high implants is placed simultaneously to maintain space under the elevated sinus membrane.

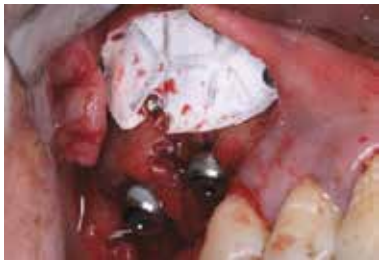


Fig. 63. The bony window is sealed with non-resorbable membrane to retain the blood in the sinus.

Fig. 64. The bony defect around dental implant is augmented with autogenous bone taken from bony portion of window and gel conditioned allograft, followed by covering with a resorbable membrane.

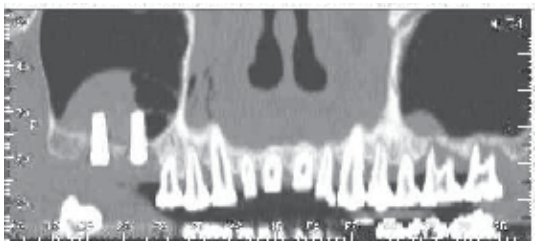


Fig. 65. Postoperative radiogram showing the elevation of sinus membrane.

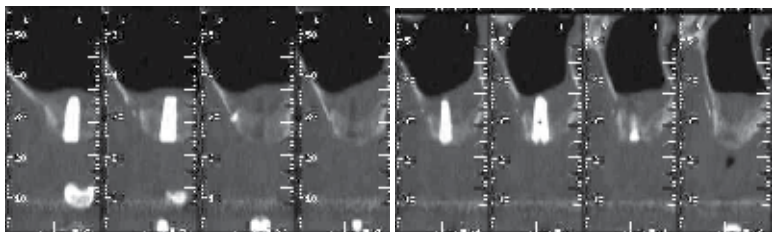


Fig. 66. (left) and 67. (right). CT scan shows bone reformation in the sinus after 6 months healing.

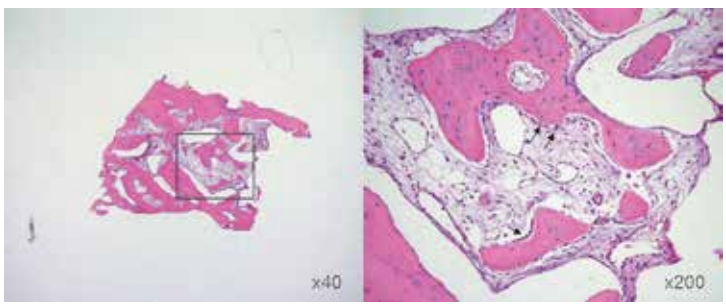


Fig. 68. Bone biopsy showing new bone formation in the sinus. Osteoblats lines along newly formed bone (arrow). (H-E stain).

Fig. 69. Final prosthesis

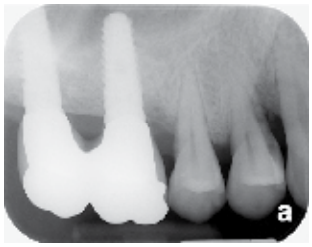


Fig. 70. Two years in function.

Surgical procedure of Group B (with replaceable bony window)

Full thickness mucoperiosteal flap was elevated to expose lateral wall of sinus cavity under local anesthesia. Saw insert with thin blade (S-saw®, BukBu Dental, Daegu, Korea) connected with ultrasonic piezoelectric device (Surgybone®) was tilted to lateral wall of sinus cavity to make replaceable bony window (Fig. 71 and Fig. 72). The bony window was carefully detached from the sinus membrane and the membrane was elevated superiorly very carefully to create new compartment to retain coagulum in the sinus. When membrane perforation was happened, resorbable collagen membrane (Tutoplast Pericardium®, Tutogen Medical GmbH, Neunkirchem am Brand, Germany) was covered over the perforation (Fig. 73 and Fig. 74). Three dental implants (Tapered Screw Vent implant, Zimmer Co) were placed at the same time (Fig. 75). The bony window was repositioned to hold blood in the sinus (Fig. 76). Postoperative care was as same as that of Group A. Postoperative radiograms and CT scans were taken immediately on surgery day and on the day of the uncovering (Fig. 77 – Fig 78). A bone biopsy was taken on the lateral access window area on the uncovering day after a six month healing period (Fig. 79). Follow up radiographic evaluation was continued after the placement of the final prosthetics (Fig. 80 – Fig. 81)

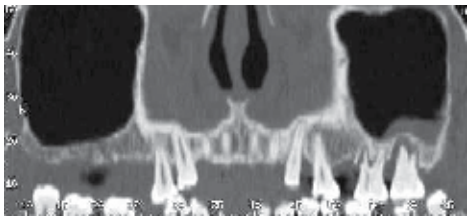


Fig. 71. Preoperative radiogram showing low bone height at right posterior maxilla.

Fig. 72. Replaceable bony window is prepared with saw insert. Tilted osteotomy is made at anterior and inferior osteotomy line. The bony window is carefully detached from the sinus membrane.

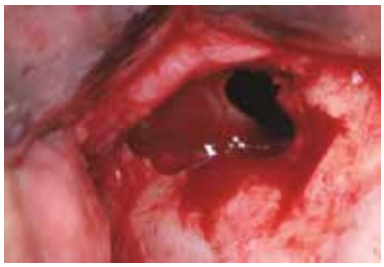



Fig. 73. (left) and Fig. 74. (right). Membrane perforation is happened during the elevation of membrane and resorbable collagen membrane is covered over the perforation.

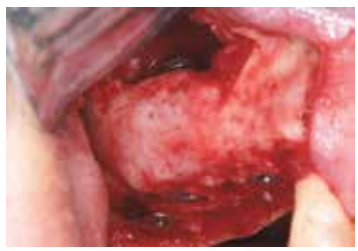


Fig. 75. Three dental implants are placed simultaneously with good stability.

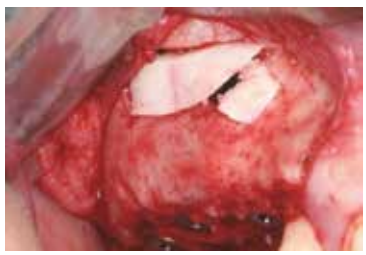


Fig. 76. The bony window is repositioned to seal the window.

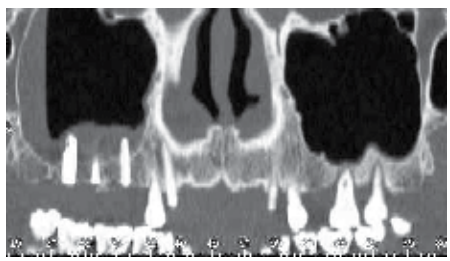


Fig. 77. Postoperative CT scan showing membrane elevation in the sinus.

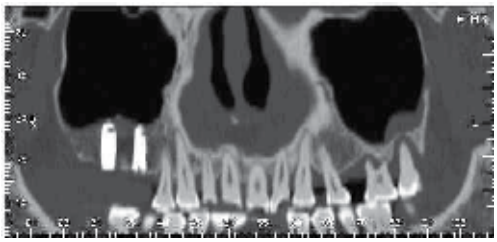


Fig. 78. CT scan shows newly formed bone in the sinus after 6 months healing.

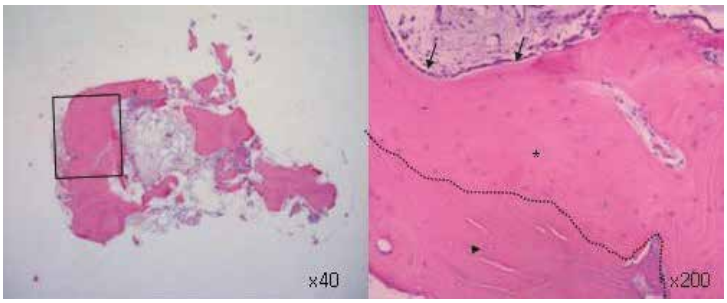


Fig. 79. Bone biopsy taken at the uncovering procedure reveales new bone formation (*) in the sinus. Arrow indicates osteoblasts and triangle indicates the bony portion of window.

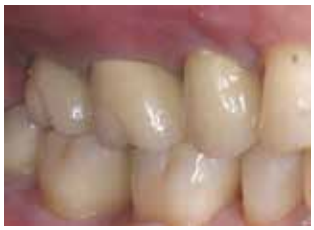


Fig. 80. Final prosthesis.

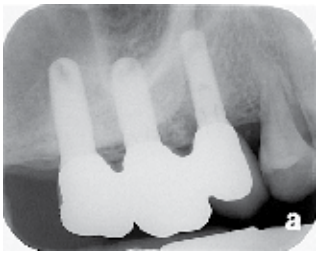


Fig. 81. Radiogram after 3 year and 8 months loading.

Results and discussion

A total of 21 implants were placed simultaneously and no implants showed osseointergration failure after the uncovering procedure. No postoperative infection was developed. All sinuses demonstrated new bone formation through clinical, radiographic

and histologic evaluations. All implant supported prosthesis was clinically stable after about 3 year follow-up. There are no differences on new bone formation between two groups. The most important factor is to induce new bone formation in the sinus is to maintain the space of new compartment under the elevated sinus membrane.

To prepare bony window, various cutting devices have been recommended. Rotary bur is a common instrument to make bony window, but high rate of membrane perforation is reported in some studies.[28-30] Some studies reported lower rate of membrane perforation with piezoelectric round tip.[31-33] Current author compared the effect of two types of piezoelectric inserts – round and saw tip – on membrane perforation during creation of lateral bony window.[33] A total rate of membrane perforation using both kinds of piezoelectric insert at 5.51%. The rate of perforation created by the piezoelectric saw (7.14%) was relatively higher than that created by the round diamond insert (2.32%) in this study. But there was no significant difference between the rates for membrane perforation for the two types of piezoelectric inserts. According to this study, the piezoelectric saw insert has some advantages over the round inserts, such as precision, minimal bone loss, and facilitation of precise replacement of the bony window. In addition, replaceable bony window acts as osteoinductive barrier to accelerate new bone formation in the sinus.[23]

4.2 New bone formation in the maxillary sinus using absorbable gelatin sponge alone

Maxillary sinus has a potential to induce new bone formation in the new compartment under the elevated sinus membrane. As demonstrated earlier, bone graft may not be prerequisite for sinus augmentation. So even though resorbable gelatin sponge is inserted in the compartment under the elevated sinus membrane for space making, bone reformation in the sinus is evident.[24,34]

Patient selection

The study consisted of nine patients treated with sinus augmentation with resorbable gelatin sponge membrane and simultaneous implant placement. Seven patients (seven men and two woman), with age ranging from 40 to 75 (a mean age of 55.2 years), were included in this study. Preoperative examinations with panoramic views and dental cone-beam computed topographic scans (Combo®: Pointnix, Seoul, Korea or Implagraphy: Vatec, Kyungi, Korea) were performed. The bone height of remaining alveolar ridge was 1.5~7.2mm (average 4.7 mm). Bilateral sinus surgery was performed in three patients and unilateral sinus surgery was performed in six patients.

Surgical procedures

Prophylactic oral antibiotics, Cefditoren pivoxil (Meiact®; Boryung Parm., Seoul, Korea) 300mg t.i.d. were used routinely, beginning one day prior to the procedure and continuing for seven days. Surgery was performed under local anesthesia through maxillary block anesthesia by using 2% lidocaine that includes 1:100,000 epinephrine. After elevating of full thickness flap, the lateral wall of the maxillary sinus was exposed. Piezoelectric saw with thin blade (S-Saw, Bukbu Dental Co., Diego, Korea), connected to piezoelectric device (Surgybone®, Silfradent srl, Sofia, Italy), was used to create the lateral window of the maxillary sinus. The tilted osteotomy into sinus cavity was performed at anterior and inferior osteotomy line to facilitate the precise replacement of the bony window as a barrier

over inserted gelatin sponge in the maxillary sinus (Fig. 82- Fig. 83) . After careful elevation of the sinus membrane, the absorbable gelatin sponge (Cutanplast®, Mascia Brunelli Spa, Viale Monza, Italy) was inserted in the new compartment under the elevated sinus membrane. Twenty two Implants were placed at the same time. The bony portion of lateral window was repositioned to prevent soft tissue ingrowth into the sinus cavity and to promote new bone formation from the lateral wall of maxillary sinus (Fig. 84 - Fig. 87). Preoperative prophylactic antibiotic therapy was continued postoperatively for 7 days and the sutures were removed 14 days postoperatively. After sinus augmentation, postoperative panoramic radiographs and cone-beam CT scans were taken immediately after surgery. An average of 6 months was allowed for the implants to integrate. The implants were then uncovered and panoramic radiographs and dental cone-beam CT scans were obtained to assess the new bone formation around the implants. Follow up radiogram after loading was taken (Fig. 88-92).

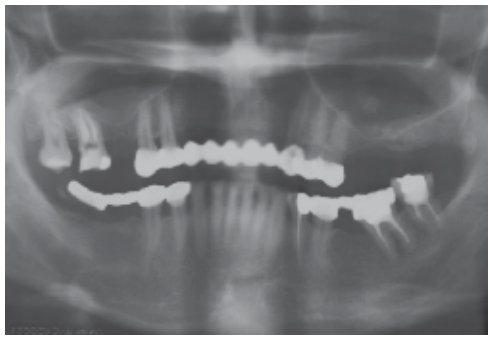


Fig. 82. Preoperative radiogram reveals pneumatization of bilateral maxillary sinus and insufficient bone height in both posterior maxilla.

Fig. 83. Replaceable bony window is prepared with piezoelectric saw insert. The tilted osteotomy into the sinus cavity was made to facilitate the precise replacement of the bony window as a barrier over inserted gelatin sponge in the maxillary sinus.

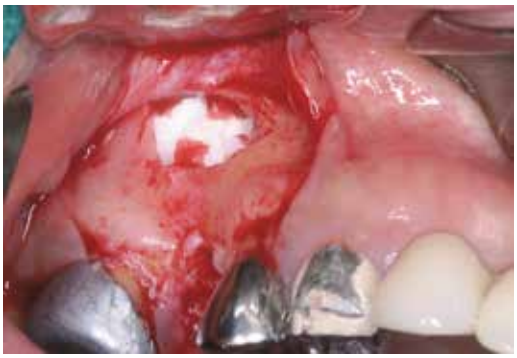


Fig. 84. After careful elevation of sinus membrane, gelatin sponge is inserted in the new compartment under the elevated membrane.

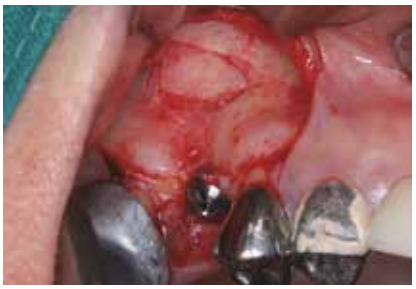


Fig. 85. Implant is placed at the same time and the bony window is repositioned. Ridge augmentation is performed at bony defect around implant.

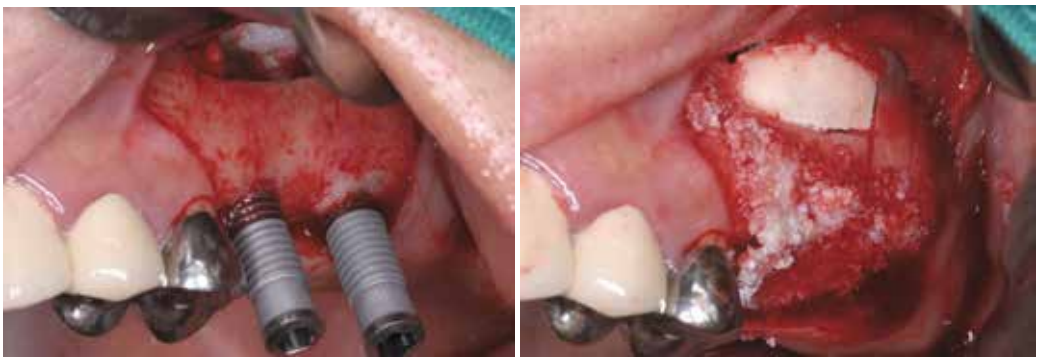


Fig. 86. and Fig. 87. Same procedure is performed in the left sinus.

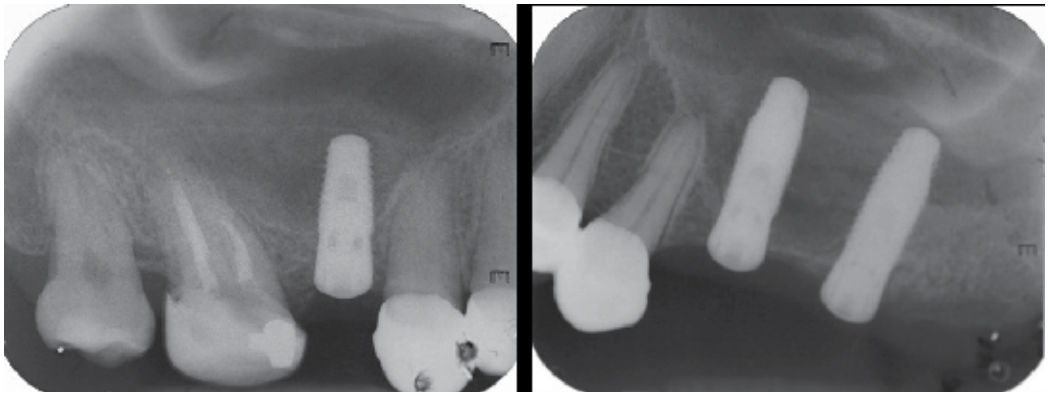
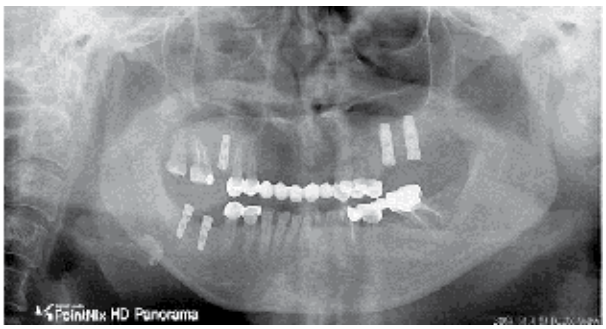


Fig. 88. Postoperative radiograms showing original sinus floor and no radiopaque image in the sinus



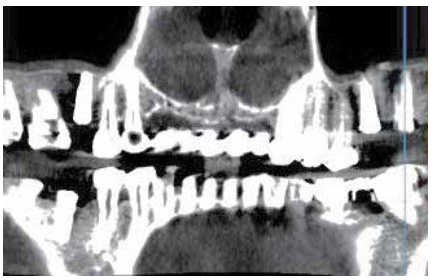


Fig. 89. (left) and Fig. 90. (right). Radiogram taken after 6 months healing reveals bone reformation in the both sinus.

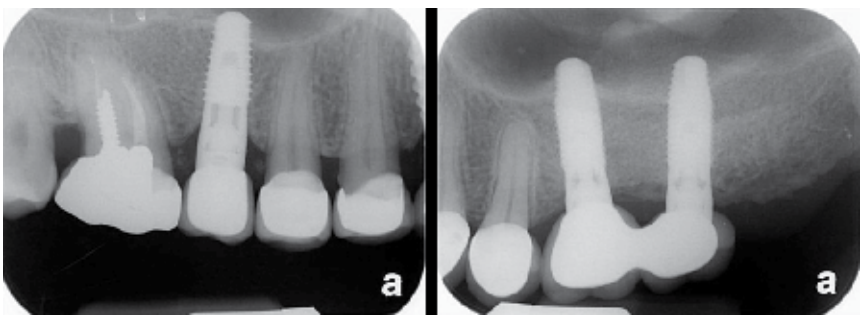


Fig. 91. A radiogram confirming bone reformation in the both sinus after 2 year loading.

Results and discussion

The membrane perforation was developed in two cases. Infection was not developed. New bone formation in the sinus was achieved in all cases using gelatin sponges alone after average six months healing period. Two RBM surfaced implants were failed after the uncovering procedure. The failures came from insufficient initial instability. This study

revealed that placement of dental implant with maxillary sinus with gelatin sponge appears to be predictable procedure for sinus augmentation and bone graft is not an essential factor for sinus augmentation.

4.3 Bone reformation in the maxillary sinus using peripheral venous blood alone

As described earlier in this chapter, bone graft is not a prerequisite for sinus augmentation when implant is placed simultaneously. According to Hatano et al and Moon et al's study, [21] The insertion of peripheral venous blood as alternative to graft material can be safely used in maxillary sinus augmentation as demonstrated in this chapter.

Surgical procedure

The surgical procedure is as same as those demonstrated above except for the insertion of venous blood in the new compartment under the elevated sinus membrane (Fig. 92 – Fig. 98)

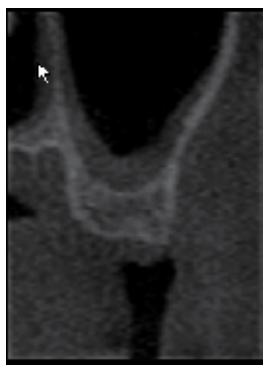


Fig. 92. Preoperative radiogram reveals approximately 6mm bone height at the site of left upper second molar.

Fig. 93. After the elevation of a mucoperiosteal flap, lateral wall of sinus cavity is exposed. The piezoelectric saw with thin blade (S-Saw, Bukboo Dental Co., Daegu, Korea), connected to piezoelectric device (Surgybone®, Silfradent srl, Sofia, Italy), is used with copious saline irrigation to create the lateral window of the maxillary sinus. The anterior and inferior osteotomy line was created out of perpendicular to the sinus lateral wall, and then superior and posterior osteotomy were made perpendicular to the lateral wall. This design of osteotomy facilitates the precise replacement of the bony window as an osteoinductive barrier over injected venous blood in the maxillary sinus. The bony window was detached carefully to expose the sinus membrane. The sinus membrane is carefully elevated from the sinus floor walls with a manual elevator.

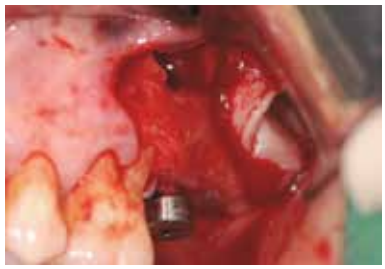


Fig. 94. A 4.1 mm wide and 13 mm high implant (SybronPRO™XRT implants, Sybron implant solution, California, USA) is placed after elevation of the sinus membrane. The collected peripheral venous blood taken from the brachial vein of patient's arm is injected to fill the new compartment of maxillary sinus.

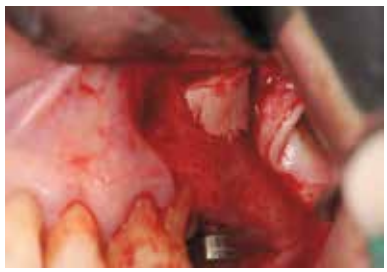


Fig. 95. The detached bony window is repositioned to prevent soft tissue ingrowth into the sinus cavity and to promote new bone formation from the lateral wall of maxillary sinus. This bony widnow acts as osteoinductive barrier.

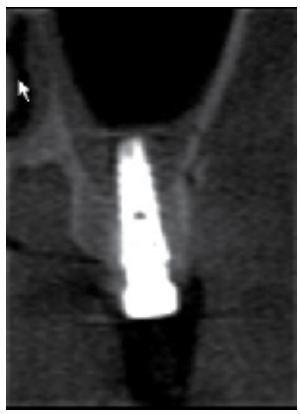


Fig. 96. A postoperative cone-beam CT scans shows venous blood filled sinus.

Fig. 97. 6 months healing is allowed for the osseointegration of implant. Cone beam CT shows newly formed bone in the sinus. During uncovering procedure, biopsy specimens is taken with 4 mm wide trephine bur at the site of repositioned bony window.

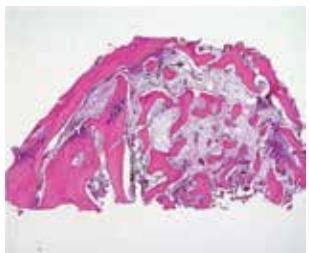


Fig. 98. Bone biopsy reveals active new bone formation. (H-E stain, x 40)

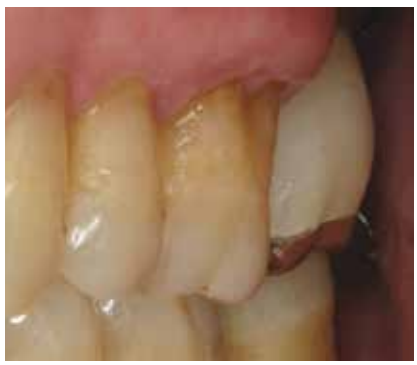


Fig. 99. Final prosthesis.

The peripheral venous blood under the elevated sinus membrane could to induce new bone formation in the maxillary sinus as shown on this case. The presence of a blood clot in healing of circumscribed bone defects was already reported .[36-37] Injection of collected venous blood could be a scaffold for new bone formation in the new compartment under the elevated membrane. The insertion of peripheral venous blood as a graft material can be a viable alternative to bone substitutes and safely used in maxillary sinus augmentation.

4.4 New bone formation in the maxillary sinus using fibrin rich gel with concentrated growth factors alone

Platelet aggregates, such as platelet rich plasma (PRP), platelet rich in growth factors (PRGF), platelet rich fibrin (PRF) and fibrin rich gel with concentrated growth factors alone (CGF) have been used to accelerate new bone formation associated with guided bone regeneration and sinus graft.[38-40] Platelet aggregates are known to contain concentrated growth factors such as transforming growth factor (TGF-β1), Platelet-derived growth factor and vascular endothelial growth factor and release slowly the growth factors. As alternative to bone filler in the sinus, the effect of fibrin rich block with concentrated growth factors (CGF) was already reported.[41] Compared to platelet rich plasma or platelet rich in growth factors, fibrin rich gel with concentrated growth factors is simple to make and doesn't require any synthetic or biomaterials to make gel condition. So it is free from the risk of cross-contamination from bovine thrombin. (Fig 100)

According to current author and colleagues' unpublished study on 61 sinus augmentations using fibrin rich gel with concentrated growth factors alone, fast new bone formation in the sinus was apparent in the all sinus radiographically and histologically.[42] Any significant postoperative complications were not developed. The success rate of implant was 98.2% after average 10 months loading. The study showed that the use of fibrin block with concentrated growth factor acts as alternative to a bone graft and can be a predictable procedure for sinus augmentation.

Fig. 100. (left). Fibrin rich block with concentrated growth factors (CGF) made by specific centrifuge (Medifuge®, Silfradent srl, Sofia, Italy). CGF is prepared with 20-60CC of patient's venous blood was taken from patients' vein in patient's forearm before sinus graft is performed. The blood in the test tubes is centrifuged at 2400-2700 rpm using specific centrifuge with a rotor turning at alternated and controlled speed for 12 minutes. The 2nd layer is fibrin buffy coat layer represented by a very large and dense polymerised fibrin block and the 3rd layer is a liquid phase containing the concentrated growth factors, white line cells and stem cells waiting for stimulation and to differentiate into specialized cell types**.** The second and third layer is known to have high concentration of growth factors. These two layers are inserted into the sinus cavity to accelerate new bone formation.

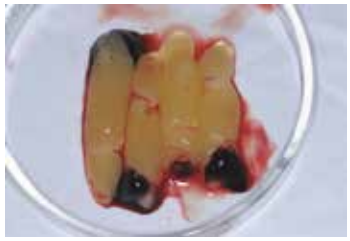


Fig. 101. This Fibrin rich block includes 2nd and 3rd layer of prepared CGF.

Surgical procedure

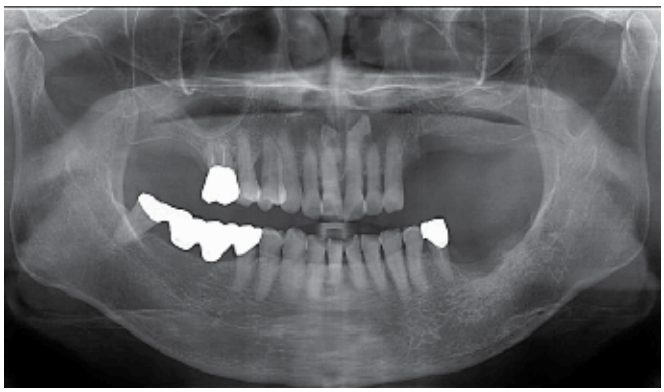


Fig. 102. Preoperative radiogram shows 5-6mm of bone height at left posterior maxilla.

Fig. 103. The piezoelectric saw, connected to ultrasonic piezoelectric device (Surgybone), is used to create the replaceable lateral window of the maxillary sinus. Sinus membrane is carefully elevated to expose medial wall of sinus.

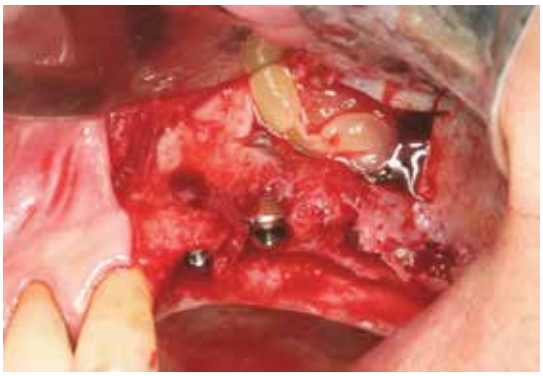


Fig. 104. Four pieces of fibrin rich gel are inserted in the new compartment between the elevated membrane and sinus floor. Three tapered design implants (Dentis implant, Dentis Inc, Daegu, Korea) is placed simultaneously

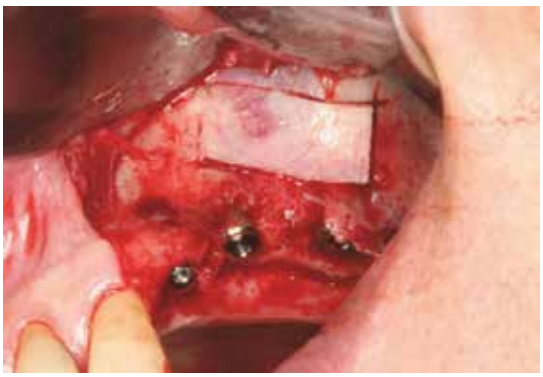


Fig. 105. The bony window was repositioned with stability to seal the window. Guided bone regeneration using mineral allograft and collagen membrane was performed to augment the bony defect around implants.

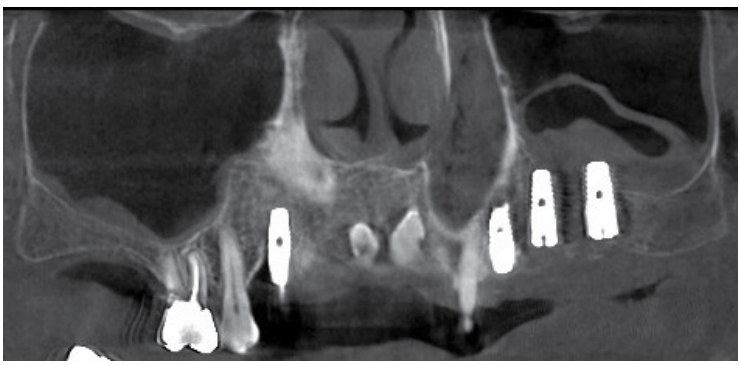


Fig. 106. Postoperative cone beam CT scans showing membrane elevation and implant placement in the left sinus.

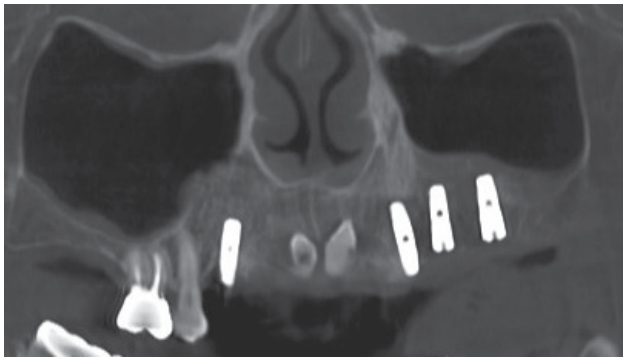


Fig. 107. Radiogram after 5 months healing. Bone reformation is evident in the left sinus

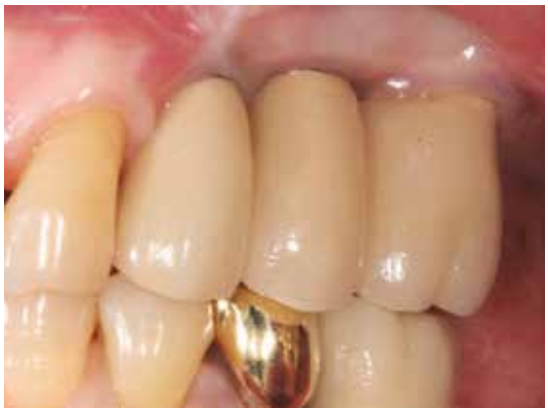


Fig. 108. Final prosthesis after 6 months loading

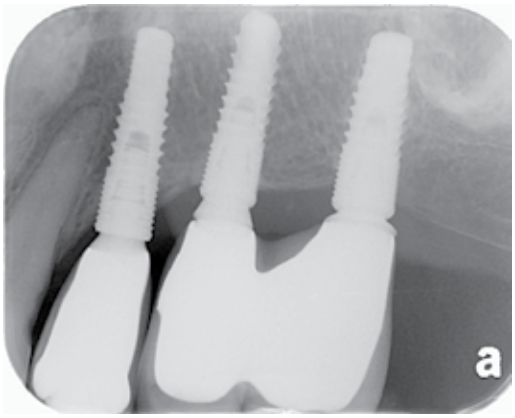


Fig 109. A periapical radiogram after 6 months loading.

As various studies on sinus augmentation without bone graft revealed earlier, bone graft may not be essential factor for sinus augmentation. The key is to maintain new space under the elevated sinus membrane.

No bone added sinus augmentation has many advantages such as

1. Infection is not reported.
2. Significant higher new bone formation is achieved than bone added sinus graft
3. Patient's morbidity is very low because the harvesting of autogenous bone is not required
4. The risk of cross contamination from bovine and human bone is eliminated.
5. Surgical cost can be reduced

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4

Bone Substitutes

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1. Introduction

In daily clinical practise we frequently encounter situations in which the bone volume is insufficient for an ideal dental implant placement. Bone regeneration can provide the structural support necessary in these cases. Procedures such as sinus lifting and alveolar ridge augmentation have reached high levels of predictability and already are of major importance in implant practise. Interest for bone substitutes for alveolar ridge augmentation or preservation appears in the early 1980's alongside the development of endoosseous dental implants. Although first studies regarding bone substitutes dates from 1920 by Albee (Albee, 1920), until 1980´s there are very few studies in reference this issue. From 1980´s until nowadays an exponential number of studies about bone substitutes have been made.

The reason for this increasing interest in bone substitutes stems from the fact that about 10-20% of the patients that need treatments with dental implants, require bone regeneration procedures before implant placement. Moreover, more than 60% of the population in industrialized countries need dental prosthetic replacements (Peterson, 2006), ideally with implants. This is the reason why the market of dental implants is experiencing an increase of approximately 15% every year.

Bone regeneration procedures are becoming an almost daily practice in dentistry all around the world as a result of the wide acceptance of dental implants as the "ideal" option for oral rehabilitation. Bone regeneration procedures are critical for the success of dental implant treatments in cases where there is a deficiency in bone width and/or height. The cornerstone in these treatments is the use of bone substitutes to create a bone mantle that covers the screw to enhance implant stability and treatment outcome. In this chapter, we will discuss the different types of bone substitutes and recent developments achieved to enhance the outcomes of bone regeneration procedures with the newest available biomaterials.

The term "bone graft" was defined by Muschler (Bauer, 2000) as: "any implanted material that alone or in combination with other materials promotes a bone healing response by providing oteogenic, osteoinductive or osteoconductive properties". An osteogenic material can be defined as one that has inherent capacity to form bone, which implies to contain living cells that are capable of differentiation into bone cells. An osteoinductive material

provides biologic signals capable to induce local cells to enter a pathway of differentiation leading to mature osteoblasts. An osteoconductive biomaterial provides a three-dimensional interconnected scaffold where local bone tissue may regenerate new living bone. However, osteoconductive biomaterials are unable to form bone or to induce its formation.
Another property that is interesting to find especially in bone substitutes is biodegradability. This is defined as the capacity of degradation of a particle by two mechanisms principally; through a passive chemical degradation or dissolution, and through active cellular activity mediated by osteoclast and/or macrophages. Moreover, the biological properties of bone substitute biomaterials are also influenced by their porosity, surface geometry and surface chemistry. The events leading to bone healing and regeneration are influenced by all the variables mentioned above. These properties are related to the biomaterial itself, however, host factors such as bone quality, vascularity of the graft bed and tobacco addiction may also influence the final outcome of a bone regeneration procedure with a bone substitute.

2. Biomaterials used for bone regeneration in implant dentistry

Bone graft materials can be divided in four large groups: Autografts, Allografts, Xenografts and Synthetic biomaterials.

2.1 Autograft

"Autograft" refers to bone tissue harvested from, and implanted in the same individual. Accordingly, autograft is a bone tissue that is separated from one site and implanted in other location in the same individual. The cellular component of trabecular bone graft includes few osteoblasts and a high number of precursor cells that survive the transplantation. These precursor cells explain the osteogenic potential of bone autograft. Autograft is considered the "gold standard" in bone regeneration due to its properties of osteoconduction, osteoinduction, osteogenicity and osteointegration. However there are major drawbacks to the use of this sort of ideal bone graft, namely the necessity of a second surgery to retrieve the bone graft at the donor site, with its associated morbidity; the increasing surgery time, the restrictions in quantity and shape of the bone graft, and the additional cost (Arrington, 1996; Giannoudis, 2005).
Autografts are subdivided in two groups: cancellous autografts and cortical autografts. *Cancellous autografts* are retrieved mainly from calcellous bone, and upon transaplantation, the majority of cells presnet in the grafts die as result of ischemia. However, the mesenchymal stem cells present in the bone marrow are resistant to ischemia and may survive the grafting procedure. The stem cells capacity of survival and proliferation after exposure to changes in the oxygen tension, pH and cytochine environment are the main reason behind the reliability of of cancellous bone autograft interventions. The incorporation of such type of autogrfraft is speed, about 8 weeks (Virolainen, 1995).
Cortical autografts are segments of cortical bone composed of necrotic bone that provides an osteoconductive support for bone formation, but does not supply significant amounts of living cells. For this reason, revascularization and integration of cortical autografts is slow. The main advantage of cortical autografts is the mechanical support that provides at the graft site (Bauer & Muchler, 2000), while its incorporation is slower than cancellous autografts.

Bone graft	Properties: Origin	Availability	Reabsorption	Graft´s form
Extraoral Autograft				
Calvaria	IM	+++	++	Block/Particulate
Iliac crest	EC	+++	+++	Block/Particulate
Tibia	EC	+++	+++	Particulate
Intraoral Autograft				
Ramus	IM	++	++	Block
Sinfisis	IM	+	++	Block/Particulate
Tuberosity	IM	+	+++	Particulate

IM: Intramembranous bone, EC: Endochondral bone

Table 1. Summary of bone autograft properties as a function of their anatomical origin

2.2 Allografts: Freeze dried bone allograft (FDBA) and demineralized freeze dried bone allograft (DFDBA)

Allograft is defined as tissue that has been harvested from one individual and implanted into another individual of the same species (Eppley, 2005).The use of cadaver bone for grafting is known as bone allograft and it is considered by some the best available alternative to autografts due its similarly characteristics. Despite the superior properties of autografts, allografts are usually preffered by patients as bone grafting material because they the problems associated to donor site surgery in autografts.. Allografts are obtained from cadaver tissue banks for mineralized freeze-dried (FDBA) or decalcified freeze-dried (DFDBA) bone.. Both FDBA and DFDBA are obtained from cortical bone of long bones due to its high content of bone inductive proteins and less antigenic activity than cancellous bone. Bone allografts come in various configurations, including powder, cortical chips, cancellous cubes, and cortical granules among others (Eppley, 2005). The granulated form is btained by milling the cortical bone under sterile conditions to obtain a particle size ranging from 250 to 750 μm. Moreover, allografts have been recently made available in different block forms; although their mechanical properties remains slightly lower than those of autograft cortical blocks.

Once the allograft is harvested they are processed through several methods including physical debridement to remove soft tissue and reduced cellular load, ultrasonic washing to remove remnant cells and blood, ethanol treatment in order to denaturalize proteins and viral deactivation, antibiotic wash to kill bacteria, and sterilization through gamma radiation and ethylene oxide for spore elimination (Khan, 2005). FDBA are washed in antibiotic twice for 1 hour, frozen at -70C^{o} and dried up to 5% of water (Kao, 2007).During

this process microfractures form along the allografts' collagen fibers, resulting in a decreased in its mechanical properties, for this reason it is advised to rehydrate allografts before use to regain some of the lost properties (Kao, 2007). Processed bone allografts do not include any living cells, and therefore, they lack osteogenic activity. Allografts are essentially osteoconductive, and depending how they are processes, they may have some osteoinductive properties (Stevenson, 1999).

FDBA: Mineralized bone matrix has no active bone morphogenetic proteins (BMPs) and therefore it lacks osteoinductive properties, although it has osteoconductive properties. Graft incorporation is qualitatively similar to autograft, but occur more slowly. Cortical allografts will incorporate and eventually resemble their autograft counterpart although with more unremodeled necrotic bone present in allografts (Stevenson, 1999). Milled forms present an open structure that facilitate invasion by blood cells, enhance graft incorporation and allows mixing with blood, platelet concentrates and other graft materials forming composites.

DFDBA: DFDBA forms are processed by acid demineralization in 0.5 to 0.6 molar hydrochloric acid as a result, 40% of the mineral content is removed leaving the organic matrix intact. This process preserves the BMPs present in bone, and therefore maintains some of the inherent osteoinductive properties (Khan, 2000). Moreover, the collagen matrix present in DFDBA acts as a scaffold that provides osteoconductive properties alone side the osteoinductive behavior. Osteoinductivity of DFDBA was first described by Urist et al, after observing endochondral bone formation on DFDBA when placed in soft tissue. It has since been discovered that BMPs are the factors responsible for the novo bone formation (Reddi, 1998). BMPs are associated with the organic matrix of bone and embedded within mineral content, so demineralised process increases its bioavailability. BMPs attract mesenchymal stem cells and induce them to differentiate into chondrocytes leading into endochondral bone formation. Endochondral bone formation is attributed to a osteoinductivity response, while intra-membranous bone formation is indicative of an osteoconductive response. Nevertheless, osteoinductivity of DFDBA has been recently questioned, since it seems that this property is highly dependent on the manufacturing procedures (Drosos, 2007)

The main advantage of allografts include easy availability, avoiding the need of harvesting a patient donor site, reduced costs in terms of anesthesia (general anesthesia is not needed) and reduced surgical time. However, the use of cadaver bone for grafting is avoided by many clinicians due to its potential risk of infectious disease. Nevertheless, allografts have been used for more than 25 years without any reported incidence of disease transmission. The risk of HIV infection through allograft implantation has been estimated to be 1 in 1.6 million, compared with the risk of 1 in 450.000 in blood transfusions (Khan, 2000). DFDBA forms may have even less risk of disease transmission than FDBA, because demineralization allows most affective removal of viruses and blood elements reducing immunological reactions (Eppley, 2005). Moreover the establishment of better equipped tissue banks has allowed an increase in the use of bone allografts, and there is a current tendency by many surgeons to replace autografts by bone allograft (Albert, 2006).

Allografts are available in the form of granules and blocks. Allograft granules' appearance is similar to other bone substitute granules, and they are ideal to fill bone cavities as alveolar bone defects and maxillary sinus. On the other hand, allograft blocks are especially useful in both vertical and horizontal bone augmentation procedures.

Bone graft	Properties			
	Structural stregth	Osteogenesis	Osteconductor	Osteoinductor
Autograft				
Cancellous	No	+++	+++	+++
Cortical	+++	++	++	++
Allograft				
Cancellous	No	No	++	+
Cortical	+++	No	++	No

Table 2. Comparison between bone autograft and allograft according to their properties

2.3 Xenografts: Anorganic bovine bone (ABB)

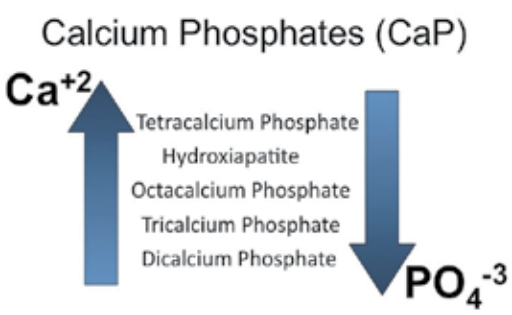
Bone xenograft is defined a bone tissue harvested from one species and implanted into a different species. One of the most commonly used xenografts is anorganic bovine bone (ABB). ABB is a biomaterial with major long-term success reports in the bone regeneration literature and it has been extensively used in the clinics for over 20 years (Frame et al., 1987). ABB has an ultrastructural composition similar to human bone, it is composed of almost pure hydroxyapatite, and it is chemically treated to remove all organic components so it can be used as a graft material without causing host immune response (Mish et al., 1993). ABB is thermally and chemically treated in order to extract organic constituents and thereby eliminating its antigenicity and potential inflammatory response by the host bone (Cohen et al., 1994). The structure consists of a wide interconnective pore system with a particle size of 0.25 to 1 mm that can easily be invaded by blood vessels resulting in osteoblastic migration. . ABB is up to 75% porous and has a high specific surface area of almost 100 m2/g that results in increased angiogenesis, enhances new bone growth (Hammerle et al., 1997; Rodriguez, 2003), and excellent osteoconduction properties. However, its highly porous consistency sometimes compromises its mechanical properties and its initial stability. ABB lacks osteoinductive properties, and its presentation in form of granules makes it difficult to hold on surgical sites. Moreover, it is non resorbable *in vivo*. Indeed, ABB might need several years (3-6 years) of implantation before showing some slow *in vivo* resorption through osteoclast activity, (Skoglung et al, reported that granules were present even after 44 months (Skoglund., 1997) . The presence of unresorbed granules within the newly formed bone is undesired because it affects the quality of the newly formed bone by interfering with its remodelling, compromising its osteointegration capacity with dental implants.

Although ABB is mostly used in form of granules, xenografts blocks design are also available. Xenogenic derived bone block have already been reported to achieve vertical bone augmentation in the mandible. However, these materials are quite brittle and fragile. This mechanical inconvenience not only complicates the surgical technique but it also hinders the bone graft healing process (Simion et al 2006; Felice et al., 2009). Other types of xenogenic

(porcine) bone block seems to show better mechanical properties and low risk of fracture while screwing. (Simion et al., 2009). Generally speaking, the use of xenogenic bone blocks is still under evaluation and at this moment there is not sufficient information regarding its *in vivo* behaviour.

2.4 Synthetic calcium phosphates

Calcium phosphates constitute synthetic biomaterials that chemically resemble the bone mineral . Calcium phosphate biomaterials are widely selected to regenerate bone tissue due to thier biocompatibility, osteointegration and osteoconductivity (Alkhraisat et al., 2008). We can make a classification of calcium phosphates in order to its Ca^{2+} and P compounds.



Calcium Phosphates (CaP)

CaP	Properties			
	Biocompatible	Reabsorbible	Osteoconductor	Osteoinductor
AP	+++	-	+++	-
CAP	+++	+	+++	-
β-TCP	++	++	+++	+
DCPD	++	+++	+++	-
BCP	+++	++	+++	+

Table 3. Summary of synthetic calcium phosphate bone substitutes arranged to their chemical composition and biological properties

Regarding this classification is interesting to know that materials which contains high levels of Ca^{2+} ions have alkaline Ph and therefore shows low resorption capability as hidroxiapatite (AP), while materials with low levels of Ca^{2+} ions have acid Ph and shows high resorption properties, as dicalcium phosphate forms.

According to their preparation, calcium phosphate could be divided into high temperature (ceramics of tricalcium phosphates, hydroxyapatite and biphasic calcium phosphate) and low temperature (cements) calcium phosphates. Such bone substitutes differ in the degradation rate in vivo, strength, alkalinity and acidity, and crystallographic structure. Generally, they are fragile materials and should be used in non-load bearing areas.

Hydroxyapatite and β-tricalcium phosphate (β-TCP) are the ceramics mostly recruited clinically to treat bone defects and voids. Biologicall, stoichiometric hydroxyapatite of Ca/P ratio of 1.67 is highly stable and its very slow degradation is mediated by phagocytosis [Constantino & Freidman, 1997). Such handicap is managed by introducing impurities like carbonate ions, silicon ions and other ionic species present in the bone mineral [Alkhraisat et al., 2008). Structurally, porous hydroxyapatite was introduced to resemble native bone architecture, improve the degradability and enhance tissue reaction of angiogenesis and new bone in-growth. This resulted in engineering apatite and calcium carbonate of live species to produce a hydroxypatite conserving the macro and microporous architecture of the source . An example of such technology is the anorganic bovine bone, coral apatite and algae apatite.

Since the experiments reported by Albee and Morrison (Albee, 1920), research and development projects have been dedicated to explore the potential of β-TCP ceramic bone substitute. β-TCP has Ca/P ratio of 1.5 and is more resorbable than hydroxyapatite. This higher degradability is related to higher solubility that aid phagocytosis to induce biomaterial replacement with new bone.

Biphasic calcium phosphate (BCP) is engineered to combine the advantages of both hydroxyapatite and β-TCP. A relation of 60% hydroxyapatite and 40% β-TCP is the most common among commercial biphasic calcium phosphate.

These ceramics are presented in porous granular and block forms and they are difficult to reshape. The granules have a size range between 0.2 mm and 1mm.

The lack of adaptability of calcium phosphate ceramics was solved by Brown and co-workers when they developed calcium phosphate cement (Brown & Chow, 1985). This cement is the mixture of calcium phosphate powders that upon reacting with aqueous phase produce new calcium phosphate. The chemical reaction that produce this new phase is termed setting reaction and the consistency of the cement is progressed from paste-like to solid structure by the entanglement of the setting product. This enable the cement to be moulded, adapt intimately to the bone defect borders and permits the development of injectable preparation for minimally invasive surgery. Such cements are biocompatable, degradable and osteoconductive (Figure 1) (Alkhraisat et al., 2008).

Calcium phosphate cements are classified according to the setting reaction end-product to hydroxyapatite and brushite cements. Hydroxyapatite cement is first developed by Brown and co-workers and since then variuos formulation have been developed and patented. Of such formulations are tetracalcium phosphate/dicalcium phosphate anhydrous (DCPA) system and α-TCP based system. The setting reaction of hydroxyapatite cement occurs at neutral pH which is biologically favourable. The hydroxyapatite as setting product is low-crystalline and the stoichiometry can be varied to produce calcium deficient-hydroxyapatite (Ca/P ratio less than 1.67). These features and the development of carbonated apatite cement improve the degradability of hydroxyapatite cement.

Since their development by Mirtchi and co-workers, brushite cements are recieveing much interest as bone substitute in the recent years [Mirtchi, 1990). These cements are obtained by various combinations, such as β-TCP + monocalcium phosphate monohydrate (MCPM) and β-TCP + phosphoric acid. The setting reaction of these cements is a continuous dissolution/precipitation mechanism at low pH values as brushite precipitates at pH <6 [Alkhraisat et al., 2010).. The relatively short setting time of brushite cements compared with hydroxyapatite forming pastes depends on both the higher solubility of the cement raw materials and the higher rate of brushite crystal growth (3.32×10^{-4} mol min^{-1} m^{-2}) [compared with hydroxyapatite (2.7×10^{-7} mol min^{-1} m^{-2}) (Zawackiet al., 1996).

The main advantage of brushite is its higher degradability compared to hydroxypatite that stems from higher solubility at physiological conditions. However, in vivo brushite transformation to hydroxyapatite is kinetically favourable and additives are patented to inhibit such transformation [Alkhraisat et al., 2010). This fact have raised the attention to the anhydrous form of brushite, monetite, that is prepared by drying brushite. Monetite is more stable than brushite due to its lower solubility and in vivo transformation to hydroxyapatite was not reported ensuring a predictable degradability (Tamimi et al., 2009).

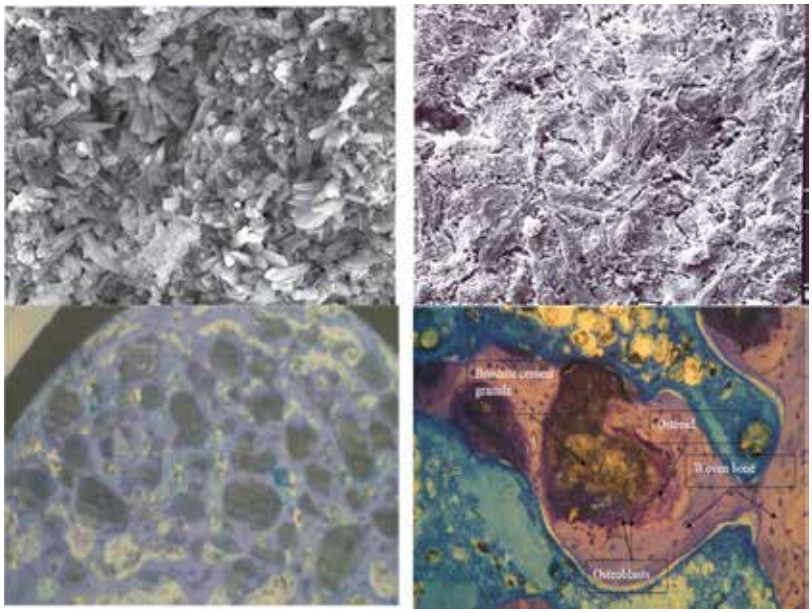


Fig. 1. The microcrystalline structure of dicalcium phosphate dihydrate cement provide support for osteoblasts growth and proliferation that permits bone formation on and between calcium phosphate granulate.

Recently, calcium phosphates are shown to be efficient as local drug and bioactives delivery system [Alkhraisat et al., 2010).and the technology is now available to prepare custom-made bone calcium phosphate based on patient's CT scan data to fit in a bone defect [Klammert et al., 2010). These biomaterials are the corner-stone in the development of 3D-porous scaffold in combination with organic components to resemble the inorganic-organic harmony of bone. Such scaffolds are designed to support the growth and differentiation of stem cells for their application in the emerging field of regenerative medicine.

2.5 Bio glass

Bioglass, also known as bioactive glass, is the commercial name for the first calcium substituted silicon oxide that was marketed as a bone regeneration material over 30 years ago. This material was developed by researchers working for the US army during the Vietnam War as a biomaterial for repairing bone loss in injured combat soldiers (Välimäki & Aro, 2006).
In plain language bioglass is a glass similar to that in the windows of your house, but with a large portion of calcium in its chemical structure. It has a large surface area that is alkaline and highly reactive to serum ions. This feature enables it to interact with serum, allowing a very fast precipitation of hydroxyapatite on its surface once implanted *in vivo*. This phenomenon is called *bioactivity*, and is one of the unique characteristics of Bioglass that allows a quick integration to bone tissue.
Bioglass is suitable for bone regeneration in dental implant surgery; moreover, it is purely synthetic therefore it does not present problems regarding transmission of infectious diseases. However, its granule format is difficult to handle due to the repulsive charges between the highly charged surfaces the granules. This renders its clinical handling more demanding than other biomaterials (Välimäki & Aro, 2006).

The critical component of bioglass is SiO2 which constitutes 45-60% of its weigth. The first bioglass developed for bone regeneration was based on 4 components: SiO_2, Na_2O, CaO and P_2O_5.However, this composition tend to crystallize, and was modified to a more stable glass composed of: Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2.In vivo experiments have shown that implantation of bioglass in bone defects causes an inhibition in bone formation during the early healing stages, but it eventually doubles the amount of bone formed when no biomaterial is used.
Moreover, bioglass experiences sever resorption during the first 2 weeks after implantation. However, beyond this point its resorption rate is stabilizes.
Upon implantation, the smaller ions present in bioglass (i.e. Na^+ and K^+) tend to leach to the extracellular fluids. This results in a rich Si layer coating the biomaterial. Ca^{2+} and PO_4^{3-} ions from the body fluids then react and precipitate on the Si rich layer, forming a thin coat of hydroxyapatite. The calcium phosphate layer adsorbs proteins. And these extracellular properties attract macrophages stem cells and osteoprogenitor cells (Välimäki & Aro, 2006).
Bioactive glass can be used in form of granules or as preformed cones designed for placement into fresh sockets to maintain the alveolar ridge (Stanley et al., 1997). It has shown clinical success in vertical bone augmentation procedures, in regeneration intra-bony defects and in the preservation of alveolar sockets (Gatti et al., 2006) .However, even though it is resorbable and promotes bone formation, its bone regeneration capacity in maxillofacial surgery has been shown to be lower than Calcium phosphate biomaterials (Santos et al., 2010).

3. Surgical procedures that require the use of bone substitutes

3.1 Sinus lift

Bone augmentation in sinus lifting procedures requires the use of bone regeneration biomaterials that would enable bone formation within the maxillary sinus. Ideally besides good osteoconductive properties, biomaterials used for sinus lifting should be easy to handle, and have a limited *in vivo* resorption. Underneath we discuss the main bone substitutes that have been employed in sinus lift bone augmentation procedures
Autograft: The use of bone autografts are recommended when treating large pneumatized sinuses and a short treatment period is required. The healing period in such situations is 3-4 months shorter than when other bone substitutes are used (7-9 months). Also the use of autografts is an option when patients reject the use of allograft or bovine bone. Several studies have reported high rate of success using autografts from either endochondral or intramembranous origin. (Daelemans et al., 1997). Intramembranous bone has been shown to resorb less readily than endochondral bone (Hardesty et al., 1990). Despite the excellent clinical results obtained with autografts, a high degree of morbidity has been reported in such procedures. In order to diminish the morbidity and also the high resorption rate of autografts, many clinicians use autografts in combination with other bone substitutes in different proportions, and obtaining promising results.
Autografts combined with ABB: Studies evaluating the use of bone autografts in combination with ABB, have shown great results in terms of percentage of newly formed bone in the sinus and subsequent implant success rate. Bone formation is higher when a major percentage of autograft is added (48%) and significantly lower amounts of vital bone forms when autograft is used in minimal propoprtions. Froum et al. observed a statistically significant increase in vital bone formation when as little as 20% autologous bone was added to ABB compared with ABB alone. Nevertheless, a high percentage of bone volume

formation within the sinus is not a crucial parameter regarding the subsequent success rate of the dental implants. Indeed, bone volumes as low as 24% have shown to be sufficient for successful osteointegration of dental implants placed in augmented maxillay sinuses (Esposito et al., 2010).

Vital bone formation with different types of graft in sinus augmentation			
Authors	*ABB:Autograft*	*Healing time (months)*	*Vital bone (%)*
Hallman et al 2002	*100 Autograft*	*6-9*	*37*
John et al 2004	*100 Autograft*	*3-8*	*53*
Mish & Krauser 2003	*20:80*	*4-9*	*48*
Mish & Krauser 2003	*40:60*	*4-9*	*36*
Mish & Krauser 2003	*60:40*	*4-9*	*38*
Galindo et al 2010	*80:20*	*6-9*	*46*
Hallman et al 2002	*80:20*	*6-9*	*41*
Froum et al 2008	*100 ABB*	*6*	*22*
Hallman et al 2002	*100 ABB*	*6-9*	*39*
John et al 2004	*100 ABB*	*3-8*	*29*

Table 4. Overview of different composite graft material in sinus lift technique

Allografts: Despite the risk of disease transmission associated with its use, approximately 350.00 to 400.000 bone allografts procedures are performed in the United States, Out of which 100.000 are dental related. The use of Allografts in sinus augmentation procedures has shown high success rates. (Avila et al., 2010). The percentage of bone volume obtained with FDBA in sinus lifting procedures is 23%. Moreover, the success rate of implants placed in the maxillary sinuses following bone augmentation with solely applied allograft has been shown to be a 97.7% in three years follow up (Minichetti et al., 2008). On the other hand, Peleg et al reported a 100% success rate over 160 implants placed in 63 grafted sinuses using as graft material autogenous graft harvested from symphysis with DFDBA combined in 1:1 ratio after a 4 year follow up (Peleg et al., 1999).

DFDBA was compared with autograft in sinus augmentation procedures; DFDBA showed 29% of new bone formation compared to a 40% achieved with autograft (Kao 11). Froum also studies the differences between FDBA and ABB, observing a high vital bone formation (28%) in FDBA grafted sinuses compared to a 12% in ABB grafted ones'. (Froum et al., 2006). No differences have been observed between DFDBA and FDBA in terms of percentage of vital bone formation in the maxillary sinus.

Anorganic bovine bone (ABB): ABB is a biocompatible and osteoconductive anorganic bovine bone (ABB), that provides an ideal scaffold for new bone formation (Hammerle et al., 1998, Piatelli et al., 1999). It has been extensively used in maxillary sinus floor augmentation (Valentini et al., 2003, Wallace et al., 2005) with high clinical success rates (Carmagnola et al., 2003). Comparative studies by Hallman et al and Valentini and Abensur observed higher survival rates for implants placed in sinuses grafted with 100% ABB compared with sinuses grafted with 100% autograft (Hallman., 2002, Valentini., 2003). Also Froum reports higher implant survival rate in sinuses grafted with ABB alone than those grafted with a composite of ABB+DFDBA (Froum et al., 2006). In a recent study Torres et al observed a 97% implant success rate in 286 implants placed in 144 sinuses grafted with ABB alone or ABB+PRP. Overall, 96.2% of ABB and 98.6% of ABB+PRP implant success was obtained during the monitoring period and differences were not found between sites grafted with and without

PRP in the 87 patients studied. (Torres et al., 2009). Regarding vital bone formation achieved with ABB in maxillary sinuses, Scarano et al compared ABB to autograft bone in maxillary sinus augmentation procedures. In this study, 6 months after the initial intervention, ABB resulted in 39% new bone formation compared to 40% with autograft. Although the results of new bone generation were very similar, 31% of the grafted Bio-Oss was still present at the graft site compared to only 18% of autograft (Scarano et al., 2006).(Fig 2)

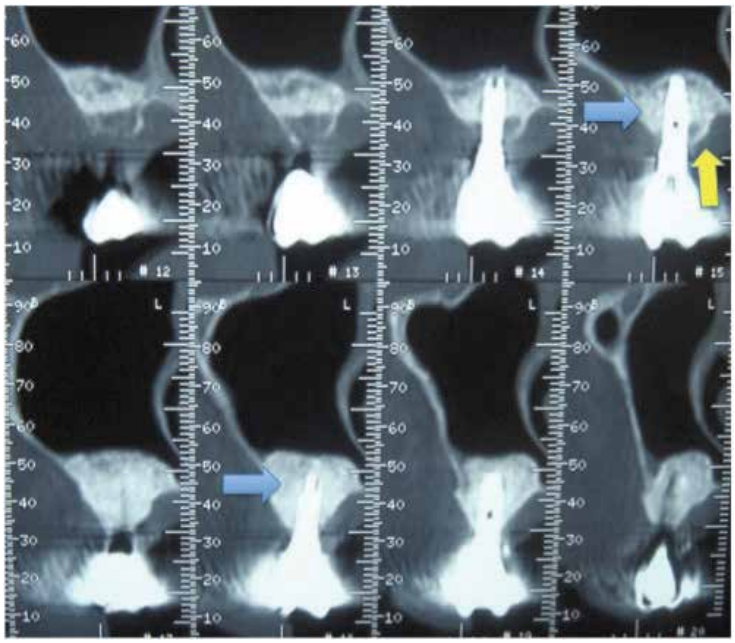


Fig. 2. Slow resorption of ABB particles could be observed after 24 months of implant´s placement. Blue arrow shows remanent ABB graft. Yellow arrow shows residual host bone

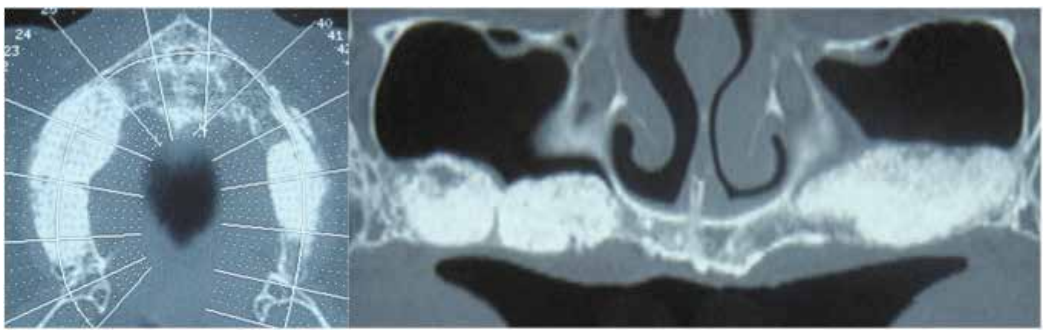


Fig. 3. Bilateral sinus augmentation performed with ABB alone as graft material.

Synthetic calcium phosphate such as β-TCP ceramic granules have also been used in sinus augmentation techniques with high rate of implant success. In a comparative study, Simunek et al showed that a higher proportion of the new vital bone was achieved with ABB (34.2%) compared to β-TCP (21.4%). (Simunek et al. 2008)

However Szabó et al in another comparative study between β-TCP ceramic granules versus autograft observe that vital bone areas were 36% and 38%, respectively.(Szabó et al. 2005)
Rapidly resorbable biomaterials are not recommended for sinus lift procedures since the maxillary sinus is a non functional site for bone to form, and in the absence of mechanical load any bone augmentation achieved is likely to be lost if the scaffold provided by the biomaterial resorbs. For these reasons the most suitable biomaterials for sinus lifting procedures are osteoconductive biomaterials with limited or non resorption properties such as granules of ABB, β-TCP, hydroxyapatite, biphasic calcium phosphate and Bioglass.
In a recent systematic review performed by Esposito, it was concluded that ABB and β-TCP might be as effective as bone autografts for augmenting atrophic maxillary sinuses. Therefore these biomaterials might be used as replacement for autogenous bone grafting (Esposito et al. 2010).(Fig 3)

3.2 Alevolar ridge augmentation
3.2.1 Onlay bone grafts

Onlay bone grafts

Onlay bone grafting is the most predictable technique available for bone augmentation of the alveolar ridge. This procedure is used to augment bone before or at the time of implant placement to ensure proper implant osteointegration (Rocchietta et al. 2008)
Onlay bone grafting is achieve by fixing a biomaterial in the shape of a block directly onto the bone surface of the alveolar ridge, and covered with the periosteum and oral mucosa. This procedure requires a biomaterial strong enough to bear direct occlusal forces and allow screw fixation. Besides, the biomaterial should also be bioresorbable and osteconductive.
Currently the best available biomaterial for onlay bone grafting is the autologous bone graft. Autologus onlay bone grafts can be harvested from extra-oral or intra-oral bones (Rocchietta et al., 2008).Onlay grafts of autologous bone can resorb once implanted, limiting the amount of bone volume available for dental implant stabilization. In this sence, intra-oral grafts are always preferred, since they tend resorb less than extra-oral bone grafts after implantation (Rocchietta et al., 2008).
The limited amount of autlogous bone available for grafting alongside the morbidity associated to the donor site has driven the need for developing alternative materials. Accordingly, alloplastic, allogenic and xenogenic onlay blocks have been recently developed and tested showing promising results. Several animals studies have shown that blocks made of either allogenic materials, hydroxyapatite, tricalcium phosphate or monetite are capable of achieving vertical bone augmentation in onlay bone graft procedures (Tamura et al., 2007, Tamimi et al .,2009, Fujita et al., 2003, Hetherington et al., 1996).Moreover, clinical studies have confirmed the great potential of allogenic bone blocks as biomaterials for onlay bone augmentation (Waasdorp et al., 2010)
In order to achieve succes with an onlay bone graft procedure, the onlay blocks have to be firmly secured to the recipient bone surfaces with osteosynthesis screws. These screws used for onlay bone grafts are made of either titanium or of a resorbable biomaterial. Titanium screws are stronger, but they require a second surgery for removal before implant placement, sometimes resulting in screw fracture during removal. Resorbable screws are weaker but do not need to be removed. Both screw systems offer predictable clinical results (Quereshy et al., 2010).

Usually, onlay bone grafts are placed without the use of barrier membranes. This fact renders the onlay sensitive to masticatory forces, and requires the patients not to wear any removable prosthesis after the regeneration procedure for a period of at least 2 months (Rocchietta et al., 2008).For this reason, recent studies have tested the benefits of covering onlay bone grafts with resorbable membranes. This novel procedure, been shown to be beneficial in preventing grafts resorption due to masticatory forces (Rocchietta et al., 2008).(Fig 4,5)

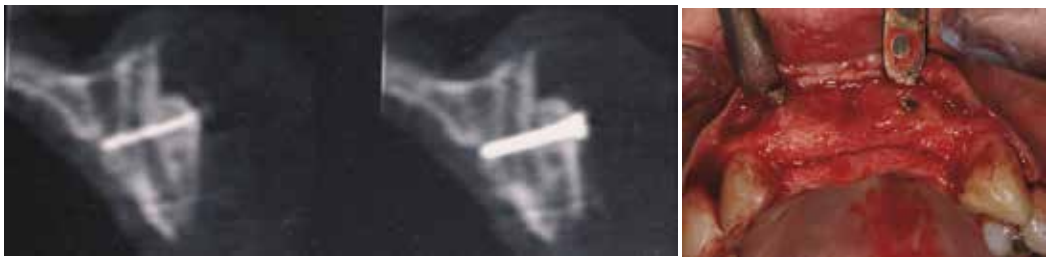


Fig. 4. Slightly resorption with autogenous intraoral bone in the most coronal part of the graft is observed in a patient where no membrane was used.

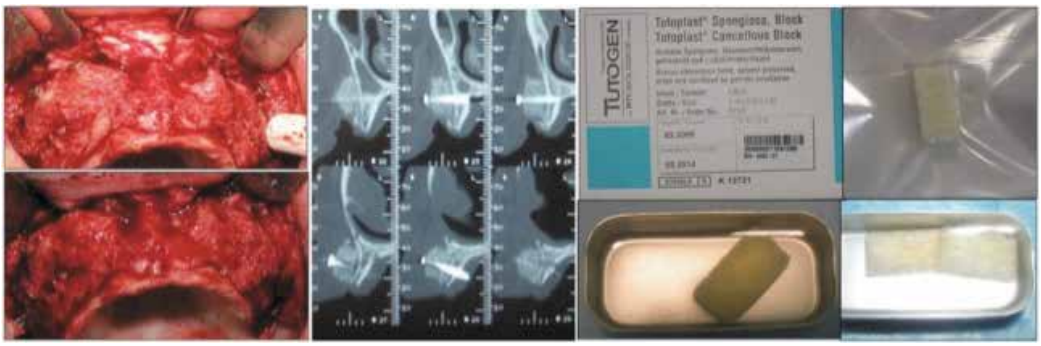


Fig. 5. Remodelling of allograft has been produced after 4 month. Grafts of right side of the patient were covered by a membrane conserving all the volume, while left side graft´s were not covered observing more resorption in this side.

3.2.2 Guided bone regeneration & titanium mesh

Guided bone regeneration (GBR) and Titanium mesh technique (Ti-mesh) provide bone augmentation of the alveolar ridge and create the conditions needed for better aesthetic and higher rate of dental implants success. In order to achieve high rates of success in GBR and Ti-mesh procedures, there are three major issues that need to be addressed: coagulum maintenance, free tension flap closure and an adequate biomaterial selection.

In the treatment of large defects autografts are considered the gold standard, since they have osteoinductive, osteoconductive, osteogenic properties and no risk of immunologic rejection. In view of mechanical properties, cancellous bone grafts still clearly surpassed by ABB, coral, or synthetic bone substitute. However, it provides a high amount of vital stem cells and proteins, such as BMPs, that result in osteogenesis and osteoinduction, therefore GBR with particulated autografts is a safe and predictable treatment (Urban et al., 2009).

Autografts combined with ABB have been used with success in GBR (Table 5). However in the last years different studies have shown that even allografts alone offer reliable results in

large defects, with less cost on the patient. In a study performed by Dahlin they conclude that reconstruction of atrophic maxillae with DFDB using the GBR technique can be performed with an equal success as iliac crest autografts (Dahlin., 2010)

Allografts, alloplasts or xenografts are considered appropriate candidates for small defects (Fig 6, 7). Allograft bone, is the most popular alternative to autogenous bone. It offers the substantial advantage of allowing the patient to avoid a second surgical procedure, with the attendant risks of pain, complications, and morbidity. Available in various shapes and sizes, allograft bone also has fewer supply restrictions than autograft material. Synthetic bone graft materials (alloplasts) constitute a third category of widely used bone-grafting materials, including such non-human, artificially produced materials as calcium sulfate, calcium phosphate, hydroxyapatite, and bioactive glass. Their advantages consist on their abundant supply, long shelf lives, and lack of potential disease transmission. Their capacity to predictably regenerate bone has not yet been demonstrated consistently. Xenografts constitute the final category of bone-grafting materials in common use. Derived from animal sources, xenografts have been developed largely in response to the donor site complications associated with autografts, as well as the limited supplies of both autogenous and allograft material. Porous bovine-derived material is by far the most common variety of xenograft in use today. Such material has been demonstrated to be highly biocompatible. Histologic analysis has found bovine bone particles to be well incorporated within newly regenerated grafted bone and a high degree of osseoconductivity

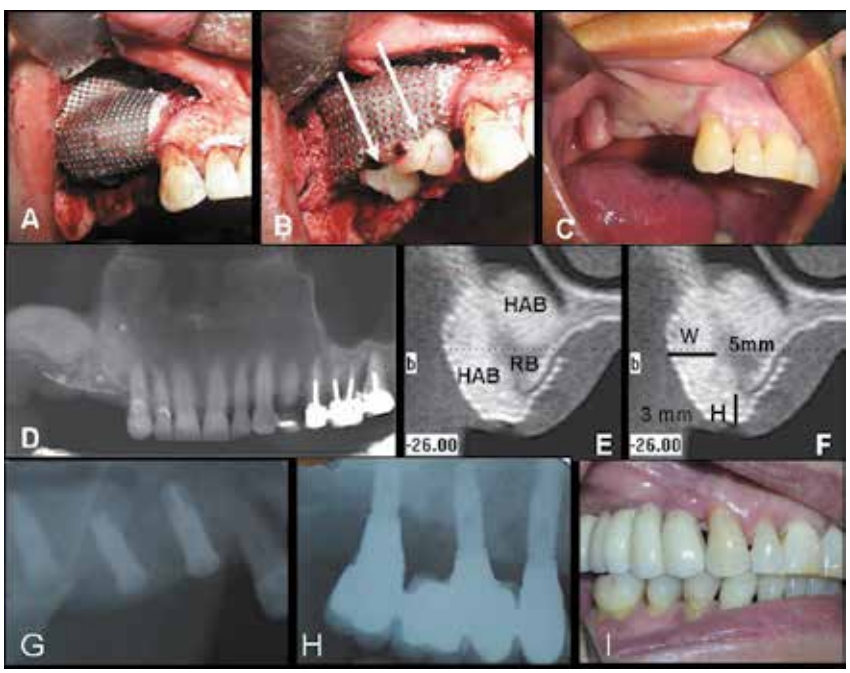


Fig. 6. A. Ti mesh augmentation procedure in upper maxilla using ABB alone as graft material. B. PRP covering Ti mesh in order to enhance soft tissue healing. C. Soft tissue healing after six month of surgery. D,E, F. TC scan images showing horizontal (5 mm) and vertical (3 mm) bone augmentation achieved.G,H,I. Implant´s placement and prosthesis evaluation radiography. I. Image of prosthesis rehabilitation.

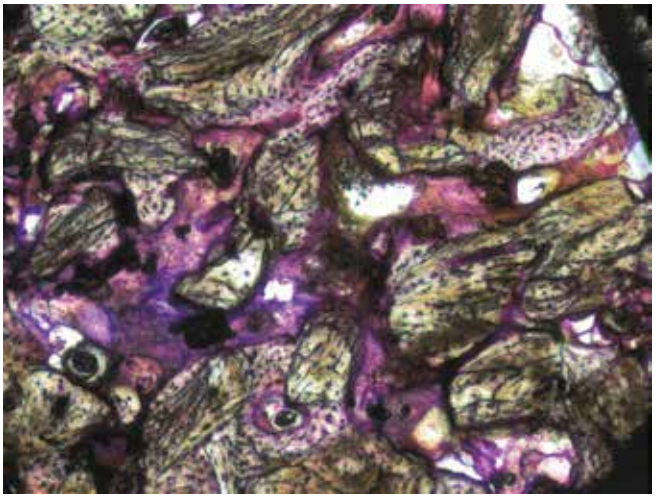


Fig. 7. ABB particles surrounded by newly formed bone in a Ti-mesh bone augmentation procedure where ABB alone was used as graft material.

Type of Graft (%)	ABW (mm)	ABH (mm)	Survival (%)	Success (%)	References
AB (100)	ID	ID	ID	ID	Von Arx et al 1996
AB (100)	5.65	ID	ID	100	Malchiodi et al 1998
AB (100) *	ID	5	ID	ID	Rocuzzo et al 2004
AB (100) *	ID	4.8	100	100	Rocuzzo et al 2007
AB/ABB (50/50)	ID	ID	98.3	ID	Maiorana et al 2001
AB/ABB (70/30)	4.16	3.71	100	100	Pieri et al 2008
AB/ABB (70/30)	ID	ID	100	100	Corinaldesi et al 2007
AB/ABB (ID)	3.71	2.86	ID	ID	Profusaefs & Lozana 2006
ABB (100)	ID	5.2	100	ID	Artzi et al 2003

Pts: patients; BAP: bone augmentation procedures; ABW: average bone width gained; ABH: average bone height gained; AB: autologous bone; ID: insufficient data;: block grafts.*

Table 5. Summary of clinical studies reporting the amount bone gained, type of graft and complications rate using the Ti-mesh technique. (Modified from Torres et al 2010)

3.3 Treating fenestrations and dehiscence

Particulate autogenous bone, anorganic bovine bone and FDBA have been proposed for treatment such defects in combination with GBR technique.

4. Summary

Since the introduction of dental implants, bone grafting has become an important procedure required for the treatment of patients with limited bone availability. Bone autograft, alone or together with other bone substitutes, has been the biomaterial of choice for clinicians

worldwide. However different xenogenic, allogenic and synthetic biomaterials have shown promising results in many bone augmentation procedures.

The bone substitute needed for each bone regeneration procedure must be selected based on the individual´s characteristics, and the surgical procedure it self. Factors such as the osteogenic potential of the host residual bone, systemic health of patients, and morphology of the defects, will delimit the ideal bone substitute for each situation. For example in sinus augmentation, allografts, xenografs and synthetic calcium phosphates have been used as alternative to autografts with high rate of implants success and survival. On the other hand, when major alveolar ridge augmentations are required autograft onlay block are the most predictable biomaterials, although autograft granules mixed in different proportions with ABB in GBR procedures have also a high rate of success.

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5

Dental Reconstruction Using Secondary Bone Graft Followed by Implant Placement in Alveolar Cleft of Patients with Cleft Lip and/or Palate

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1. Introduction

Alveolar cleft is the major problem during the formation of the ideal dental arch and dental reconstruction in cleft lip and/or palate (CLP) patients. In 1972, Boyne and Sands introduced secondary autogenous particulate cancellous bone and marrow (PCBM) grafting for the treatment of alveolar and residual palatal clefts (Boyne and Sands, 1972) Nowadays, a golden standard protocol for the dental reconstruction of patients with CLP is to perform bone grafting (BG) before canine eruption and subsequent orthodontic closure of the dental arch without using prosthesis, as described in previous reports. (Abyholm, et al., 1981; Enemark, et al., 1985; Bergland, et al., 1986). However, because of the excessively long treatment period or a wide interdental space resulting from several congenitally missing teeth, prosthodontic treatment such as bridgework or denture is sometimes necessary.
Verdi et al. firstly reported the use of osseointegrated implant for dental rehabilitation in patients after the repair of the alveolar cleft (Verdi et al., 1991). Since then, numerous reports indicated that the use of dental implants placed in grafted alveoli after repair of an alveolar cleft using a secondary BG with particulate PCBM is a viable option for the dental reconstruction of patients with cleft lip or palate (CLP) (Ronchi et al., 1995; Takahashi T et al., 1997; Jasma et al., 1999; Härtel et al., 1999; Dempf et al., 2002). Since 1993, we have used this treatment procedure in patients with graft alveolar clefts. (Takahashi et al., 1997, 2008). In this chapter, dental reconstruction using secondary BG followed by dental implant placement in alveolar cleft of patients with CLP is described. The main purpose of this chapter to demonstrate the surgical procedure, long-term clinical outcome, and the impact on the preservation of the grafted alveoli after implant placement of this treatment procedure.

2. Use of dental implants for dental reconstruction in clp patients with grafted alveolar cleft

2.1 Secondary alveolar bone grafting for osseous reconstruction of the alveolar and palatal cleft

The management of the dentoalveolar cleft is a significant challenge for the surgeon. Timing is generally described as “primary,” “secondary,” and “delayed.” Primary bone grafting is

generally defined as that taking place before eruption of the primary dentition or before 1 year of age. Secondary bone grafting is that performed after developing of the permanent dentition, and delayed bone grafting takes place after eruption of the permanent canine. Historically, primary bone grafting was used from 1950 to 1968, and was found to inhibit normal growth and to impede later treatment using orthopedic forces of arch expansion. Nowadays, early secondary (5 to 6 years of age) or secondary (7 to 11 years of age) before canine eruption is recommended for osseous reconstruction followed by orthodontic dental arch closure. The erupting tooth stimulates alveolar and graft bone growth and produces a more normal-appearing canine eminence. Late bone grafting is not recommended because root resorption and graft failure sometimes observed when bone grafts are placed after eruption of the canine and when the bone graft is placed in contact with exposed tooth root surfaces. As bone graft source, the standard bone graft is autogenous particulate cancellous bone and marrow (PCBM) from the ilium. This bone is highly cellular, making it both resident to infection and able to heal rapidly. The graft materials are clinically and radiographically indistinguishable from alveolar bone by 3 months after operation and function as alveolar bone. Orthodontic treatment, thus, could be started 3 months after bone grafting. The purposes of bone grafting to the alveolar cleft are (1) closure of the oronasal fistula; (2) stabilization of the expanded arch, and in the case of bilateral clefts, of the premaxilla; (3) bone to support the canine eruption; (4) improved bone and periodontal status for the central incisor and lateral, if present; (5) support for the ala of the nose; and (6) a more normal appearance of the alveolar process, teeth, and gingival of the anterior maxilla. Now, another important purpose of bone grafting should be added to the list. Seventh purpose of bone grafting to the alveolar cleft would be to give osseous support for dental implant placement.

2.2 Problems related to the dental reconstruction of the grafted alveolar cleft

Although golden standard procedure of dental reconstruction in CLP patients is to perform bone grafting between the ages of 7 and 11 years, followed by orthodontic closure of the cleft dental gap without the use of a prosthesis, CLP is often associated with congenital absence of the lateral incisor adjacent to the cleft. Many patients therefore require prostheses such as a denture or bridge, particularly when the cleft-dental gap might not result in a good occlusion relationship between the upper and lower jaws. For such patients, we propose the option of dental reconstruction with dental implants after repair of the alveolar cleft by autogenous PCBM grafting.

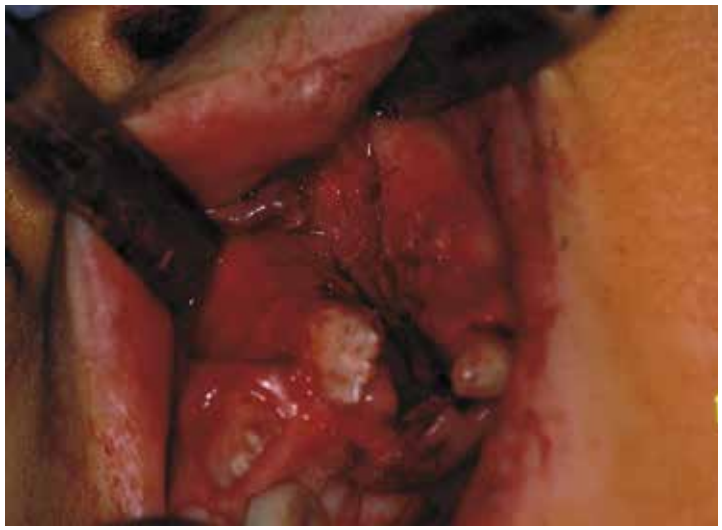
2.3 Subjects and methods

2.3.1 Subjects

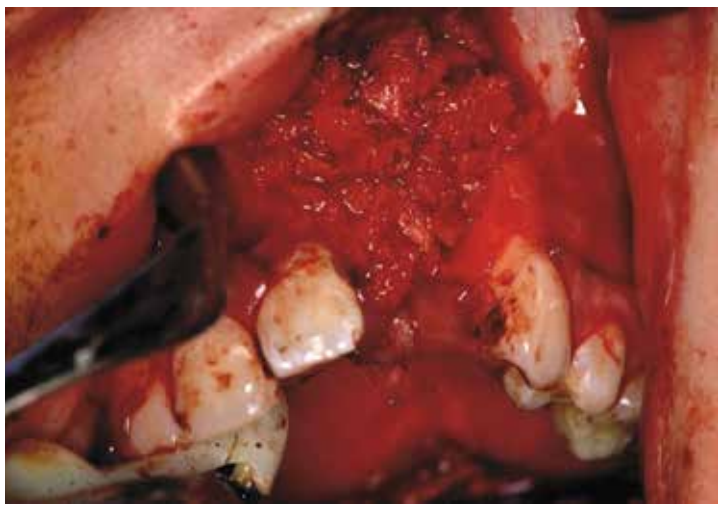
Twenty-one (7 male, 14 female) patients with CLP underwent implant placement between February 1993 and May 1995. Thirteen of the patients had unilateral cleft lip and palate (UCLP), and three had unilateral cleft lip and alveolus (UCLA). The mean age at first implant surgery was 19.1 ± 4.7 years (range: 13.9 to 33.6 years).

2.3.2 Bone grafting

Surgical procedure was shown in Fig 1 (Fig. 1a and 1b).



a: closure of the nasal mucosa at the cleft site



b: PCBM filled in the cleft gap

Fig. 1. Secondary bone grafting of alveolar cleft

All patients received autogenous particulate cancellous bone and marrow (PCBM) grafts from the ilium beaten 8.3 and 31.7 years of age under general anesthesia. Epinephrine 1:00, 000 is administered in the labial tissues to reduce bleeding. Incisions are made, and the labial flaps are reflected on either side of the cleft and these two incisions connected by sharp and blunt dissection deep to the orbicularis oris muscle on the nasal mucosa. This dissection is extended superiorly to the level of the floor of the nose. The nasal mucosa is reflected from the bone of the cleft and extended to the palatal tissues. Subperiosteal reflections of the palatal tissues on either side of the cleft are performed from the gingival margin to the depth of the palate. The reflection of mucosa through the alveolar cleft is completed. The nasal mucosa that passes through the cleft and is attached to the palatal tissues is separated from the palatal mucosa to the depth of the palate. If a palatal fistula

extends further posteriorly, the separation and reflection are carried to the posterior end of the fistula. Then, nasal mucosa is closed with 4-0 Vicryl. The edges of he palatal fistula are freshened and closed with 4-0 Vicryl. Specifically, the very end of the fistula is closed using through-and-through suture using 4-0 Vicryl. PCBM is packed firmly from the level of the floor of the nose to the crest of the ridge. Releasing incisions are made through the periosteium of the posterior buccal flap, allowing anterior and inferior advancement. The labial and palatal flaps are closed with sutures. For the subsequent implant placement, the most important point of BG bed preparation is the level of the floor of the nose. The level of the floor of the nose at the BG bed should be the same level of the contra-lateral side. If inferior nasal turbinate is obstructive for the preparation of the floor of the nose, it should be removed. Usually, inferior nasal turbinate is easily removed without any bleeding.

2.3.3 Evaluation of the grafted alveoli around the implants

The marginal bone level around the implants was evaluated radiologically as previously described. (Takahashi et al., 2008). Using the reference points of the dimension of the implants, especially the fixture length and screw pitch (0.6 mm), the marginal crest level of the bone was calculated relative to the baseline (Fig. 2).

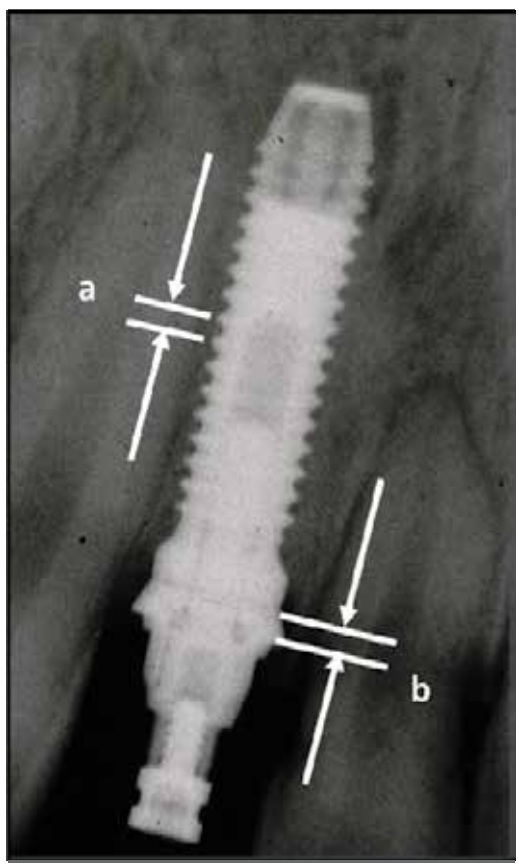


Fig. 2. **Measuring the marginal bone level**. a, A screw pitch (0.6 mm); b, baseline to bone level (marginal bone level) (Takahashi T, et al. Oral Surg Oral Med Oral Pathol Oral Radiol Endod2008: 105: 297-302)

An average of the mesial and distal values was used. All assessments were performed at three stages after abutment connection, at 1 (stage I), 3 (stage II), and 6 (stage III) years after abutment connection.

2.3.4 Evaluation of the marginal interdental alveolar bone height (IABH)

The interdental alveolar bone height (IABH) was estimated and was indicated by 4 score values (Fig. 3). (Enemark, et al., 1987).

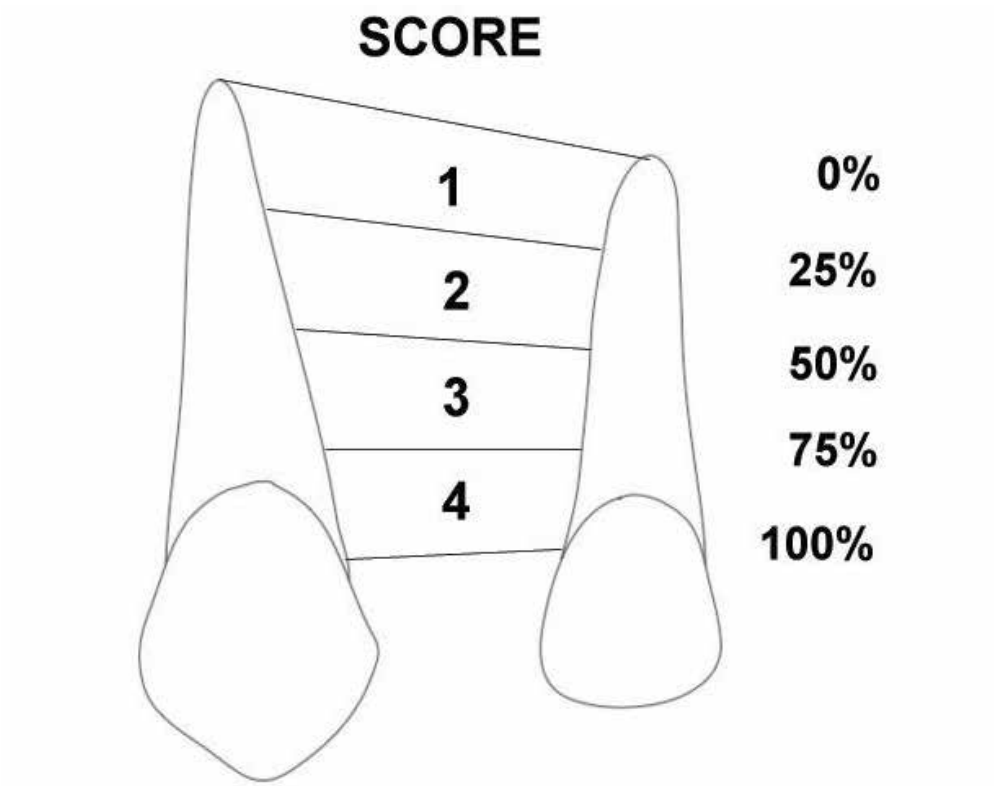


Fig. 3. Score of interdental alveolar bone height (IABH)

Briefly, The extent of the vertical bone height was determined in relation to the interdental bone height and assessed on a 4-point scale: score 4= 0 % to 25 % bone loss, score 3= 25 % 50% bone loss, score 2= 50 % to 75 % bone loss, score 1= 75% to 100 % bone loss. All assessments were performed 1, 6, 12, 24, 36, and 72 months after implant placement.

2.4 Clinical outcomes in a long-term follow-up

A total of 23 implants were placed in bone-grafted alveoli in 21 patients between February 1993 and May 1995. One patient was lost to follow-up, two implants were lost in one patient, and three implants in three patients were still temporary restoration because of the prolonged orthodontic treatment. The implant length ranged from 10 to 18 mm; the most frequently used length was 15 mm. The duration from bone grafting to first implant surgery ranged from 1.4 to 10.2 years (mean: 5.0 ± 2.7 years). In five patients with insufficient IABH,

which was evaluated as Score 1 or 2, a chin bone-onlay graft (CBOG) was used during implant placement as described later. Two of these patients had wound dehiscence, and the exposed chin bone was partially lost. Ultimately, however, all five implants were osseointegrated, and the alveolar bone height was increased in these patients. The follow-up period ranged from 7.2 to 9.4 years (mean period: 8.6 ± 0.6 years). Twenty implants placed in 19 patients survived, and the overall rate of implant survival was 90.9%. IABH was reduced in 2 of 16 (12.5%) of the implant-placed grafted alveoli, in which score 4 was reduced to score 3, and score 3 was reduced to score 2, 6 years after implant placement, respectively. In other 14 cases, there was no change in score of IABH up to 6 years after implant placement (Fig. 4). These results clearly suggested that placement implants in the grafted alveoli would maintain alveolar bone height in the region.

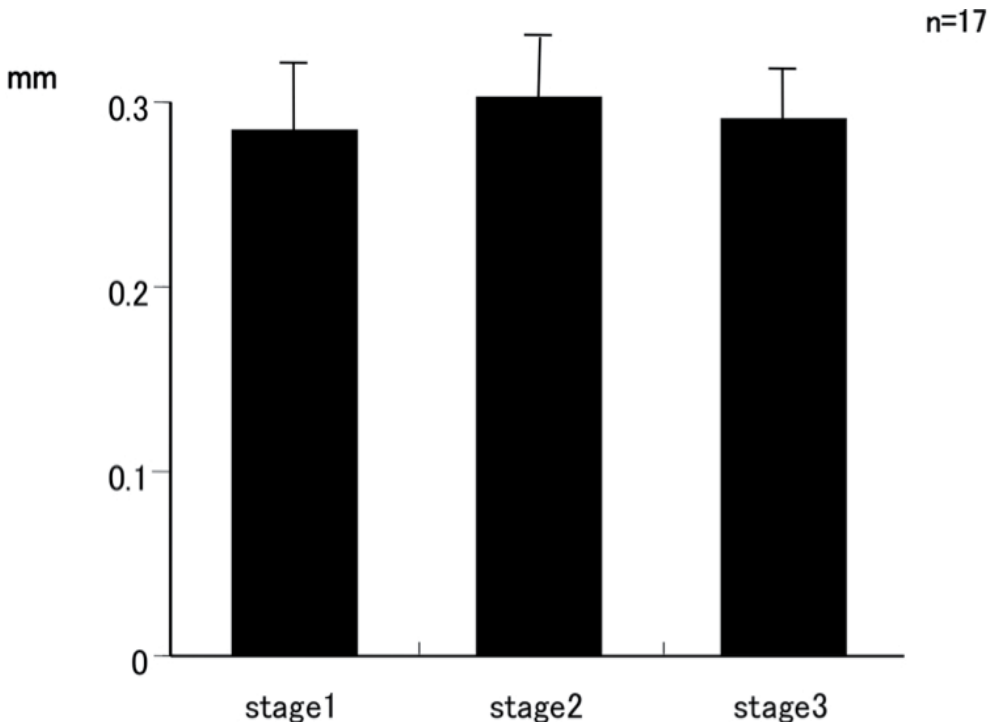
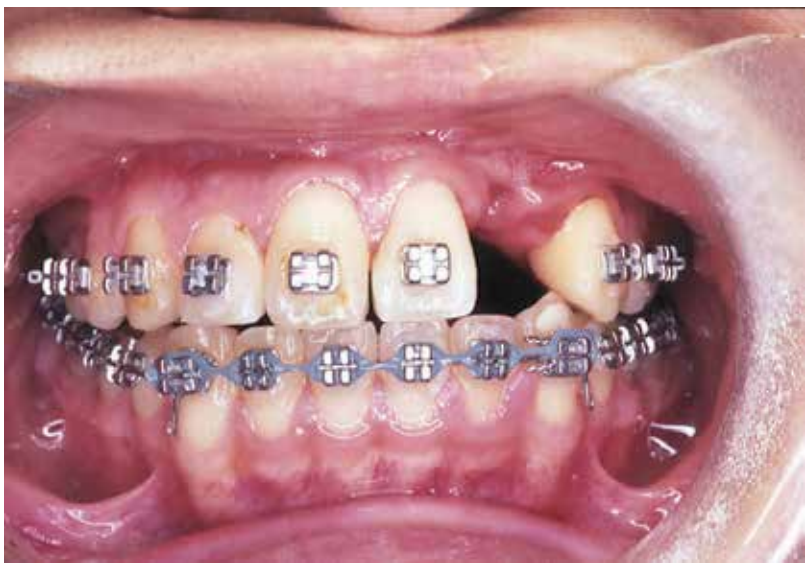


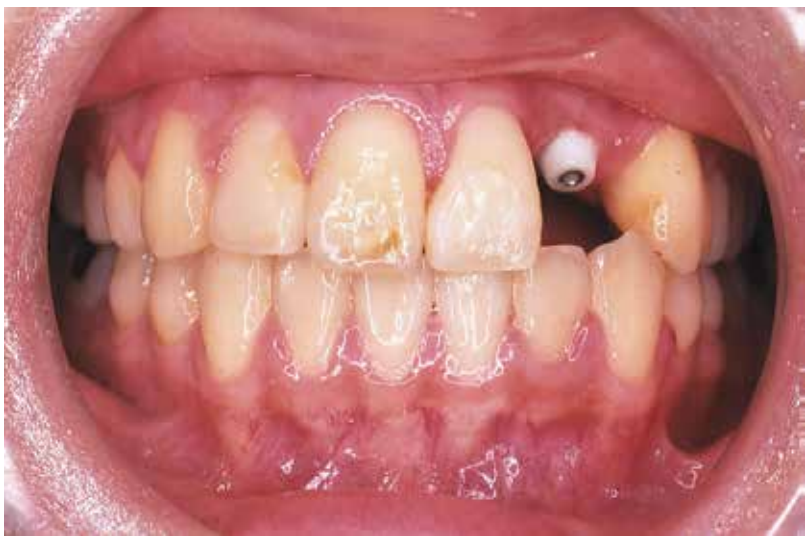
Fig. 4. Marginal bone loss around the implant

The mean marginal bone levels were 0.29 ± 0.18, 0.29 ± 0.19, and 0.28 ± 0.15 mm at stages I to III, respectively. In addition, there was no mobility of the implants and was no pain, swelling, or inflammation around the peri-implant tissue. Clinically, all the implants were functioning in excellent condition 7.2 to 9.4 years after implant placement. A long-term follow-up of a typical case was shown in Figure 5a-c and Figure 6a-c. These data satisfied the implant success criteria according to Albrektsson et al., (Albrektsson, et al., 1986), who stipulated that the vertical bone loss should be less than 0.2 mm annually after the first year of implant service. In addition, our long-term follow-up study showed that the use of dental

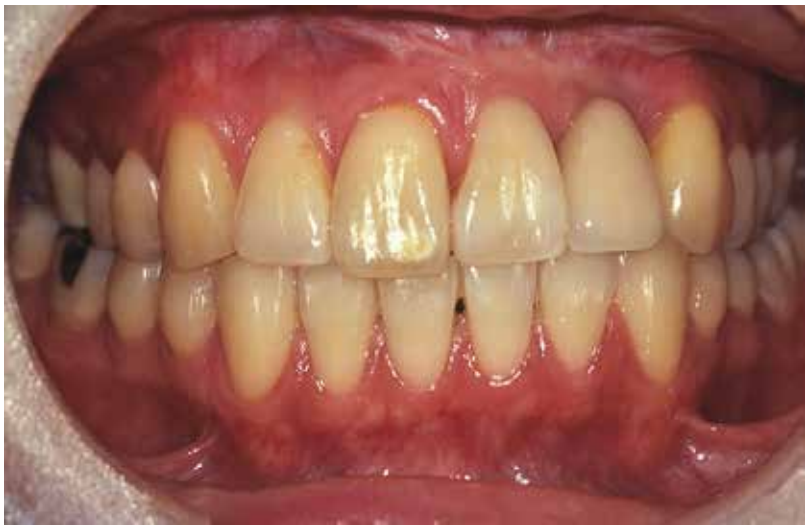
implants placed in the grafted alveoli is an excellent treatment modality for the dental rehabilitation of patients with alveolar clefts and congenitally missing teeth.



a: After orthodontic alignment and space making for an implant placement of the grafted alveolar cleft.

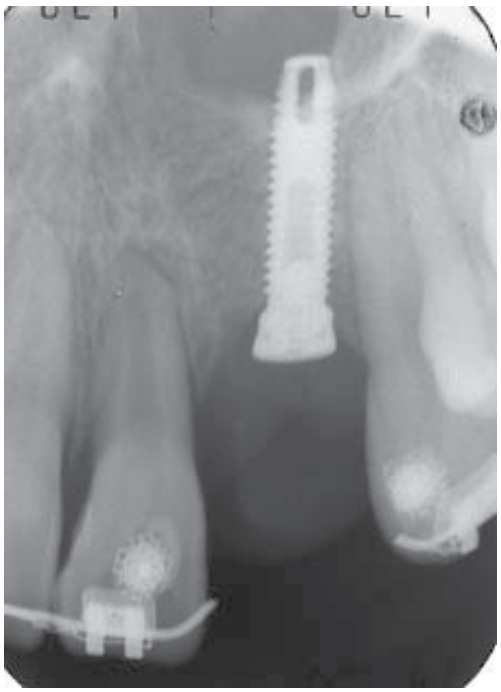


b: Postoperative view after implant placement

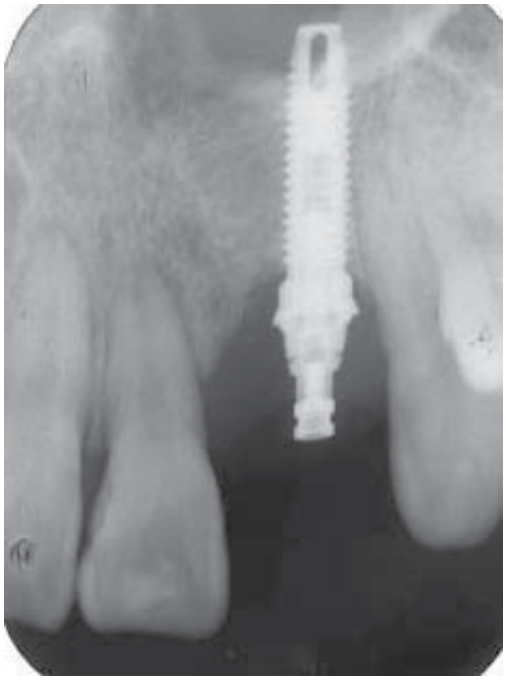


c: 13 years after implant placement

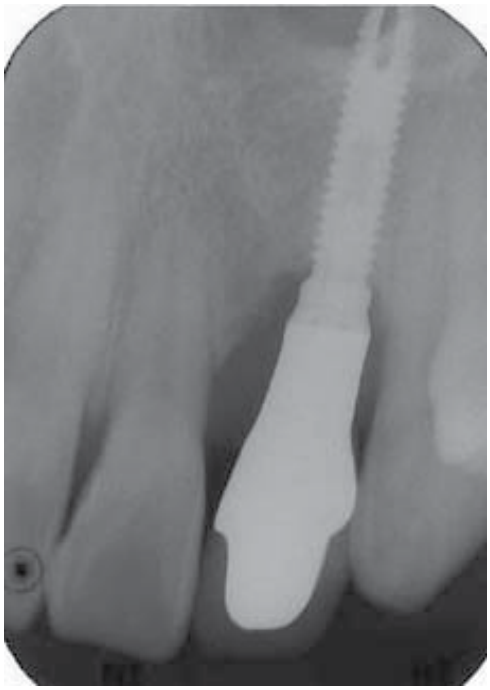
Fig. 5. A Long-term follow-up in a unilateral cleft lip and palate (UCLP) patient.



a: Periapical radiograph immediately after implant placement



b: Periapical radiograph years after implant placement (marginal bone level: -1.0 mm).



c: 13 years after implant placement (marginal bone level: -1.0 mm)

Fig. 6. Radiological evaluation of the long-term follow up in a UCLP patient.

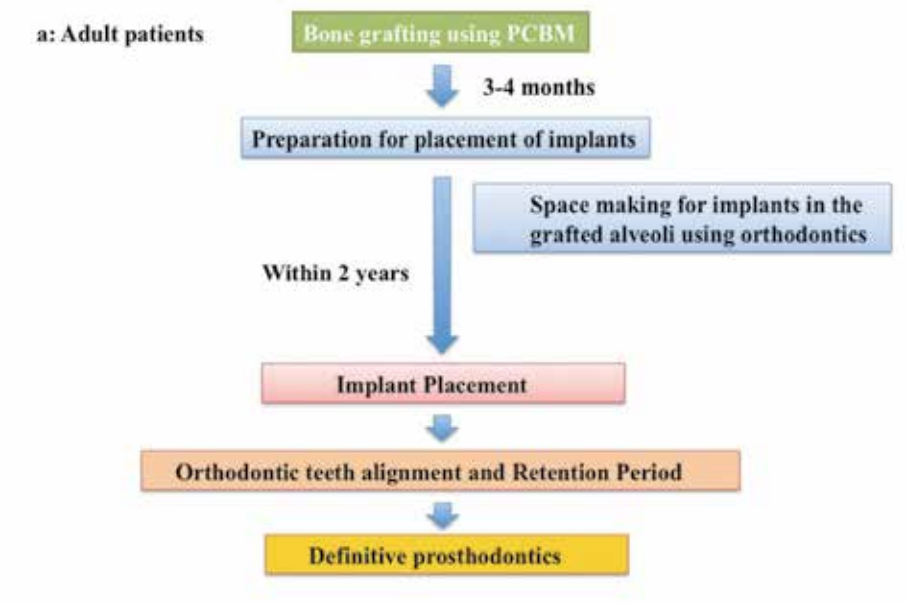
2.5 Timing for BG and for implant placement

Generally, secondary BG is recommended to perform before canine eruption. (Enemark, et al., 1985; Bergland, et al., 1986). The reasons for this are the better clinical results seen in younger individuals, and the greater osteogeneic activity in younger than older patients. It was shown that there was a tendency to an inverse correlation between IABH and age at the time of BG (Takahashi, et al., 1999). Therefore, from the clinical point of view, secondary BG for alveolar clefts should be performed when the patient is young. On the other side, generally implant placement before the growth spurt is assumed to be contraindication. (Lekholm, 1993) because implants are immobile similar to ankylotic teeth (Adell, et al., 1981; Shapiro and Kokich, 1988) and do not accompany other parts of the jaw bone during rapid growth in the adolescence. Adhering to this rule, the time lag between BG and implant placement is required. Actually, the mean duration from BG to implant placement was 4.6 ±2.5 years. It is well known that the grafted alveoli undergoes resorption 3-dimensionally (Van der Meji, et al., 1994), and the interdental alveolar bone height (IABH) also decreases (Takahashi et al., 1999). The mean net bone height 1 month after BG was approximately 17 mm, and the mean width 1 month after BG was 12. 9 mm, both of which were enough for the implant placement. However, almost half of the grafted alveoli required another bone graft within 24 months after the original bone graft to increase IABH level for implant placement (Takahashi et al., 1999).

None the less, the grafted alveoli with PCBM are suitable for implant placement. If the grafted alveoli do not have sufficient bone volume and IABH, another bone augmentation procedure such as onlay graft or guided bone regeneration (GBR) should be considered. The flow chart of the dental reconstruction of alveolar cleft using PCBM BG and implant placement was shown in Fig. 7. According to this flow chart, BG with PCBM should be performed followed by orthodontic teeth movement and space making for an implant bed in adult CLP patient with un-repaired alveolar cleft. Subsequently, implant placement should be performed within 2 years after BG to avoid another bone augmentation procedure (Fig. 7a). If the patient is young and is on the line of multi-disciplinary team approach, secondary BG to the alveolar cleft should be performed between 7 to 11 years of age before canine eruption. Orthodontic dental arch closure without prosthodontic treatment should be the primary choice as golden standard procedure for dental reconstruction of the grafted alveolar cleft. However, in cases of excessively long treatment period or a wide interdental space resulting from several congenitally missing teeth, use of dental implant placement in the grafted alveoli should be considered after the growth spurt (Fig. 7b) as an alternative treatment protocol. Please keep in mind that another bone augmentation procedure may be required for implant placement in the grafted alveoli as will be discussed later.

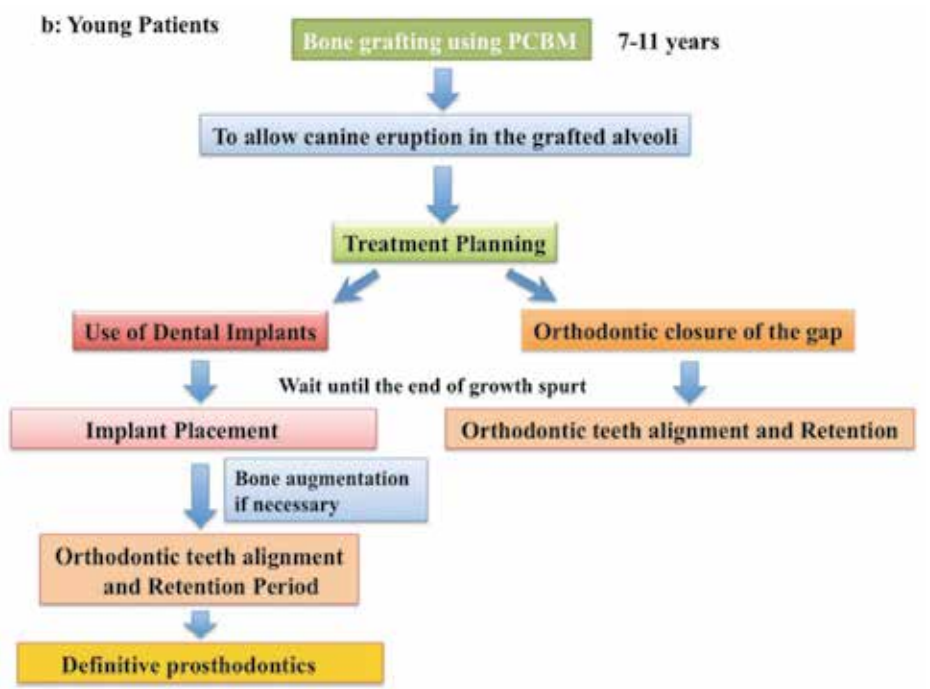
2.6 Bone augmentation procedure for implant placement in grafted alveoli

For the dental reconstruction of the grafted alveoli, insufficient bone volume and IABH seems to be a limiting factor for implant placement. Therefore, another bone augmentation procedure is sometimes necessary. Generally speaking, the treatment modality depends on the shape and the volume of the residual grafted alveoli. Small defect could be repaired by GBR (Buser et al., 1990) as a simultaneous augmentation procedure with implant placement. Sometimes however, greater bone volume is necessary for esthetic and functional dental reconstruction in the maxillary anterior region of the grafted alveoli. A various augmentation procedures including onlay bone graft (Fukuda et al., 1998, 2000), titanium mesh and particulate bone graft (Von Arx, et al., 1996; Miyamoto et al., 2011) and alveolar distraction osteogenesis (ADO) (Buis, et al., 2001) could be available for bone augmentation.



Flow Chart of Dental reconstruction of the alveolar cleft using BG and dental implant

a: Adult patients



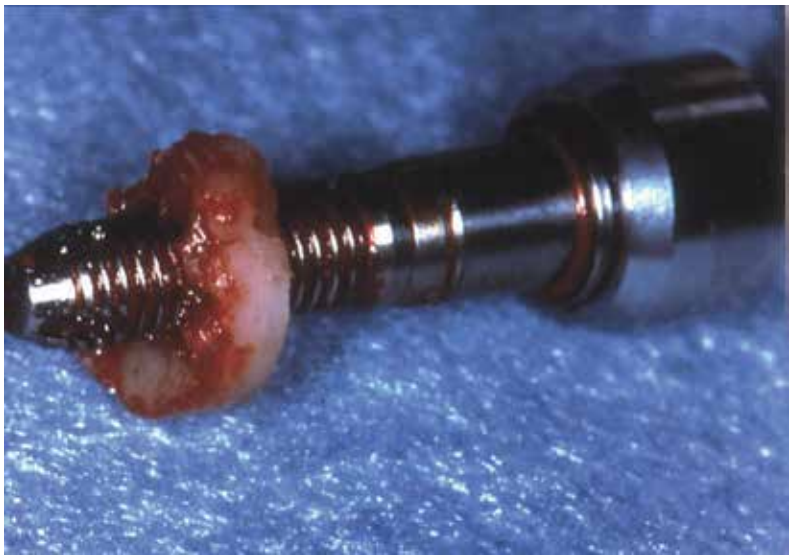
Flow Chart of Dental reconstruction of the alveolar cleft using BG and dental implant

b: Young patients

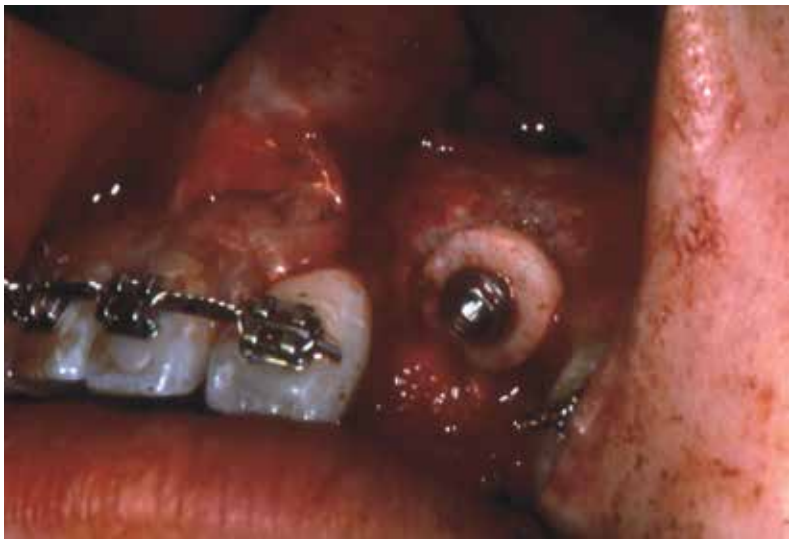
Fig. 7. Flow Chart of Dental reconstruction of the alveolar cleft using BG and dental implant

2.6.1 Chin bone onlay graft

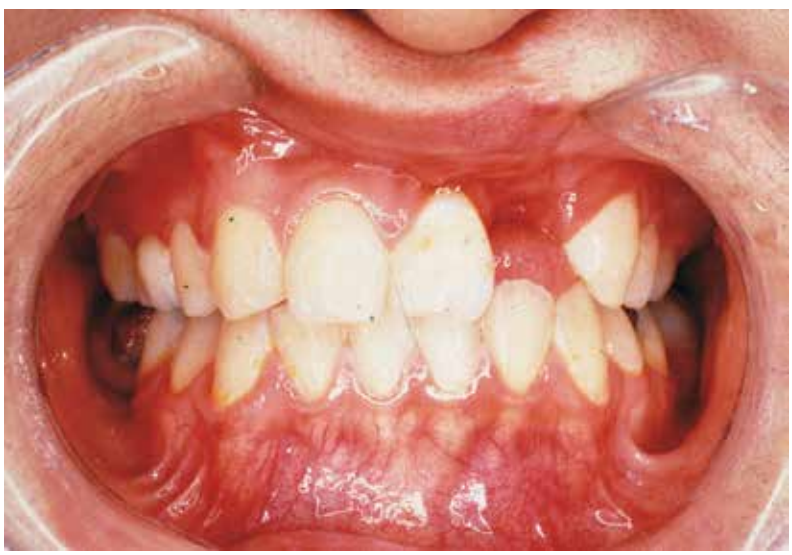
Onlay grafting provides for appropriate alveolar bone height and width in patients with alveolar local bone defects. We have been using chin bone onlay grafting with simultaneous implant placement in patients with insufficient IABH (Fukuda et al., 1998 and 2000). Surgical procedures are as follows. At the grafted alveolus with insufficient IABH, the gingival on top of the alveolar ridge is incised. Mesially and distally to the alveolus, releaf incisions are made buccally. A buccal mucoperiosteal flap is raised to expose the nasal floor. The thickness of the chin bone graft needed, i.e. the height from top to bottom of the inter-dental alveolar bone margin is calculated using calipers. Drilling for implant installation is performed following a surgical protocol, without perforating the nasal mucosa. In the mandibular symphysis region, a vestibular sulcus incision is made through the mucosa. The periosteium is elevated to allow a hand-driven instrument (Leibinger® Fritsch™ Bone Graft Set; Leibinger GmbH, Freiburg, Germany) to be introduced. The tip of a self-tapping implant is inserted into the chin bone without penetration of the lingual cortical bone. The instrument is rotated around the placed implant perpendicularly to the chin bone surface until the labial cortical bone plate has been passed through to the cancellous bone. The implant and monocorticocancellous bone complex are removed by rotating the instrument. This complex (Fig. 8a) is then placed at the prepared recipient site, and the implant is inserted in the cortical bone of the nasal floor (Fig. 8b). Small gap between the complex and the alveolus are filled with cancellous bone chips obtained from the donor site. The periosteium of the mucoperiosteal flap is incised to allow this flap to cover the complex without any tension, and the wound is closed. Six months later, the abutment connection was performed, and prosthetic rehabilitation was completed with a single-unit implant-supported prosthesis. In 7 patients with CLP, simultaneous chin bone onlay graft (CBOG) was performed for implant placement. Although four of the seven patients had an uneventful course, three had some wound dehiscence and exposed chin bone underwent partial (2 cases) or total necrosis (one case). Ultimately, all seven implants were integrated, and the alveolar bone height had increased in all patients except one. A typical case is shown in Fig. 8 (Fig. 8a-d) .



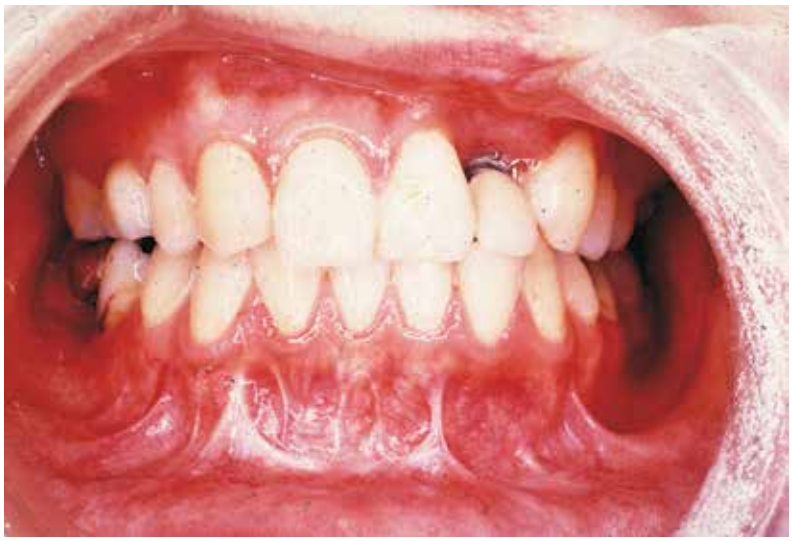
a: Fixture and corticocancellous chin bone complex



b: Clinical appearance after placement of complex



c: Intra-oral view after secondary BG with PCBM followed by completion of orthodontic alignment of maxillary arch.

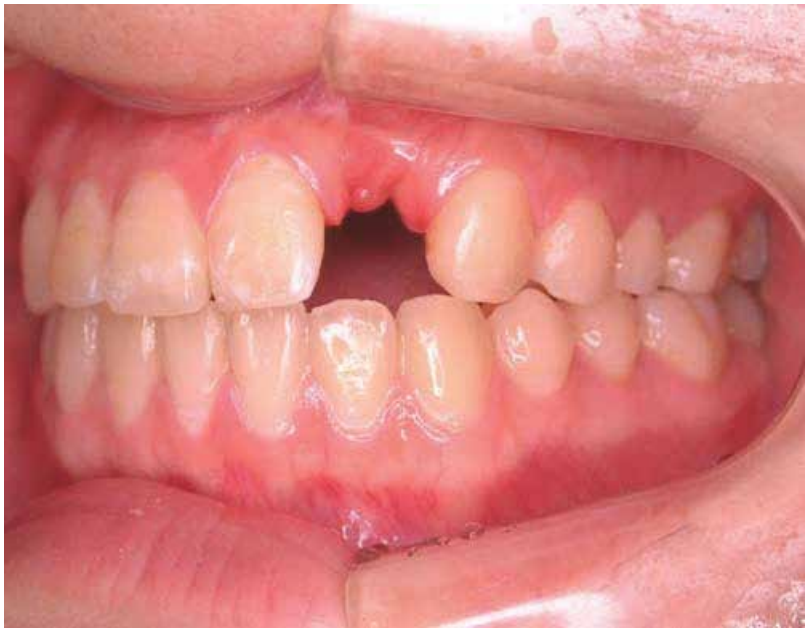


d: Occlusal view showing final prosthesis rehabilitation. Interdental alveolar bone height (IABH) was increased by CBOG. (Takahashi T, et al. J Oral Maxillofac Surg 1997: 55: 576-583)

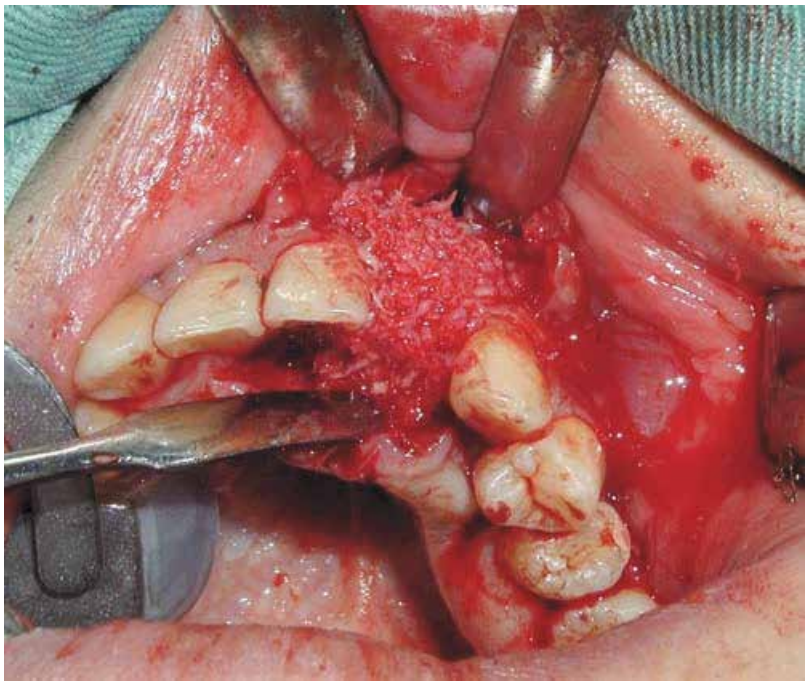
Fig. 8. Augmentation procedure using chin bone onlay graft (CBOG) for dental implant placement

2.6.2 Titanium mesh and particulate bone graft

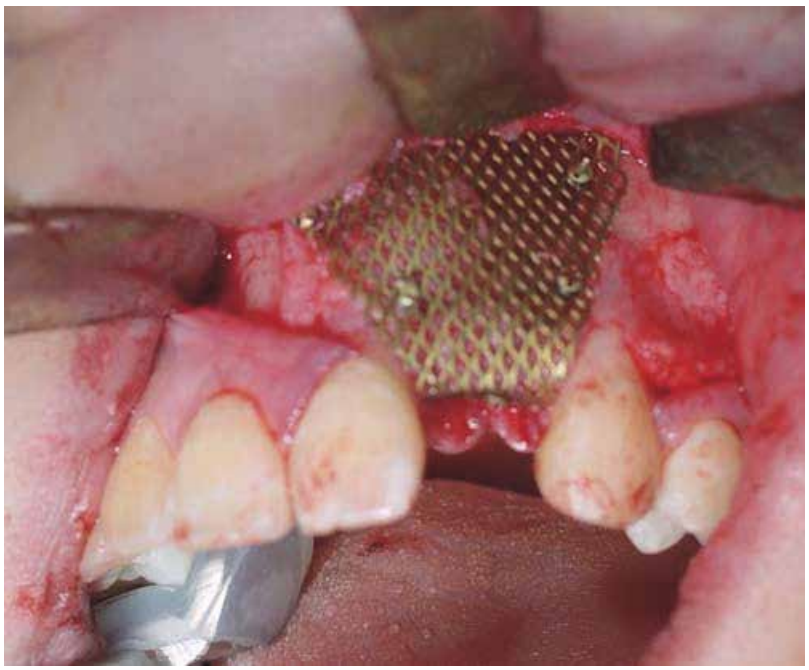
The titanium mesh technique is one alternative, based on bone grafting with a stiff occlusive titanium membrane. Titanium meshes were used according to the shape of the defects. Harvested bone was set on the defects and a shaped titanium mesh was fixed with small titanium screws. A typical case is shown in Fig.9a-e. In this case, grafted alveolus underwent resorption and bone volume as well as IABH was decreased. For the surgical procedure, at the grafted alveolus with insufficient IABH, crestal incision was always used. Mesially and distally to the alveolus, relief incisions are made buccally. A buccal mucoperiosteal flap is raised to expose the nasal floor. Decortication of the drill holes was performed by using round burr to ensure vascular nutrition of the grafted bone. Titanium mesh (0.1- or 0.2-mm thickness; M-TAM, Stryker Leibinger GmbH & Co., USA) were trimmed to ensure to cover the bone grafts. Autogenous particulate bone grafts were obtained from intraoral, mainly from mandibular retromolar region with a scraper (mx-grafter; Maxilon Laboratories, Inc., Hollis, NH, USA). Bone grafts are filled in the recipient site. Then, the titanium mesh was stabilized with several small titanium screws buccally and palatally. With sufficient saline irrigation for a clean surgical field, tension free 5-0 nylon sutures were placed across the incision on the periosteal membrane over the mucoperiosteal flap. A staged approach was used for implant installation.



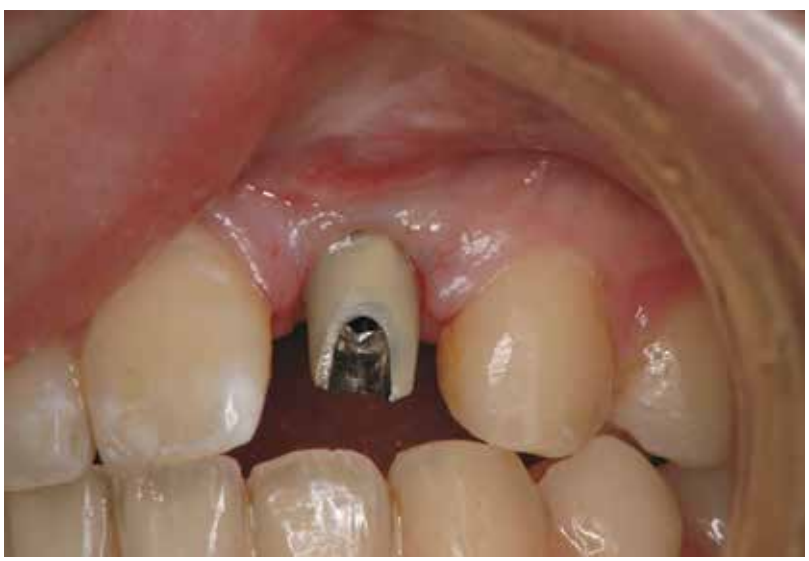
a: Preoperative view after secondary GB with PCBM followed by completion of orthodontic alignment of maxillary arch.



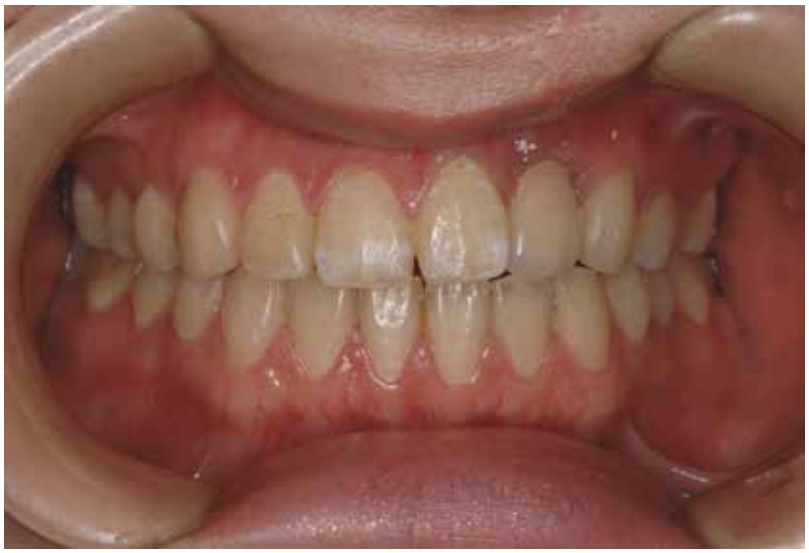
b: Bone graft (BG) using particulate bone harvested from mandibular ramus.



c: Intra-oral view after secondary BG with PCBM followed by completion of orthodontic alignment of maxillary arch.



d: After implant placement and abutment connection



e: Occlusal view showing final prosthesis rehabilitation. IABH was increased by titanium mesh and BG.

Fig. 9. Augmentation procedure using titanium mesh for dental implant placement

A major complication of the titanium mesh technique is mesh exposure during healing period. Mesh exposure would result in infection, which can jeopardize the results. Therefore, two-stage approach is strongly recommended. Usually, implant placement is suitable 6 months after the titanium mesh and particulate autogenous and bone graft.

2.6.3 Alveolar distraction Osteogenesis

Alveolar distraction Osteogenesis (ADO) is another alternatives of augmentation in the insufficient grafted alveoli. The advantages of ADO include avoiding autogenous bone harvest, and simultaneous soft tissue expansion. However, ADO is very technically sensitive, and many complications are reported. Disadvantages of DO are difficulty in vector control, the exposure of distraction device, necessary for long treatment time. Nonetheless, since the mucosa around the grafted alveoli is abundant in scar tissue, and is difficult in soft tissue cover, the soft tissue expansion by ADO seems to be a big advantage. Clinical data concerning the use of ADO in the grafted area are lacking, and further study will be necessary for ADO.

2.7 Preservation of the grafted bone after implant placement

Our study demonstrated that the IABH did not change for up to 6 years after implant placement. Actually, in this study, IABH was reduced only in 2 of 16 (12.5%) of the implant-placed grafted alveoli. Furthermore, the marginal bone level at 6 years averaged 0.28 mm, showing extremely low resorption around the implant. Although no linear measurement of the IABH bone loss of the grafted alveoli was made in this study, these results clearly demonstrate that implant placement in grafted alveoli maintain the grafted bone after secondary PCBM.

The reason of the maintenance of the implant-placed grafted alveoli in a long-term follow-up may be achieved through functional loading of the grafted bone. Dempt et al. suggested

that the endosseous implants into the grafted alveoli not only closed the gap, but exert functional stimulation of the transplanted bone by mastication (Dempf, et al., 2002). Our data support this hypothesis.

3. Conclusion

This study further confirmed that the use of dental implants placed in the alveoli after PCBM grafting is an excellent treatment modality for the dental rehabilitation of patients with alveolar clefts and congenitally missing teeth and that it is a great tool for preventing grafted bone resorption after cleft repair with secondary BG. However, the grafted alveoli undergo bone resorption in time-dependent manner. Therefore, the loss of width and height of the bone bridge must also be considered for implant installation. If the grafted alveoli do not have sufficient bone volume and IABH, another bone augmentation procedure such as onlay graft, GBR, or titanium mesh and cancellous bone graft technique should be considered.

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6

Bone Substitutes and Validation

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1. Introduction

This chapter will focus on the review of the various bone grafting materials in the market for implant dentistry with much emphasis on the fact that they are all second-hand bones (bone substitutes) when compared with the autogeneous bone graft which is the gold standard to which all bone substitutes are compared.

Bone grafting implies to the application of autogenous bone or other bone substitute obtained from natural or synthetic source to an area of with boney defect. Bone grafting is a procedure and should not be confused with bone regeneration which is the actual formation of new bone in the grafted defect. Bone grafting does not necessarily lead to bone regeneration. It is so important that the clinician and the patient are aware of the difference in terminology because of the clinical, scientific and medicolegal implications.

The use of bone substitutes or bone replacement source has increased tremendously in implant dentistry today and will continue to be so because of the unavailability of autogenous bone from the intra-oral site in most situation and patients are becoming more and more tolerant to clinicians harvesting bone from the extra-oral site such as the iliac crest or the tibial tuberosity.

The mechanisms available for bone regeneration will be fully described and classification of bone substitutes under these mechanisms will be attempted so as to assist the surgeon make a decision regarding which bone substitute to be used for pre-implant, intra-implant surgery and post-implant bone grafting and regeneration.

Theses previously mentioned bone regeneration mechanisms are actually positive mechanisms (osteogenesis, osteoinduction and osteoconduction). The author will introduce a newly discovered mechanism called the osteo-obstructive mechanism as a negative bone regeneration mechanism. This osteoobstructive mechanism was accidentally discovered by the author on single photon emission computerized tomography (SPECT) with histologic correlation during animal experiment to validate bone grafting technique and substitutes in the Ogunsalu sandwich bone regeneration technique. This osteoobstuctive mechanism has been histologically confirmed to be due to foreign body reaction.

In this chapter bone grafting will be mentioned distinctly from bone regeneration, similarly bone substitute (second -hand bone) will be distinctly separated from autogeneous bone graft.

The best GTR-membranes which will be preferably used with bone substitutes will be mentioned against the background of a new bone grafting technique called the Ogunsalu sandwich technique.
Finally the only available method for the qualitative and quantitative validation of bone substitutes and their comparism with one another will be described, in conjunction with histologic correlation. This method utilizes SPECT as a dynamic way for assessing osteoblastic activity after bone grafting and during bone regeneration.

1.1 Classification, types and source

This has been dealt with poorly in most standard textbook and as such I would attempt to adjust the existing classifications and sources. The source of bone graft could be autogenous or non autogenous with the autogenous bone source being the gold standard by which the non autogenous sources are to be compared for efficiency in effecting bone regeneration in the desired site. The autogenous bone can be derived from both intraoral or extra-oral sites. The intra-oral site includes the, chin, maxillary tuberosity, the body of the mandible , the ramus of the mandible, zygomatic buttress or even the exostosis including the oral tori (Fig. 1)

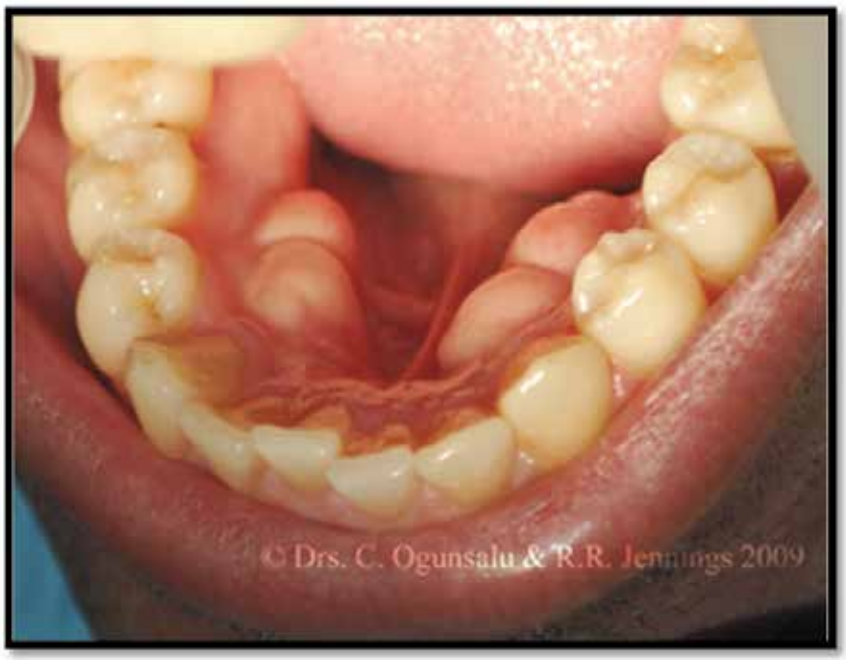


Fig. 1. Bilateral, multilobulated oral tori of the mandible.

The non-autogenous source are therefore second-hand in comparism to the autogenous source, for this reason I will call them the second-hand bones or bone grafting material(bone substitutes). Autogenous bone used for bone grafting should as such not be called bone grafting materials but rather a bone graft source.

The second hand bones are basically the allograft, alloplast of which the commercially available xenograft and the synthetic graft materials are generally considered a subgroup of the alloplastic bone grafting source.
The figure 2 shows the classification of bone grafting sources taken from the Glossary of implant dentistry II, published by the international congress of oral implantologists. I would however suggest that the classification shown in figure 3 be considered a reasonable variation of the former classification.
The origin of the bone graft will dictate the mechanism of its action with the understanding that none of them is osteogenic in action like the autogeneous bone graft source, which is an organic bone source harvested from the patient. This autogeneous bone also additionally forms bone by osteoinduction and osteoconduction, the two mechanisms ascribed to the second hand bones.

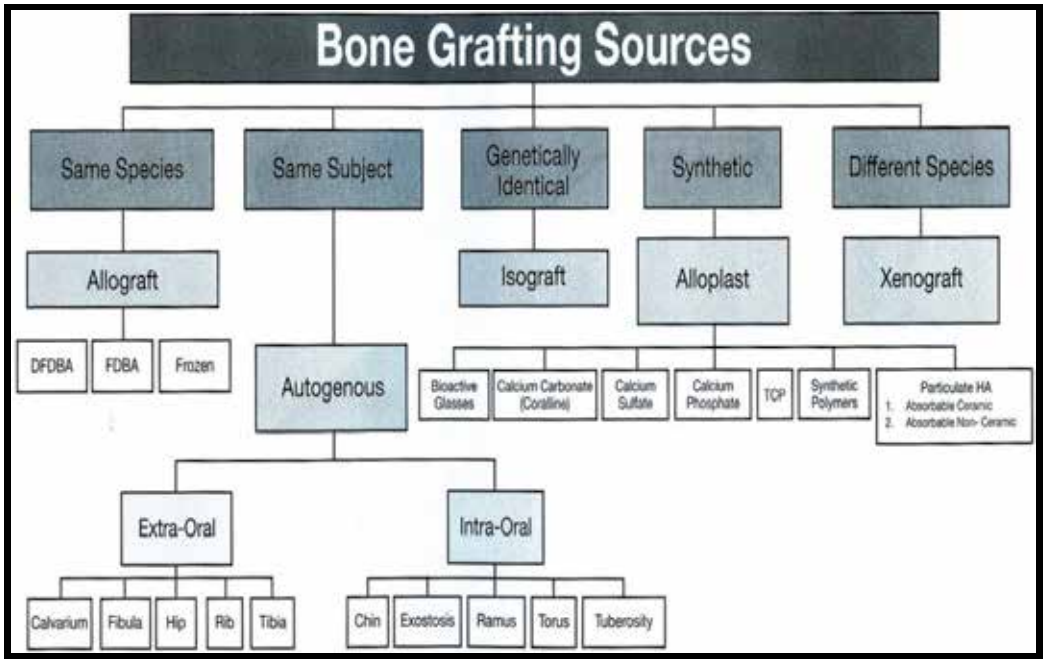


Fig. 2. Showing a reasonable classification of bone grafting source

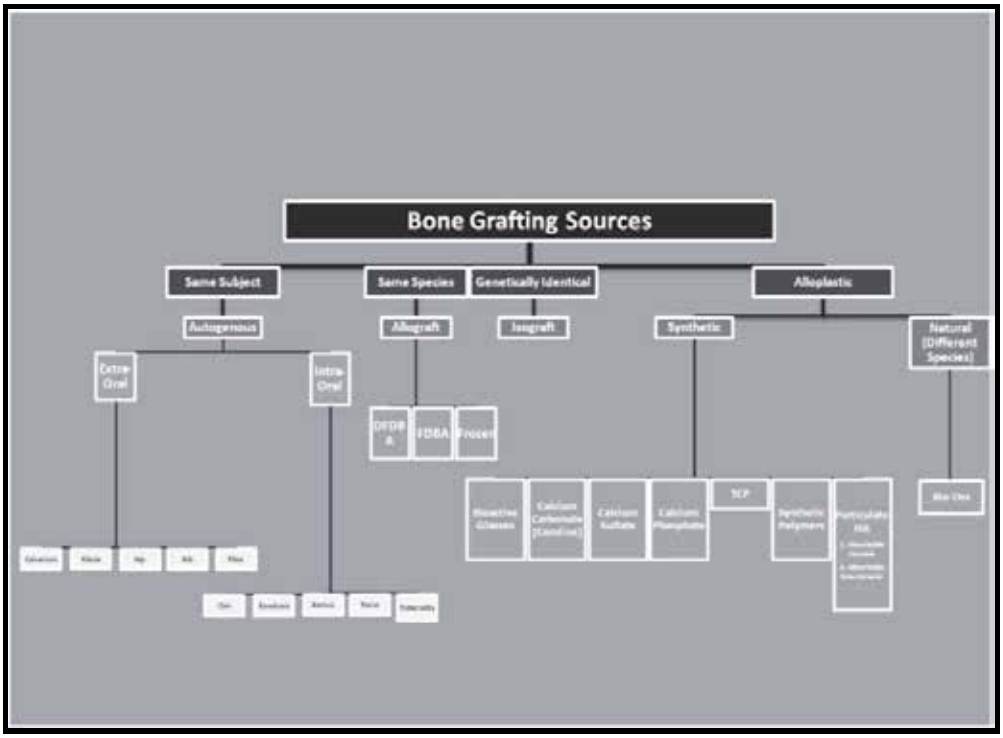


Fig. 3. Showing a much better classification of bone grafting source

1.2 Mechanism of action of bone substitutes

Bone grafts can effect bone replacement through three different mechanisms: osteogenesis, osteoinduction and osteoconduction (Misch and Dietsh 1993). Osteogenesis refers to organic material capable of forming bone directly from osteoblast (Misch and Dietsh 1993 and Marx and Saunders 1986). An osteogenic graft can therefore be said to be derived from or composed of tissues involved in the natural growth or repair of bone. It is for this reason that they can even encourage bone formation in soft tissues or activate more rapid bone growth in bone sites (Garg 2004, Wood and Moore 1988). Osteoinductive materials are capable of inducing the transformation of undifferentiated meseneymal cells into osteoblasts or chondroblast and enhance bone growth or even grow bone where it is not expected (Misch and Dietsh 1993). Urist (Urist 1980 and Urist 1965) recognized the mechanism as dependent upon many factors which includes specific proteins (e.g. bone morphogenic proteins [BMPs] located primarily in cortical bone. Osteoconduction is characteristic of a material (often organic) which permits bone apposition from existing bone and requires the presence of bone or differentiated mesenchymal cells (Rejda, Peelen and deGroot 1977 and Jarcho 1981). Osteoconduction provides a physical matrix or scaffolding suitable for the deposition of new bone. Osteoconductive graft are conductive to bone growth and allows bone apposition from existing bone, but do not produce bone formation themselves when placed within soft tissue (Garg 2004, Wood and Moore 1988). The healing of dental implants with a direct bone contact has been described as an osteoconductive process (Albrektsson 1985, 129-143).

Transplanted osteogenesis is another term for bone grafting. This term emphasizes that bone is dynamic and forms by cellular regeneration, which produces osteoid that becomes mineralized. A graft is not a solid bone block that heals into place (Garg 2004 and Marx and Garg 1998). Bone grafting is accomplished through osteogenesis, osteoinduction and osteoconduction (Lane 1995, Frame 1987, Pinholt, Bang and Haanaes 1991, and Lancet 1992). Osteogenesis refers to the formation and development of bone by osteocompetent cells. Osteogenic graft materials which are derived from or comprised of tissue involved in the natural growth and repair; it can encourage bone formation in soft tissues, and stimulates faster bone growth in bone implant site, whereas osteoinduction is the process of activating osteogenesis by recruiting cells from the surrounding natural bone that then differentiate into bone-forming cells. Osteoinductive grafts can enhance bone generation, sometimes even resulting in the extension or growth of bone where it is not normally found (Marx and Garg 1998). Osteoconductive grafts are those that act as nonviable scaffold on to and within which the patient's own natural bone grows. They are conductive to bone growth and allow apposition from existing bone but do not produce or trigger bone formation themselves when placed in soft tissue.
The table below shows the properties of the various types of bone graft sources in terms of mechanism of action with regards to bone regeneration.

Properties of various types of bone graft source.

	Osteoconductive	Osteoinductive	Osteogenic
Alloplast	+	–	–
Xenograft	+	–	–
Allograft	+	+/–	–
Autograft	+	+	+

Table 1. Showing the properties /mechanism of action for various bone source

It is important to note that all bone grafting materials have one or more of these three modes of action. Mixing of bone grafting substitutes can assist in bringing about a desired combination of modes of action for bone formation. For example Bio-Oss which is basically osteoconductive can be mixed with allograft as a deliberate or circumstantial cocktail to effect both osteoconductive and some osteoinductive properties. Because of making up the quantity of bone required in defects that definitely will benefit more from autogenous bone graft, any bone substitute can be mixed with autogenous bone graft to additionally effect all the mechanisms of action of bone regeneration attributed to autogenous bone graft.
Osteogenesis can form bone more rapidly and in conditions which have the least amount of bone. Autogenous bone is suggested even when additional operating time and surgical site preparation is required when limiting factors exist. Osteoconductive materials require the most ideal condition to grow bone, yet are the easiest material to obtain and manipulate. The amount of remaining host bone in the region and mode of action and physical characteristic of available graft materials must be considered prior to the selection of any one type or combination for use in implant dentistry (Misch and Dietsh 1993).
Osteogenesis, osteoinduction and osteoconduction are all positive mechanisms of bone regeneration with Osteogenesis being the fastest and most reliable. Bone substitute that regenerate bone via the osteoconductive mechanism are the least efficient.

In his classical experiment using animal model, Ogunsalu et al accidentally discovered the negative mechanism that will prevent bone regeneration (Ogunsalu 2009). This new mechanism called osteobstruction will be discussed in detail towards the end of the chapter.

2. Review of the literature on bone replacement source

The three primary types of bone graft material are autogenous bone, allograft and alloplast of which commercially available xenografts are generally considered a subgroup (Garg 2004). The mechanism by which these graft materials work normally depends on the origin and composition of the material (Misch and Dietsh 1993 and Lancet 1992). Autogenous bone, an organic material harvested from the patient, forms new bone by osteogenesis, osteoinduction and osteoconduction. Harvested from the cadavers, allografts which may be cortical or trabecular, have osteoconductive and possible osteoinductive properties (Garg 2004), but definitely they are not osteogenic. The alloplasts, which may be composed of natural or synthetic material, are typically only osteoconductive (Garg 2004).

In determining what type of graft material to use, the clinician must consider the characteristics of the bony defect to be restored (Misch and Dietsh 1993). In general, the larger the defect to be restored, the greater the amount of autogenous bone required. For small defects and for those with three to five bony walls still intact, alloplast may be used alone or with allografts. For relatively large defects or those with only one to three bony walls intact, autogenous bone must be added to any other type of graft material being considered. One of the complications during augmentation procedures with any grafting material is soft tissue ingrowths; it is for this reason that guided bone regeneration (GBR) using resorbable or non-resorbable membrane is to be employed (Schopper, Goriwoda, Moser, Spassova, Watzinger and Ewers 2001).

With regards to Maxillary sinus lift procedure, various materials including autogenous bone (Kent and Block1989, Jensen, Simonsen and Sindet- Pederson 1990, Reghoebar, Browne, Reintsena and Van Dort 1993, Adell R, Lekholm, Grondahl, Branemark, Lindstorm and Jacobsson 1990, Kahnberg, Nystrom and Bartholdsson 1989 and Nystrom, Kahnbourg and Gunne 1993), bone allograft (Misch and Dietsh 1993,Lane 1995, Lancet 1992, Wood and Moore 1988, Rummelhart, Mellonig, Gray and Towle 1989, Mellonig 1987, Tatum et al. 1993 and Tatum 1996) and alloplasts such as tricalcium phosphate (TCP), resorbable and non resorbable hydroxyapatite (Misch and Dietsh 1993, Rummelhart et al. 1989, Fetner, Hartigan and Low 1994, Schepers et al. 1993 and Smiler et al. 1992) bovine bone derivative (bovine-derived mineralized deprotinised bone) (McAllister et al. 1999) and bioactive glasses are used. It is important to note that an ideal graft is non-toxic, non-antigenic, non-carcinogenic, strong, resilient, easily fabricated, able to permit tissue attachment, resistant to infection, readily available and inexpensive (Wagner J 1989).

Autogenous bone which has long been considered the gold standards of grafting materials is currently the only osteogenic graft material available to clinical practitioners. When utilized for bone grafting autogenous bone heals into growing bone through all these modes of bone formation; these stages are not separate and distinct, but rather, overlap each other (Misch and Dietsh 1993). Autogenous bone can be harvested from extraoral sites such as the iliac crest or tibial plateau and intraoral sites sites such as the mandibular symphysis, maxillary tuberosity, ramus or exostosis (particularly the oral tori) (Misch and Dietsh 1993, Koole, Bosker, van der Dussen 1989 and Garg 1996).

It is well documented that less resorption is associated with the use of mandibular bone graft than with iliac crest grafts (Koole, Bosker, van der Dussen 1989). The use of expanded polytetrafluoroethylene (e-PTFE) membranes or slowly resorbable collagen membranes has been documented to enhance bone grafting (Buser, Dula, Hirt, Schenk et al.1996). Furthermore, bone graft obtained intraorally would generally result in less morbidity; however intraoral bone sites provides a significantly smaller volume of bones than do extraoral sites such as the iliac crest or tibeal plateau. The volume and type of regenerated bone needed for the site, will dictate the optimal donor site. The posterior iliac crest provides the greatest amount of bone (Koole, Bosker, van der Dussen 1989).

As previously stated, the autogenous bone graft is highly osteogenic and best fulfils the dental grafting requirement of providing a scaffold for bone regeneration (Hislop, Finlay and Moos 1993). Significant disadvantages associated with the use of autogenous bone include the need for a second operative site, resultant patient morbidity and in some cases the technical difficulties relating to obtaining a sufficient amount of graft material (this particular disadvantage relates to intraoral donor sites). In fact, it is these disadvantages and limitations that have lead to the development of less suitable alternatives such as allograft and alloplasts (Lane 1995, 36, Rummelhart, Mellonig, Gray and Towle 1989).

Autogenous bone graft forms the rigid scaffold which supports teeth and implants. It is composed of organic and inorganic structures. Resilience, toughness, and continuity are related to collagen, of the organic component. Stiffness, hardness and rigidity are characteristics of the inorganic aspect; a crystalline, ceramic-like material which is primarily hydroxyapatite (HA). This inorganic matrix contains organic components of osteocytes, osteoclasts, osteoblasts, osteogenic signaling proteins and various amount of mesenclymal tissue. Without any doubt, autogenous bone is the only osteogenic material (Misch and Dietsh 1993) and the various sites for harvesting autogeneous bone include intra-oral sites such as the chin, ramus, body of the mandible, maxillary tuberosity, oral tori and other exostosis, zygomatic buttress. The extra-oral sites are; tibial tuberosity, iliac crest (Misch and Dietsh 1993 and Garg 2004). Banked debris during implant osteotomy preparation is also another source of autogenous bone graft which is usually omitted by various authors (Misch and Dietsh 1993, Garg 2004, Koole, Bosker, van der Dussen 1989 and Garg 1996).

As shown in Figure. 4, grafted autogenous bone heals in three phases. During the first phase, the surviving cells are responsible for the formation of osteoid by osteogenesis. They are most active within the first four weeks after bone grafting (Marx and Saunders 1986, 347-428). The blood vessels from the host bone and the connecting tissue invade the graft. Bone cells from the host tissue follow the blood vessels and remodel the graft by a coupled resorption and formation phenomenon as reported by Roberts et al (Roberts et al. 1987). The BMP derived from the mineral matrix of the grafted bone through the resorbing action of osteoclast, acts as a mediator for the second phase (Urist 1980 and Urist 1965). The BMP and other proteins must be released prior to the osteoinduction cycle. Phase three occurs as the inorganic component of bone acts as a matrix and source of minerals during replacement of the matrix by the surrounding bone and resembles an osteoconductive mode of action. The three phases overlaps in the time sequence and are not separate phases of growing bone from the grafted autogenous material.

Grafted autogenous bone can be trabecular (cancellous), cortico-cancellous or cortical. The cancellous portion of grafts provides the cells for osteogenesis and survives best when a blood supply from the host bone is readily available. Cortico-cancellous block grafts permit

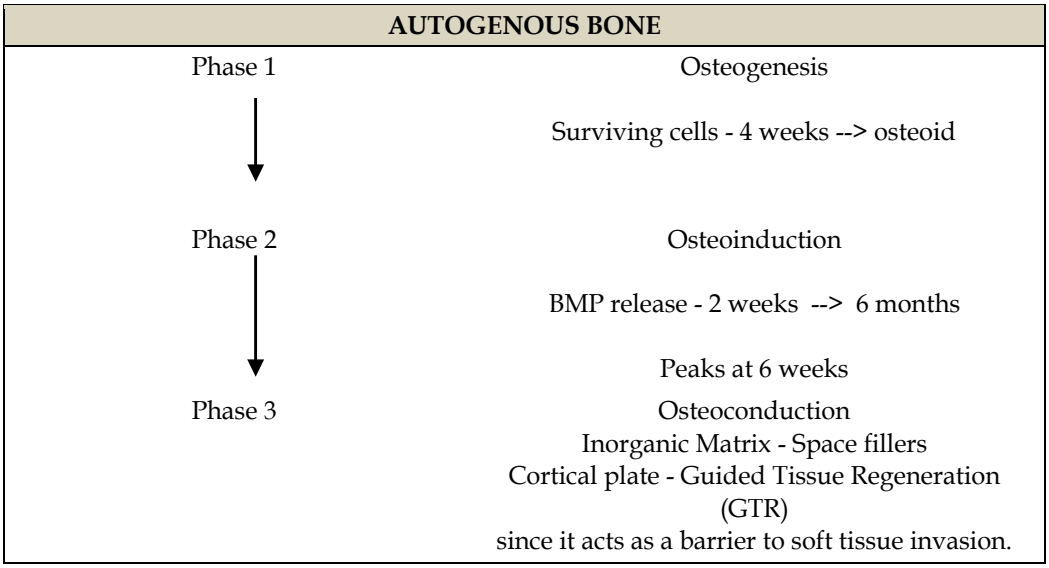


Fig. 4. Grafted autogenous bone healing, depicting the three phases of bone healing.

contouring and adaptation of the graft to the recipient bed anatomy. It is important however to quickly point out at this stage that the Ogunsalu sandwich bone regeneration technique (Ogunsalu 2009) will permit the cancellous portion of the autogeneous bone graft to be contoured and adapted to the recipients bed anatomy also. The trabecular portion is placed on the host bone and the cortical aspect is positioned on the surface of the graft. The cancellous portion is primarily responsible for the living bone cells and osteogenesis and therefore placed closest to the new blood vessels which arrive from the host bone and enter the graft at a rate of 0.5mm/day (Marx and Saunders 1986, 347- 428). The cortical graft supports osteogenesis only from the surviving cells (fewer than trabecular bone) and also provides more of the BMP compared with trabecular bone for the second osteoinductive phase (Longacre, Converse and Knize 1977). The cortical aspect also provides a more resistant scaffold for the third osteoconductive phase. In addition, it may act as a barrier to soft tissue invasion (thus excluding the need for a GTR membrane) and provide an extended period for blood vessels to enter the graft from the host bone (Misch and Dietsh 1993).

Allografts are obtained from cadavers or from patients' living relatives or non-relatives. Basically these bone grafts are of the same species but different genotypes. After processing, they are stored in bone banks. The advantages of allografts are availability, elimination of the donor site in the patient, decreased anesthetics and surgery time, decreased blood loss, and fewer complications. However, it is associated with some disadvantages which relate to bone tissues coming from another individual. Consequently the medical history must be thoroughly checked to eliminate donors with history of infection, malignant neoplasm's, degenerative bone diseases, hepatitis B or C, sexually transmitted diseases, autoimmune disease and other problems which affects the quality of the bone and the health of the recipient (Fonseca et al. 1986).

There are four main types of bone allografts: frozen, freeze-dried (lyophilized), demineralized freezed dried bone (DFDB), mineralized deproteinized and irradiated allograft. Fresh allografts are the most antigenic; freezing or freeze-drying the bone

significantly reduces its antigenicity (Lancet 1992). Allografts are not osteogenic and so, bone formation takes longer and results in less volume than can be achieved with autogenous grafts (Misch and Dietsh 1993). Allograft is said to form bone by osteoinductive effect on surrounding undifferentiated mesenclymal cells in the soft tissue over the graft as the blood vessels grow into the graft. It may also form bone by the osteoconduction phenomenon when the host bone resorbs the material and grows into its scaffold.

Freeze dried bone allograft (FDBA) can be used in either a mineralized or a demineralized (DFDBA) form. Demineralization removes the mineral phase of the graft material and possibly exposes the underlying bone collagen and growth factors such as the bone morphogenic proteins (BMPs) which has been implicated as a factor that increases the osteoconductive capabilities of allografts (Lane 1995, Acil et al. 2002 and Wikesjo et al. 2002). Freeze dried bone allograft hardens faster than DFBA because it is mineralized. Clinical studies have shown that grafting of the sinus with DFDBA alone results in the presence of dense connective tissue after six months whereas grafting with FDBA results in the presence of new bone formation (Meffert 1998). The clinical and histological study conducted by Feuille, Knapp, Brunsvold et al in 2003 showed that sites grafted with FDBA and subjected to GTR by coverage with an e-PTFE barrier can yield predictable result when augmenting alveolar ridges prior to placement of implants (Feuille et al 2003). MTFC (Dentsply friadent Ceramid, Lakewood Co.) and puros (Zimmer dental, Carlsbond, CA) are examples of manufacturers' allografts. The MTF is an allogenic freeze-dried bone that is available in both mineralized and demineralized forms. The FDBA is more effective than DFDBA in the following situations: repair and restoration of fenestrations, minor ridge augmentation, fresh extraction site filler, sinus lift, bone grafting and in the repair of dehiscence's and failing implants. The Puros is an allogenic graft material that has been subjected to a well-tested processing method to reduce antigenicity and to minimize any cross infection with HIV or Hepatitis virus (Masullo 1995). Puros which is solvent-preserved (in comparison with the freeze-drying) to extract the water component has been demonstrated to osseointegrate as effectively as cryopreserved material and to be equally biotolerable (Gunther et al 1996). This material has been very promising with regards to good bone formation and repair (Sener et al. 1998, Becker et al. 1996, Dalkyz et al. 2000 and Alexopoulau et al 1998).

Moreover, because the water component is removed by solvents rather than by cryo-dehydration, which can alter the mineral as a result of volume expansion that occurs during the transition from the liquid to the solid phase, the mineral matrix is said to remain intact (Gunther et al 1996). This mineral also has both the mineral and collagen phases of allogenic tissues.

The use of DFDBA as a graft material continues to be questioned because of various reports showing that it is unpredictable in regenerating new bone. In one study in humans, for example, the DFDBA particles were found to be surrounded by uninflammed connective tissue (Brugnami et al. 1996). However, a more recent study (Feuille et al 2003) showed positive result with the use of DFDBA and a cell occlusive membrane. Incorporation of the DFDBA particles was observed in new bone that contained lacunae with osteocytes (Brugnami et al. 1996). The use of FDBA in this study instead of DFDBA might have yielded a more favorable outcome in terms of new bone regeneration. It is believe that BMPs and other non-collagenous protein in the expressed matrix are responsible for the osteoconductivity of DFDBA (Garg 2004). The osteoconductivity however, depends on the

quality and quantity of the bone matrix in the graft material (Zhang, Powers, and Wolfinbarger 1997). Furthermore, studies have shown that different samples from the same bone bank and also different samples from different bone banks of the DFDBA can display different osteoconductive activity (Schwartz et al 1996).

To date, there are no widely acceptable tests or guarantee to ensure that DFDBA materials meet any minimum standards for osteoinductive properties; it is for this reason that this graft material had been avoided by many surgeons when bone grafting is considered. Invitro and in vivo assays have been utilized to a limited extent to assess the osteoconductivity of DFDBA (Zhang, Powers, and Wolfinbarger 1997).

DFDBA can be combined with other materials that have the potential to enhance bone growth. For example, the use of tetracycline with a DFDBA allograft has been studied; however, no benefit was derived from reconstituting the DFDBA particles in tetracycline hydrochloride during grafting of osseous defects (Masters et al. 1996). Osteogenin, a bone-inductive protein isolated from human long bones has been combined with DFDBA and studied in the regeneration of intrabony periodontal defects. Although this combination generated new attachment apparatus and component tissues more positively, it did not have any additional positive effect on new bone regeneration (Bowers, Felton and Middleton 1991).In another study, which compared Osteofil (Regeneration Technologies, Alachula, FL) a DFDBA with Grafton (osteotech, Eatontown, NJ) another DFDBA which, forms bone via osteoconductivity, suggested that the graft processing methods could represent a greater source of variability than do differences among donors (Takikawa et al. 2003).

Irradiated cancellous bone (Rocky Mountain Tissue Bank, Denver, Co.) has also been used as a substitute graft material for autogenous bone (Tatum, Lebowitz, Tatum and Borgner 1993, Tatum 1996). This is trabecular allograft obtained from the spinal column and treated with between 2.5 and 3.8 megarads of radiation. It has been shown that among all available allograft, irradiated bone is most similar to autogenous bone in terms of demonstrating rapid replacement and consistent establishment of a reasonable ratio of new bone with less expense and morbidity than that associated with autogenous material (Tatum, Lebowitz, Tatum and Borgner 1993 and Tatum 1996). Unfortunately, because of lack of further work in this area, the use of this material is not recommended (Garg 2004).

Gendler (Gendler 1986) in 1986 demonstrated by experiments that perforated demineralized bone matrix was a new form of osteoinductive material. Osteoinduction which is defined as transformation of non osseous connective tissue cells into osteogenic and chondrogenic cell, is an important biological process whose contribution to the physiology of bone remodeling and fracture healing at that time had only began to be appreciated (Mckibbin 1978 and Peck 1981).

In his unique experiment, Gendler demonstrated that subcutaneous implantation of perforated decalcified bone matrix (PDBM) induced multiple centers of endochondral osteogenesis with subsequent resorption of bone matrix and replacement by new bone. He therefore suggested that PDBM should be a useful research model to study osteoinduction and in the clinical management of orthopedic and reconstructive surgery for the filling of bone defects and stimulation of fracture healing (Gendler 1986).

Alloplast, xenografts and tissue-engineered materials are another group of bone graft substitutes. These include the deorganified bovine bone, synthetic calcium phosphate ceramics (e.g. hydroxyapatite, TCP) and calcium carbonate (e.g. Coralline). These ceramics form the new bone strictly by osteoconduction (Misch and Dietsh 1993 and Meffert et al. 1985) with the new bone formation taking place along their surface.

Basically alloplastic materials for bone growth are synthetic or deorganified biocompatible materials developed to cover a broad range of clinical applications for bone growth or soft tissue support. They come in a variety of textures, sizes and shapes and readily available and are mostly ceramics (Misch and Dietsh 1993).
Ceramic alloplasts may be bioinert or bioactive. Inert ceramics do not bond with the host bone. The relationship consists of an intimate mechanical contact which permits force transfer. They are rarely used as bone augmentation materials, but often are used as endosteal implants (e.g. aluminum oxide [$Al_2 O_2$] and titanium oxide [TiO_2]) as previously mentioned; the mode of bone formation for these ceramics is osteoconduction. Sub categories of bioactive calcium phosphate ceramics includes synthetic TCP and dense HA and those derived of natural origin (corallin or deorganified bovine and human bone). A chemical contact between the host bone and grafted material may be developed as well as possible stimulus for bone activity (Le Geros 1988).These materials exhibit good compressive strength, but poor tensile strength (similar to bone) (Le Geros 1983). Additionally, particle size, porosity, chemical structure and composition of the bioactive ceramics greatly influence the resorption rate of the material and may be another method of describing bioactive materials (Misch and Dietsh 1993). The bioactive ceramics differ greatly in resorption properties. Although difference in the biologic response of implanted bone substitute occurs, all have been recommended for augmentation (Masters 1988 and Le Geros 1988). TCP can be used with osteogenic or osteoinductive materials to improve the handling characteristic of the graft during placement (Misch and Dietsh 1993). Both hydroxyapatite and TCP are safe and well tolerated.
Cerasorb (Curasan, Kleinostheinon, Germany) is a beta-tricalcium phosphate (beta-TCP) material that has been certified for use in bone defect regeneration in the entire skeletal system. It is also certified in Europe as a synthetic carrier of the patient's own platelet rich plasma. This material is resorbed completely and is a generally replace by natural bone in three to twenty four month period, depending on the type of bone.
Hydroxyapatite (HA) is the principal inorganic component of the calcified tissues in the human body and has calcium to phosperous ratio of 10:6. Its crystallographic similarity to the bone mineral apatite allows bone growth and contact when implanted in hard tissue (Misch and Dietsh 1993). Various types of HA can be distinguished according to physical or chemical characteristics. Physical properties are the surface area or form of the product (block, particle), porosity (dense, macroporous, microporous) and crystallinity (crystal or amorphous), chemical properties are related to the calcium-to- phosphorous ratio, elemental impurities (such as carbonate) ionic substitution in HA and the PH of the surrounding region (ToFe, Watson and Bowerman 1991). These properties all play a role in the rate of resorption and clinical application of HA material (ToFe, Watson and Bowerman 1991). The larger the particle size, the longer the material will remain at the augmentation size. Thus 75um particle will resorb more rapidly than 3000um particles. The porosity of the calcium phosphate also impacts on the resorption rate. Tofe et al (ToFe, Watson and Bowerman 1991) reported on the porosity of dense, macroporous and microporous HA. Dense HA may lack any macro or microporousity within the particles (Misch and Dietsh 1993). The longest resorption rate occurs with the dense HA type since osteoclasts may only attack the surface and cannot penetrate the dense material (Misch and Dietsh 1993). The greater the porosity, the more rapid the resorption of the graft material. The crystallinity of HA also affects the resorption rate of the material. The highly crystalline structure is harder for the body to alter and resorb. The crystalline form of HA has been found to be very stable over the long term

under normal conditions while the amorphous structures are more likely to exhibit resorption, The less crystalline the material, the faster its resorption rate (Le Geros 1983).
The purity of HA bone substitute may also affect the resorption rate. The resorption of this bone substitution requires living cells, similar to the modeling/ remodeling process of living bone with the coupled resorption/ formation process. A solution-mediated resorption permits the dissolution of the material by a chemical process. Impurities in bioactive ceramics, such as calcium carbonate, permits solution-mediated resorption which then increases the porosity of the bone substitute. It is said that corallin HA does not demonstrate micropores around the larger holes, the HA has carbonates incorporated within the material, which hastens the resorption process (ToFe, Watson and Bowerman 1991).
The pH in the region in which the bone substitute is placed also affects the rate of resorption of HA. As the pH decreases (e.g. from the infection in the bone), the HA components of living bone and phosphates resorbs by the solution-mediated process. Bone, dense HA, macroporous HA, microporous HA, crystalline HA or amorphorous HA may all resorbs within a two week period.
Physical properties should determine the type of HA selected for residual ridge augmentation. Dense, crystalline, large particle size HA can be used for ridge augmentation. Dense HA particles may also act as space filler or modifier of soft tissue contours under pontics of a fixed partial denture or around implants (Jarcho 1981). Dense crystalline HA cannot be easily cut and should not be placed in bone defects when the insertion of endosteal implants is planned in the future, in addition when an implant is in contact with dense crystalline HA, the material cannot grow into or attach itself to the implant surface. As a result, less percentage of inert bone implant bone implant contact occurs and compressive forces cannot be transmitted as well to the HA particle-implant interface. This factor will increase the amount of force generated to the remaining bone contacting the implant.
As the resorption rate of macroporous HA is generally greater than thirty six months, it is used where a more long-term matrix is desired (e.g. ridge augmentation or subantral augmentation). The resorption rate of microporous HA (six to twelve months) is compatible with applications where scaffold is needed within bone during the first several months of healing, but where living bone is desired in the near future.
Tricalcium phosphate (TCP) has calcium to phosphorous ratio of 3:2, and is intended to provide a scaffold for initial bony proliferation. TCP has been reported to act as short-term biologic filler which is resorbed over time by osteoclasts and replaced by living bone cells which grow directly in contact with the material without any encapsulation process (ToFe, Watson and Bowerman 1991 and Heimke and Griss 1983). The resorption of TCP and its replacement by new bone occurs through various mechanisms. The process seems to be very dependent upon the material characteristics, primarily chemical structures, porosity and particle size. These characteristics are closely related to manufacturing processes (Swart, Rejda and de Groot 1979).
TCP is prepared by sintering processes. It is very sensitive to heat and sterilization, which may change its chemical structure and alter its properties, including resorption rate (Le Geros 1988). It can be used in combination with osteogenic and or osteoinductive materials because it provides improved handling characteristics to the graft during placement. In addition to Cerosorb®, which is mentioned above as β-TCP, other commercially available TCP products are; Calciresorb® (Ceraver Osteal, Paris, France), Synthograft (small size and dense) and Augmen (larger size and dense) (Miter, Warsaw).

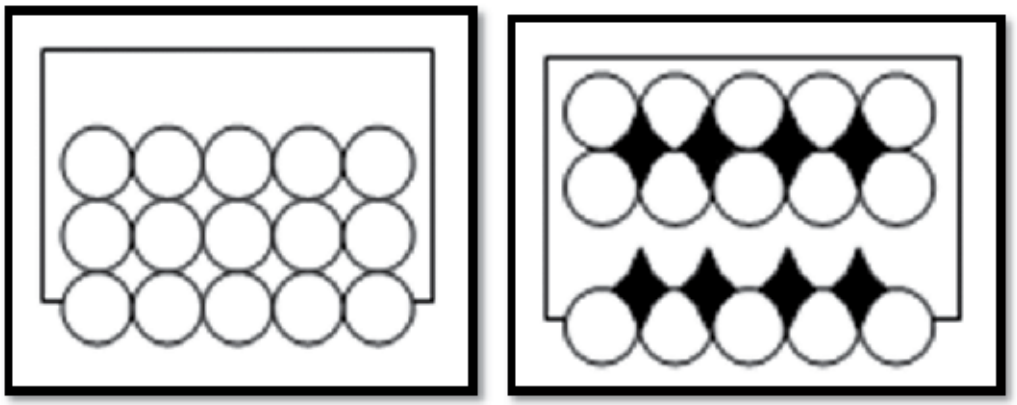
Bovine-derived anorganic bone matrix materials (xenogenic alloplast) are utilized for bone grafting. An example of this is Bio-Oss (Osteohealth, Shirley, NY) which is anorganic bovine bone that has been chemically treated to remove its organic component.
After the Bio-Oss is sterilized, it can be used as a graft without causing a host immune response (Hislop, Finlay and Moos 1993). Bio-Oss is osteoconductive (Hislop, Finlay and Moos 1993 and Pinholt, Bang and Haanaes 1991) and over time, the graft undergoes physiologic remodeling and becomes incorporated into the surrounding bone. This type of bone graft can be used alone or in combination with barrier membrane in periodontal defects, dehiscences and fenestrations around implants and in small sinus osteotomies. In large alveolar ridge deficiencies, anorganic bone can be combined with autogenous bone for successful augmentation. Anorganic bone has been utilized in the treatment of intra bony defects, and for maxillary sinus augmentation and treatment of peri-implantitis (Garg 2004).
Bovine bone substitutes are widely used for treating osseous defects, however, there is a risk of transmitting bovine spongiform encephalopathy to human (Will 1999, Scott et al. 1999 and Vedrager 1999). As these materials are routinely and successfully utilized in surgical dentistry and orthopedic surgery, careful risk assessment has to be done. Bio-Oss and osteograft are bovine derived bone substitute which are processed from veterinary certified cows from the USA, a country that is known to be free of BSE-cases. Consequently it is unlikely that the starting material for the manufacturing of Bio-Oss or osteograft contains prions. However, it has recently been questioned whether the U.S.A. can still be considered as a BSE-Free country.
(http://europa.eu.int/com/dgs/healthconsumer/library/press/press66_en.html)
The issue has become more urgent as many biomaterial scientists, dental and orthopedic surgeons are getting more concerned about the bio-safety of biomaterials from bovine origin. There is an increasing interest in the analysis of the risk of transmitting BSE through grafting materials derived from the bovine bone. The theoretical risk assessment has been done according to a model proposed by the German Health Authority (Bundesgesundheitsamt 1994 and Bundesgesundheistamt 1996). This was done before for osteograft /N based on a model published in 1994 (Bundesgesundheitsamt 1994). Wenz *et al* in their publication of 2001 on the analysis of the risk of transmitting bovine spongiform encephalopathy (BSE) through bone grafts derived from bovine bone concluded that theoretical and experimental data indicate that the use of these materials does not carry a risk of transmitting BSE to patients (Wenz, Oesch and Horst 2001).
PepGen P-15 (Dentsply Friadent Ceramed) is another form of bovine-derived hydroxyapatie which is enhanced and contains an added synthetic short-chain peptide p-15. This component mimics the cell-binding domain of type I collagen which is responsible in natural bone for cell migration, differentiation and proliferation (Similer 2001). PepGen P-15 may provide the benefit of a synthetic graft containing an inorganic and an important organic component that together may mimic autogeneous bone in graft sites. This material has been reported to provide enhanced bone formation in a shorter time compared with the other bovine-derived hydroxyapatite plus DFDBA graft material traditionally used for sinus augmentation (Krauser, Rohrer and Wallace 2000). Another study indicated that enhanced bone formation and faster particle resorption can occur with Pep-Gen P-15 flow (PepGen p-15 particles suspended in biocompatible inert hydrogel consisting of sodium carboxymethyl-cellulose, glycerol and water) compared with the pep-Gen P-15 particles (Hahn, Rohrer and Tofe 2003).

Recently the bioactive glass ceramics have emerged as a bone grafting material. Bioglass (US Biomaterials, Jersey City, NJ) is composed of calcium salts and phosphate in a proportion similar to that found in bone and teeth, as well as sodium salts and silicon, which are essential for bone to mineralize. Bioglass is an amorphous material which is deliberately not manufactured in the crystalline form (to strengthen the material) because the developers foresaw that degradation of the material by tissue fluid and subsequent loss of the crystal could cause a loss of integrity. This material is not porous and as such, tissue and blood vessel in-growth is prevented. The biologic impact of this non-porous nature is not known, and only few studies support the use of this material in periodontal and maxillofacial procedures (Garg 2004).

Bioactive glass ceramics have two properties that contribute to the successful results observed with its use: (Misch and Dietsh 1993) a relatively quick rate of reaction with the host cell and (Marx and Saunders 1986, 347- 428) an ability to bond with the collagen found in connective tissue (Kirsh and Garg 1994). It has been documented that the high degree of bioactivity may stimulate the repair process and induces osteogenesis (Wilson 1993). Because the bioactivity index is high, reaction layers develop within minutes of implantation. As a result osteogenic cells in the implantation site may colonize the surface of the particles and produce collagen on these surfaces, osteoblast then lay down bone material on top of the collagen; an action which may supplement bone that grow by osteoconduction from the alveolus. In their seminal clinical trial and subsequent publication of 1993, Schepers *et al* (Schepers, Ducheyne, Barbier and Schepers 1993) reliably demonstrated that bioactive glass granules of narrow size range constitute a valuable material to aid in the repair of dental bone lesions.

The phenomenon of osteogenesis guided by bioactive glass particles with a narrow size range has been explained. The glass particles and the surrounding tissue fluids result in the formation of a silica gel, which is quickly covered by a calcium-phosphorous-rich layer. The particle size of the glass is such that the entire granule is transformed into silica gel (i.e. it is gelated). Phagocytosing cells penetrate the silica gel by means of small cracks in the outer calcium-phosphorous layer and partially resorbs the gel. This resorption leads to the formation of protective pouches in which primitive mesenchymal cells acquire phenotypic characteristics of osteoblasts. These osteoprogenitor cells adhere to the inner surface of the pouch. When the primitive cells are immobilized on this inner calcium-phosperous-rich layer (a bone-like surface), differentiation of these cells into osteoblasts occurs. In this way islands of new bone tissue are formed without the need for osteoblastic proliferation from the preexisting bone (i.e. the cavity walls (Schepers, de Clercq, Ducheyne and Kempeneers 1991 and Schepers, Ducheyne, Barbier and Schepers 1993). The size range of bioglass particles must be narrow (300 to 360 *u*m for the glass composition selected) due to several critical considerations (Schepers, de Clercq, Ducheyne and Kempeneers 1991 and Schepers, Ducheyne, Barbier and Schepers 1993), the phenomenon of preferential resorption of the gel is restricted to this size range. Particles with a size exceeding this range do not corrode throughout and therefore are not resorbed into their centers. Hence recruitment of primitive cells exhibiting osteoblastic differentiation throughout the bone defect does not occur and healing is slow since it must proceed from the pre existing bone tissue walls. Particles with smaller diameter are fully resorbed and cannot act as a substrate for enhancement of mesenchymal cells. Glass granules preparations that contain the critical 300 to 360mm size range and also smaller particles are not active as described. Another critical aspect related to

small size range is the packing of the particles (Schepers, de Clercq, Ducheyne and Kempeneers 1991 and Schepers, Ducheyne, Barbier and Schepers 1993). When granules have uniform dimensions, dense packing will still leave space between the particles. With a wider size range, smaller particles fill up the spaces in between the larger particles. Such a granular mixture will extensively fill a defect space and leave very little room for tissue infiltration and regeneration (Fig. 5).



A B

Fig. 5. Difference in packing when a limited size range of particles is used (A) as opposed to a larger size range (B)

The endosseous ridge maintenance implant (ERMI, US Biomaterials) is another Bioactive glass bone substitute. It is a cone-shaped device made of bioglass that is placed in the extraction site (Kirsh and Garg 1994). This bone implant system can be used for maxillary and mandibular premolars and anterior teeth. It can also be used for preserving the contour of the alveolar ridge following tooth removal. This bone implant acts under a time-dependant kinetic modification of its surface after placement within one hour of implantation; a chemical bond appears to form within the bone tissue (Kirsh and Garg 1994). Based on a study (Kirsh and Garg 1994), denture wearers showed a retention rate of approximately 90% for up to seven years when the Bioglass implants were used for alveolar ridge maintenance (Kirsh and Garg 1994).

Perioglass (Mova Bone, Alachu, FL) is a synthetic particulate form of Bioglass that bond to both bone and certain soft connective tissues (Wilson 1993). Perioglass is composed of calcium, phosphorous, silicon and sodium (Fetner, Hartigan and Low 1994). The quality and quantity of new bone deposition may increase with the use of perioglass particles compared with hydroxyapatite crystals (Oonishi 1994). Perioglass is indicated for the treatment of intrabony defects and the criteria for successful perioglass use includes pre-treatment planning, debridement of the defect, preservation of soft tissue vascularity and infection control (Quinones and Lovelace 1997).

Perioglass has demonstrated two favorable characteristics: ease of compactability and ability to promote hemostasis (Fetner, Hartigan and Low 1994). When well packed into osseous defects, it becomes strongly adherent and hardens into a solid mass incapable of being disturbed by a suction tip or hand piece. Fetner, *et al* concluded that by bonding to both bone and connective tissue, perioglass achieved improved grafting results (Fetner, Hartigan and Low 1994).

Biogran (3i, Implant Innovations, Palm Beach Gardens Fl.) is another resorbable bioactive glass bone grafting material which have granules that are chemically identical to perioglass and are composed of calcium, phosphorous, silicon, and sodium. The difference between perioglass and biogran is the size range of the particles- 300 to 355 *u*m for biogran and 90 to 710 *u*m for perioglass. Biogran is hydrophilic and slightly homeostatic; it stays in place in the defect when bleeding occurs. Once in contact with the patient's blood, it forms a cohesive mass that is shaped into the defect (Garg 2004). Bone transformation and growth occurs within each granule. This osteogenesis which is guided by bioactive glass particles occurs at multiple sites, rapidly filling the osseous defects with new bone that continuously remodels in the normal physiologic manner (Rummelhart, Mellonig, Gray and Towle 1989, Schepers 1993 and Duchenyne et al 1992). This bioactivity permits material and bone transformation to occur simultaneously.

Bioplant HTR Polymer (Bioplant, Norwalk, CT) is a microporous composite with a calcium hydroxide graft surface (Ashman 1993 and Ashman 1984). This polymer resorbs slowly and is replaced by bone after approximately 4 to 5 years. This bone grafting material has been reported to be effective for ridge maintenance post extraction, ridge augmentation (immediate or delayed) and repair of periodontal and other bone defects. Boyne (Boyne 1995) concluded that in the HTR-implanted defects, bone tended to become lamellated (cortical) in the area of the particulate grafted implant particle over a long period of time. This lamellated bone structure suggests that such crestal regions should resist resorption in clinical areas. Porous bone material (Bio-Oss, Switzerland) that slowly resorbs and remodelled slowly also has been shown to confer a similar resistance to resorption on edentulous ridges in clinical situation (Boyne 1991). HTR is non-resorptive and may offer the same type of ridge support against resorption. The ability of HTR to produce an environment that resists resorption should be determined on a long-term basis by controlled clinical studies of conventional and root form implant supported prosthesis.

Garg (Garg 2004) argues that the use of autogenous bone, allografts and alloplasts or tissue engineered materials, alone or in combination should be based on the osteogenic potential of the recipient site. The decision should be based on the individual's systemic healing ability, considering among other things age, systemic illness affecting healing, (such as diabetes or autoimmune disorders like scleroderma and lupus), previous surgeries to the area, previous radiation treatment or chemotherapy, and irradiated tissue bed. Local osteogenic potential of the defects (e.g. defect size, ratio of host bone to graft material, number of walls of the defect, soft tissue bed, adjacent scar tissues, health of adjacent periostium, stability of the graft material, soft tissue closure, use of interim restorative device over and around grafted site should also be considered. The osteogenic potential of the graft, the surgeon's skill and the time available for graft maturation are also critical to the decision.

It is as such important at this stage to indicate that the membrane sandwich technique allows us to enhance, combine, and maintain osteogenic potential at the required site.

3. Application of bone grafting materials and selection criteria

The application of bone grafting materials and selection criteria should be done against the background of the understanding that autogenous bone graft is the gold standard for utilization in any peri-implant or periodontal bony defect, because it forms bone by osteogenesis and additionally by osteoinduction and osteoconduction.

For the predictable long-term success of dental implants it is important to appreciate the available bone (Misch 1990). When available bone is inadequate, bone substitute represent a viable treatment modality. The extraction sockets, ridge defects and sites where bone volume is inadequate may be filled to maintain or improve ridge anatomy, improve esthetics and function, and/ or prepare the site for endosteal implants. Bone graft substitutes can also be found beneficial in the treatment of peri-implant defects which may occur after or during implant placement. It is important that the composition of the graft used to fill defects correspond to the mode of action of the graft material, and the number of walls of host bone remaining in contact with the graft (Misch 1993).
With regards to defects in the oral cavity that may require bone grafting, we have; five-wall defects, four-wall defects, two or three-wall defects and one-wall defects; each defect requires a particular type or combination of bone grafting substitute for optimum healing. It is for this reason that in this Section; I have dealt with bone grafting requirements per defect.

3.1 Five-wall defect (with one wall missing)

An extraction socket can be compared with a five-walled pocket (Fig. 6), similarly a cystic cavity is comparable to a five-walled pocket. This pocket is expected to fill with new bone by appositional growth. In order to maintain the width of the extracting socket and to improve the chance of success of future dental implants placement, an extraction socket can be filled with inexpensive resorbable calcium phosphate material to prevent the usually documented percent to 60% of the width of the ridge resorption which primarily occurs from the facial dimension (Khan et al 1981).

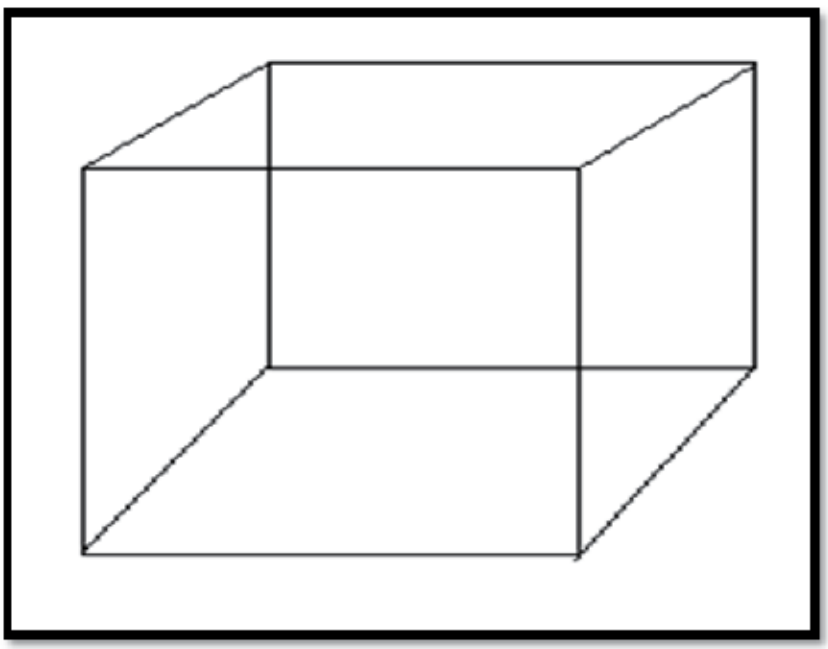


Fig. 6. Five-wall defects such as at tooth extraction socket or cystic cavities are filled with inexpensive resorbable calcium phosphate material to maintain ridge width. Autogenous bone is least indicated, but may always be used when readily available, since it is an osteogenic material and has no cost.

Autogenous bone may be used in any defect. It is often readily available without any cost to the clinician and forms bone by osteogenesis. When the harvesting site for bone is difficult to access, the five-wall defect may be grafted with any resorbable material, and then covered with a collagen or synthetic membrane to contain the resorbable material, particularly in situations where complete approximation of the soft tissue is not possible. With this procedure, it is advisable that 4 to 6 months elapse before re-entry and implant placement (Misch and Dietsh 1993).

3.2 Four-wall defect (defect with two walls missing)

If the host bone site has lost an additional wall of bone (usually the labial wall), it is called a four-wall defect. Additional active elements are beneficial in this graft since bone does not surround the defect. In such cases the addition of DFDB to the alloplastic calcium phosphate is recommended in order to compensate for the lack of labial bone and additional soft tissue in approximation to the graft (Roberts et al. 1987). It is suggested that the calcium phosphate be mixed with DFDB and over packed or contoured beyond the defect and this material is covered further with DFDB. This will allow the DFDB to be intact with the overlying soft tissue to modify the undifferentiated cells into osteoblast (Fig. 7).

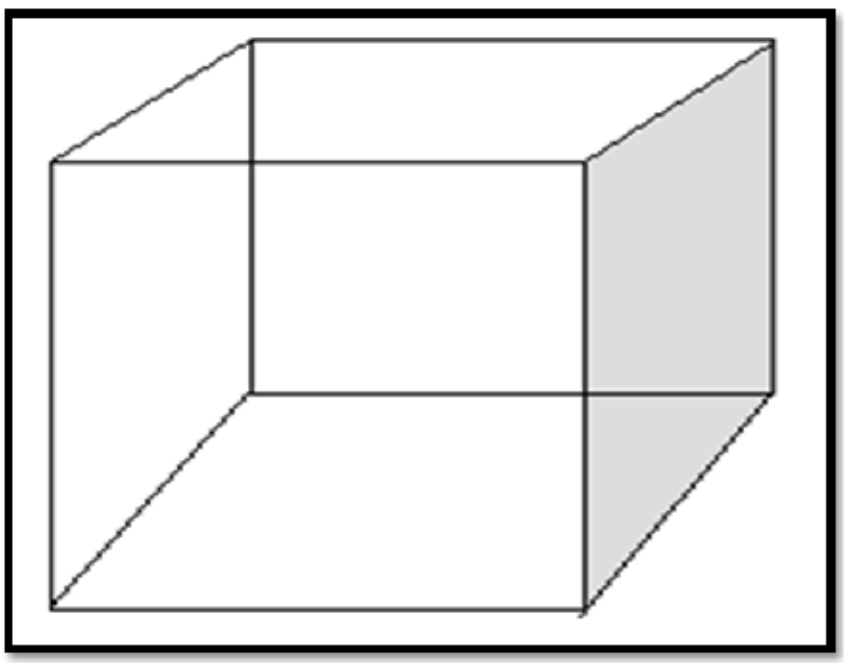


Fig. 7. With four remaining walls (usually labial and occlusal walls missing), calcium phosphate mixed with DFDB is placed over the host bone to retard soft tissue ingrowth. DFDB is placed on top of the calcium phosphate mixture so it is close to soft tissue for an osteoinductive effect.

The calcium phosphate acts similar to a barrier (due to its bulk) to retard the amount of soft tissue in growth within the graft and allows more bone formation in the region. DFDB when used alone in defects has not yielded much satisfactory results since it is eliminated too

rapidly to permit a predictable volume of bone formation in the defect with the four- wall defect as such a healing period of at least 6 months should elapse before implant placement (Misch and Dietsh 1993).

3.3 Two or three-wall defect (defects with 3 or 4 walls missing

The loss of three or four bony walls will create a two or three wall defect. This type of defect requires the use of autogenous bone. The autogenous bone can be harvested intraorally from the maxillary tuberosity or with a trephine drill under the roots of the mandibular incisors and is positioned in the defect in contact with the host bone. Such placement allows for blood supply from bone to be established, to maintain trabecular cell survival. DFDB is laid over the autogenous bone chips to begin the osteoinduction process. Calcium phosphate and DFDB are added on top and the entire graft is covered with a membrane. A resorbable membrane is preferred to prevent the need for early re-entry and to reduce the risk of infection. Overall this approach allows guided tissue regeneration technique to impair epithelial in growth into the graft which would otherwise impair the healing process. Although the two-three wall defects are larger, the healing time is more rapid with the autogenous bone component. In approximately six months the implant can be inserted (Misch and Dietsh 1993) (Fig. 8).

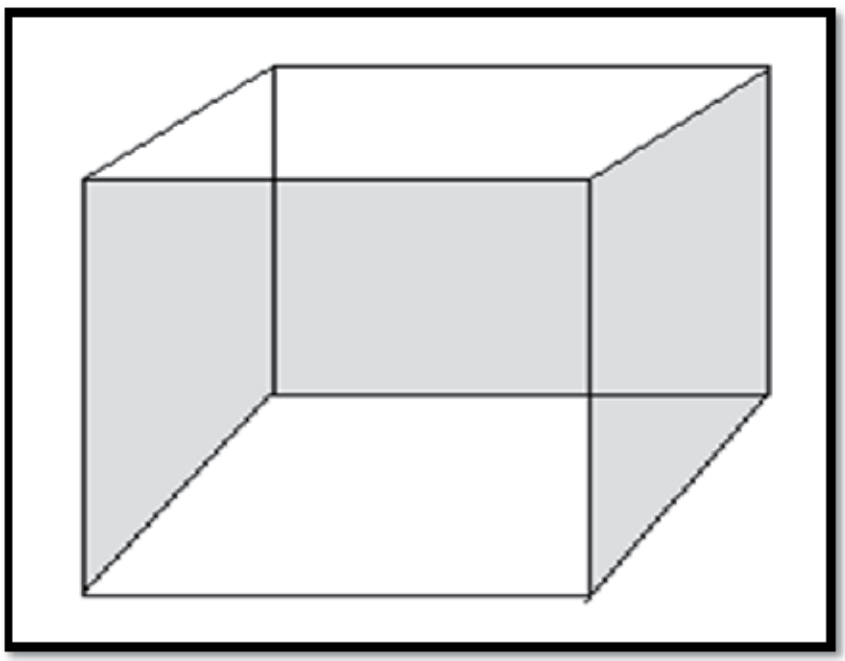


Fig. 8. The loss of three to four bony walls (a two or three wall defect) necessitates the use of autogenous bone

3.4 One-wall defect

Defects with five missing walls (one-wall defect) warrant the use of an autogenous corticotrabecular bone to regenerate a good volume of bone in the recipient site. A block graft is therefore the most preferable approach. The cortical aspect of the block is placed

superiorly to act as a barrier to the invergination of the soft tissue within the graft. A mixture of chips of autogenous bone, then DFDB and then calcium phosphate and DFDB can be used to fill any defects around the block of the bone (Fig. 9).

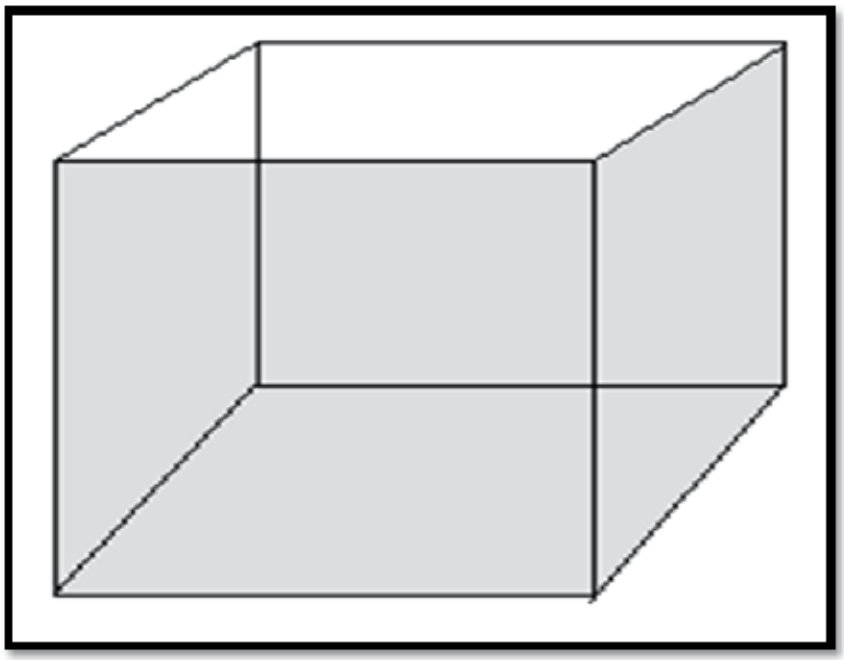


Fig. 9. Showing a one-wall defect

A healing period of four to six months is adequate to permit ridge reconstruction since autogenous bone is the major component of the graft (Misch and Dietsh 1993 and Misch 1993). If the cortical bone is present on the superior aspect of the autogenous graft, the area may be covered with a thin DFDB sheet or small pore membrane to prevent soft tissue ingrowth.

4. The indications for uses of bone substitutes

In implant dentistry the indications for use of bone substitutes can be divided into:

a. Pre-implant bone grafting needs such as:
   i. Ridge augmentation
   ii. Maxillary sinus lift procedure
   iii. Extraction socket for delayed implant placement
   iv. Extraction socket for ridge preservation
   v. Intrabony defect
   vi. Furcation involvement of teeth adjacent to implant site
   vii. Recession
   viii. Pneumatized sinus
b. Intra-implant surgery bone grafting needs such
   i. Intra-operative per-implant placement (precautionary of mandatory)
   ii. Extraction socket during immediate implant placement

iii. Repair of cortical bone plate dehiscence
iv. Alveolar or mental nerve protection
v. Conventional maxillary sinus lift procedure
vi. Osteotome technique for maxillary sinus lift and simultaneous implant placement
vii. Ballooning technique for maxillary sinus lift and simultaneous implant placement
viii. Intra-operative ridge preservation
ix. Furcation involvement, recession and intrabony defect of teeth adjacent to the implant site.

c. Post –implant bone grafting needs such as;
i. Implant failure
ii. Peri-implantitis

The repair of defects around previously inserted endosteal implants can also be performed with bone substitutes. At the time of uncovering, defects and bone resorption are best identified around the implant with a full-thickness reflection. The defect filled with soft tissue can be curetted and filled with autogenous bone. The defect is then covered with DFDB since such defects are similar to three or four wall defects (Misch and Dietsh 1993).
Subantral augmentation after sinus elevation in the posterior maxilla is the most predictable region of the oral cavity where the atrophic ridge can be augmented in height with the use of allografts and alloplastic material (Misch and Dietsh 1993). Autogenous bone is an excellent material for this procedure, but the quantity of bone necessary to fill the antrum often requires harvesting host bone from an extraoral site. The procedure has been further modified to permit the use of less autogenous bone and incorporation of both allografts and alloplastic materials. Results from structures show that the subantral region is similar to a three or four-wall defect and the graft should include autogenous bone, DFDB and TCP or microporous HA to combine osteogenic, osteoinductive and osteoconductive modes of bone regeneration (Misch and Dietsh 1993).
The fact that the last fifteen years have seen the introduction of several bone substitutes. Those materials can modify the bony structure of the patient prior to implant treatment, during implant treatment and after dental implant treatment. It is as such very important to understand the characteristics of the different materials in reference to crystallinity, porosity, particle size, chemical structure, and PH in order to be able to select the most appropriate type or combination to achieve a predictable result.

5. Validation of bone substitutes (second-hand bone)

Bone substitutes are best validated utilizing single photon emission computerized tomography (SPECT)TO disclose in a dynamic way the osteoblastic activity and calculated index around the bone grafting site over a period of time . It is the intention of this section to explain SPECT and its various applications and then to describe the SPECT experiment to be used to a compare second hand bone such as Bio-Oss with autogeneous bone (both of which are contained in the Ogunsalu sandwich unit).

5.1 Single Photon Emission Computerized Tomography (SPECT)

The literature is replete with the various applications of single photon emission computerized tomography (Khan et al. 1980, Ell et al. 1981, Flood and Russel 1998, Lima et al. 2004, Van der Wall and Fogelman 2007, Horger and Bares R. 2006, Schafers and Stegger 2008, Dasgeb Mulligan and Kim 2007, Sarikaya, Sarikaya and Holder 2001, Ozyurt et al.

2008, Kalita et al. 2008 Crespo et al. 2008, Massardo et al. 2008 and Ellis et al. 2008), however, there is a dearth of information on the applications of SPECT in relation to implant assessment and osseointegration .Reports in the medical literature on the radiologic evaluation of per-implant bone changes in the context of osseointegration are limited to one-dimensional quantitaions of heights of the defects. Despite the fact that digitized radiography and computerized tomography can facilitate quantification of bone changes, these methods generally reflect morphologic changes but may fail to detect the dynamics of osteoblastic activity (Massardo et al. 2008, Ellis et al. 2008, Alberto 1998 and Galasko 1975).

Bone scintigraphy which is a well established imaging technique that accurately reflects osteoblastic activity (Ogunsalu et al. 2008) can be utilized to radiographically assess osseointegration. In most clinical situations, the data from planar or conventional radiographic views are usually sufficient for diagnosis, but accurate quantitative analysis may not always be possible because of interference by superimposed structures. The single photon emission computerized tomography (SPECT) provides an additional refinement to planar imaging, it allows accurate quantitation common to most tomographic techniques by removing regions not of clinical interest.

Although the SPECT technique is well established and successful in clinical application for the study of many organ systems, (including skeletal system) (Khan et al. 1980, Ell et al. 1981, Flood and Russel 1998, Lima et al. 2004, Van der Wall and Fogelman 2007, Horger and Bares R. 2006, Schafers and Stegger 2008, Dasgeb Mulligan and Kim 2007, Sarikaya, Sarikaya and Holder 2001, Ozyurt et al. 2008, and Kalita et al. 2008), its application by clinicians and manufacturers alike have been very much non- existent in the clinical or experimental assessment of osseointegration of bone grafts and implant systems. Recently however, Ogunsalu and co-workers have utilized SPECT to successfully assess osseointegration of a new bone grafting/ regeneration technique (Ogunsalu et al. 2008 and Ogunsalu et al 2008) and also for comparative assessment of osseointegration relating to implant systems (Ogunsalu et al. 2008 and Ell et al. 1982).

The underlying principles of SPECT are common to most tomographic imaging techniques. When a radiopharmaceutical agent containing a single gamma-photon emitting radionuclide such as technetium 99m is injected intravenously it is possible to obtain a three-dimensional representation of the distribution of radioactivity within an organ or an area of interest in which the radiopharmaceutical agent is localized by using radiation detectors and rotating gamma cameras which detects the emitted radioactivity as the camera is rotated around the clinical or experimental area of interest. The acquired data can then be processed by a computer which will initially provide a cross-sectional (trans-axial) representation of the distribution of radioactivity. The transaxial data can additionally be used to reconstruct sagittal and coronal images.

It is the ability for multiplane image reconstruction with SPECT which confers greater diagnostic accuracy to SPECT. Additionally, SPECT permits accurate volumetric measurement and it is thus possible, for quantitation of the distribution of radioactivity in terms of Mc_i per unit volume of tissue (Khan et al. 2000). This unique ability to quantitate physiologic events, by using a bone-seeking radiopharmaceutical sets SPECT apart from other tomographic techniques such as computerized tomography (Khan et al 2000). CT Scans (computerized tomography) is able to provide excellent morphological details but unlike SPECT, it is unable to provide functional data.

In a highly significant publication, Khan et al (Khan et al 2007 and Ogunsalu 2007) described SPECT as capable of accurate quantitation of bone changes, before and after titanium dental

implants in edentulous patients. "A novel approach that has the value in imaging bone changes dynamically and further offers an objective method for monitoring such dynamic changes before, during and after implantation" (Khan et al 2007).

6. Experiment

6.1 Materials and methods

Experimental Animals

Seven pigs 4 months old and weighing between 25 and 30 kg (Fig.10) were used. Pigs were used for this experiment because of the similar metabolism to human and the ease of ethical approval which should have been problem if dogs were used for obvious reasons. The pigs were all obtained from the same swineherd and, as far as possible, from the same litters. The pigs were housed at holding pens (Fig. 10) in the School of Veterinary Medicine for a of 2-3 week period of acclimatization prior to the surgery.

On the day of surgery, each pig was pre-anaesthetised with Azaperone (Stresnil,) and Butophanol at the dose rates of 6mg/kg and 0.2mg/kg respectively, induced with 5% Thiopentone at 10 mg/kg, intubated and maintained with isofluorane in oxygen. An Omicron Plus Multiparameter monitor was used to evaluate the vital parameters, including ECG, heart rate, pulse rate, invasive arterial blood pressure, respiratory rate, $Sp0_2$, and end tidal CO_2. The anaesthetized pig was placed in dorsal recumbency and the mandibular area was prepared for surgery by clipping the hair, thorough washing with chlohexidine surgical scrub solution (Hibitane) followed by two alternating applications of povidone iodine and surgical (70%) alcohol.

Fig. 10. Showing a 4 month old pig weighing approximately 30kg being cleaned and prepared for the operating room

Fig. 11. Showing the operating room set-up and operator at work on the appropriately draped animal.

Surgical procedure

The animal was then drapped as shown in Figure 11 prior to making an incision (approximately 6 cm long) along the ventro-lateral aspect of the mandible just cranial to the masseter muscle. The incision was extended to the subcuticular muscles to expose the mandible without damaging the facial artery. A self-retaining retractor was used to allow adequate exposure of the bone. An area measuring approximately 17 mm by 16 mm was marked on each mandible (left and right) using a template, and a block of bone measuring approximately 17 x 16 x 4 mm was removed from each mandible using an Elcomed implant surgical motor and a surgical fissure burr at a speed of 18000rpm (NI). The appropriate graft (with or without the sandwich) were placed in the appropriate mandibular defect. Subcutaneous tissue was then closed with Vicryl (0), and the skin closed with #3 Vetafil.

The Sandwich

The sandwich is prepared as shown in Figure 12 below. Two sheaths of restorable membrane (Bio-Gide) are utilized and tailored with a restorable suture material into a pillowcase before the particulate bone grafting material (Bio-Oss) is placed in it. The tailoring was completed by suturing the fourth side with the same suture material to produce a closed sandwich unit ready to be implanted in the surgical site.

Fig. 12. The creation of the sandwich unit with Bio-Gide, Bio-Oss and resorbable sutures.

Evaluation of bone regeneration and ossification

Bone regeneration and osseointrgration were evaluated by (a) Computer Assisted Tomography scan (CT scan), (b) Single Photon Emission Computerized Tomography (SPECT), and (c) Histological and Histomorphometric techniques.

Single Photon Emission computerized tomography (SPECT):

SPECT studies focused primarily on evaluating the osteoblastic activities, especially the vascularization in and around the site of the graft or region of interest (ROI) At the end of each implantation period, the pig was anaesthetized and given an intravenous injection (into the ear vein) of 740MB_q (20 mci) technetium 99m-methylene diphosphate. The pig was subsequently euthanized two and half hours after the injection and then the mandible was removed. Tomographic images of the mandible in the region of interest (ROI) were acquired within 30 minutes of removal, using a Semens Orbiter II rotating large field-of-view gamma camera equipped with a low energy high resolution Collimater (Siemens Medical System

Inc, Erlangan, Germany). A total of 64 projections images (205/images) were acquired over 180 degrees in a 128 by 128 matrix with a dedicated nuclear medicine computer (Siemens ICON computer).

Fig. 13. The nuclear imaging team at work around the gamma camera and pigs mandible. From the left, Dr John Watkins, Mr Anthony Archibald and Dr Christopher Ogunsalu.

7. Results

7.1 Study I

SPECT Evaluation

The SPECT images demonstrated higher radioactivity on the right mandible compared to the left mandible, this indicates higher take-up of the radioactive material, which translate to higher osteoblastic activity. As such, on can conclude that the side with the autogenous graft (right side) has higher osteoblastic activity as shown in Figure 14. This result is presented graphically in Figure 15 which shows higher peak on the autogenous bone sandwich side compared with the xenograft sandwich side (left side). The average counts were 99.7 pixel and 78.1 pixels respectively (table 2) and a calculated relative activity ratio of 1:20.

SITE R L	COMPONENT OF SANDWICH UNIT	SIZE PIXEL	AVG COUNT	SUM
1. Right mandible	a. Membrane – Bio-Gide ® b. Bone substitute – autograft	112	99.7	11167
2. Left Mandible	a. Membrane – Bio-Gide ® b. Bone substitute – xenograft (Bio-OSS®)	129	73.1	9425

Table 2. Showing the comparasim of the osteoblastic activity between the xenograft and autograft sandwich

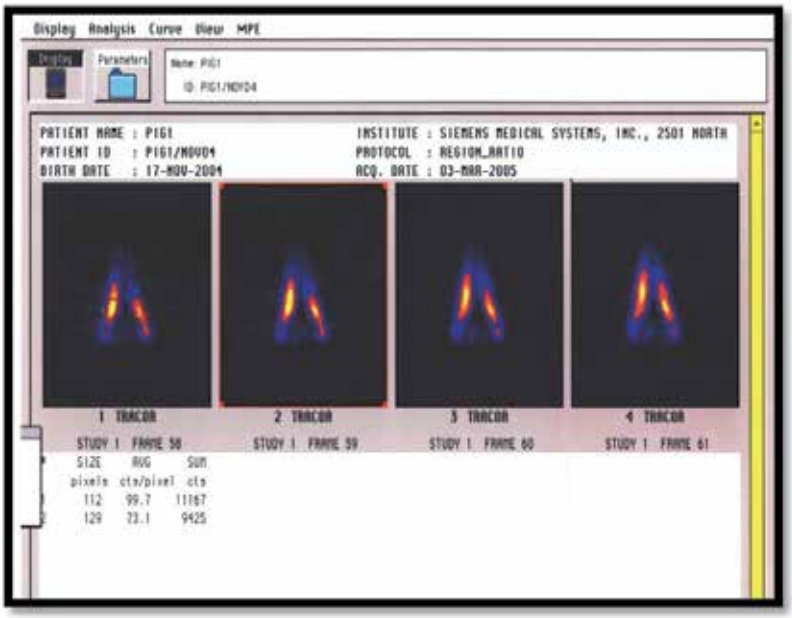
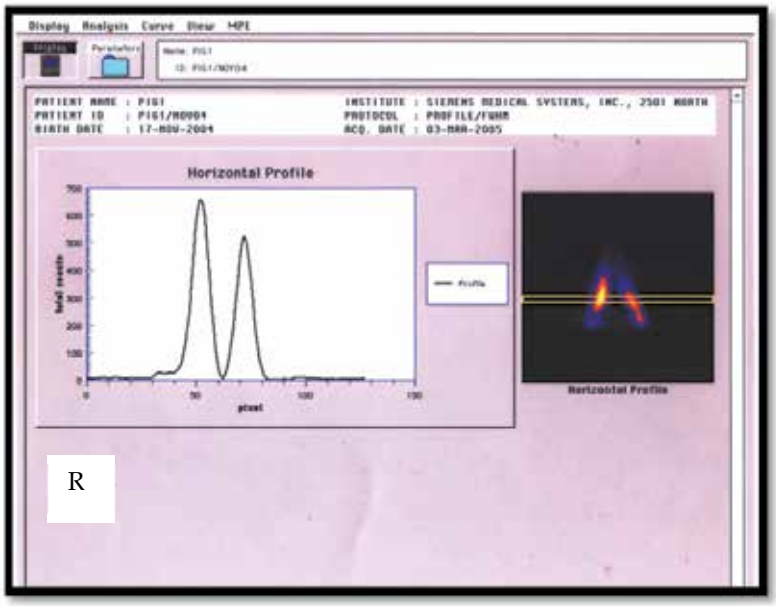


Fig. 14. Illistration of osteoblastic activity for Pig 1 at 14 weeks.

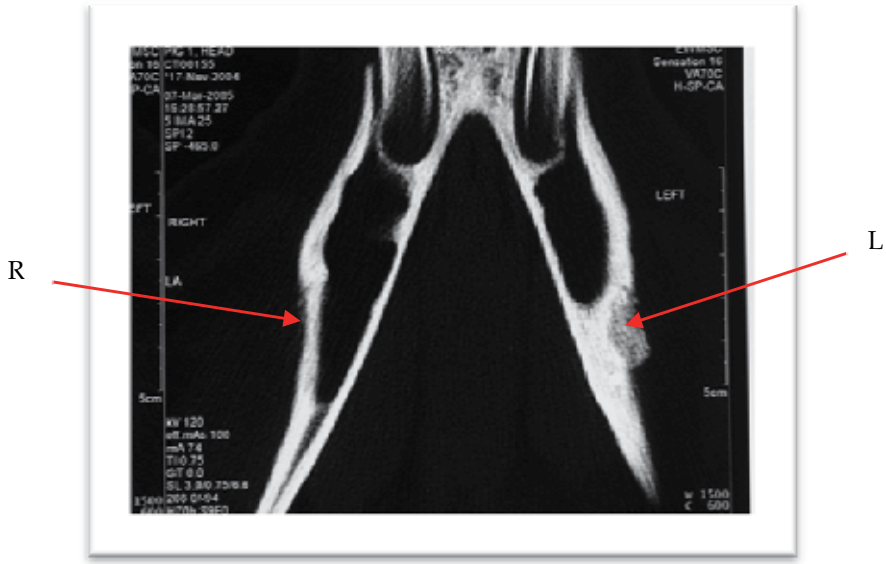


R = Right side

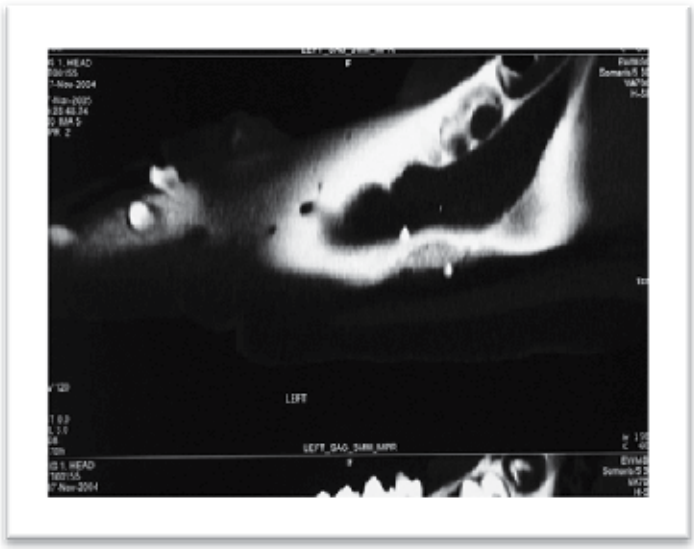
Fig. 15. Showing graphically the profile of osteoblastic activity of the autogeneous sandwich side (right side) versus the Bio-Oss sandwich side (left side), together with the activity ratio. The autogenous sandwich side obviously has more activity than the Bio-Oss sandwich side (note that the actual left side of the pigs jaw is represented by the right side of the profile).

CT–Scan evaluation

The CT-scan evaluation also depicted the morphological state of the bone regeneration site with the side which utilized the autogenous bone with the sandwich side having much more bone formation with obliteration of the marrow space (Figure 16 a and b)



A



B

Fig. 16. (a- top and b- bottom): CT Scan showing the transverse saggital slices in the area of bone regeneration. Note the regular recortication on the side with autogenous sandwich (right side), compared with the side with Bio-Oss sandwich (left side), which shows less regular recortication with obliteration of the adjacent marrow space (see arrow).

8. The proper use of bone replacements

As mentioned earlier on in the chapter, bone replacement can be either autogenous in origin or non-autogenous and as such called second-hand bones or bone substitutes.
The proper use of the autogenous bone begins with its harvesting either as particulate, block or core graft to be placed in the site that requires bone to be regenerated. Recently the disposable bone scrappers became available in the market for the harvesting of particulate autogenous bone (Fig. 17). The attached CD will assist the clinician who is not knowledgeable with the use of the disposable bone scrapper to practice such utilizing a cadaver.

Fig. 17. Disposable bone scrapper for harvesting of particulate bone

Because of the technique sensitivity ascribed to the use of particulate bone substitute and the fact that it is the most common forms of bone substitute used in implant dentistry today, I will in stages describe the use of Bio-Oss (Osteohealth, Shirley, NY) particulate bone substitute in a furcation involvement in the mandibular molar region.
Bio-Oss (Fig. 18 and Fig. 19) is a xenogenic second hand bone obtained from bovine source and distributed in sterile packs and more important is the fact that it is sold only to practioners with current annual practicing license.

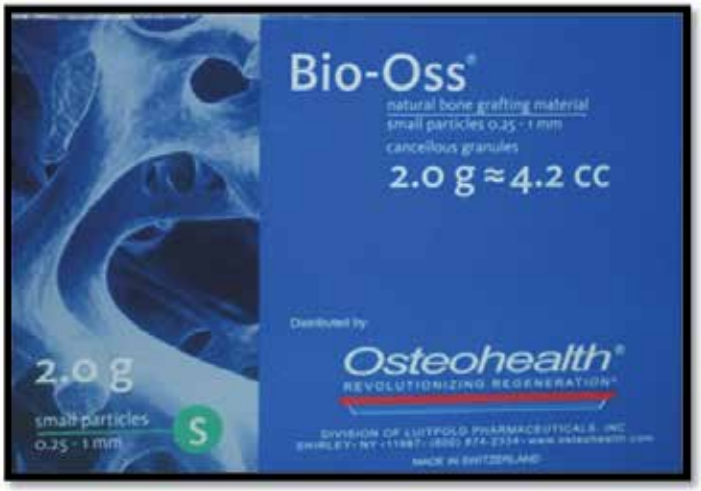


Fig. 18. Packing for Bio-Oss from Osteohealth

Fig. 19. Bottle of Bio-Oss particulate bone substitute(Xenograph)

In implant dentistry or in periodontal bone reconstruction or regeneration, it is best used with the resorbable membranes (Fig. 20 and 21)consistent with the guided tissue regeneration (GTR) technique.

Fig. 20. Bio-Gide resorbable membrane(smooth side)

Fig. 21. Bio-Gide resorbable membrane(rough side)

Once the surgical site has been exposed and all granulation tissue has been removed from the periodontal pathologic pockets and furcation area as shown in Figure 22 and 23.

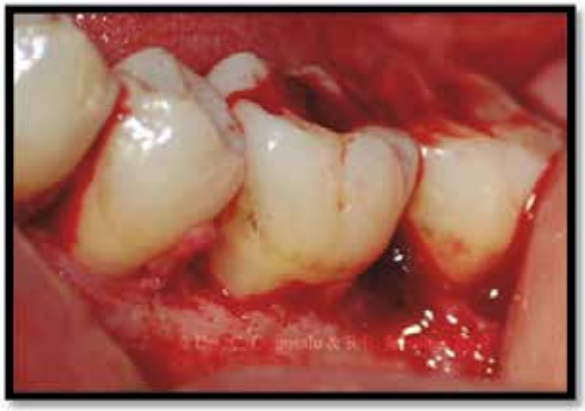


Fig. 22. Surgical site with some granulation tissue mesial to first molar tooth

Fig. 23. Surgical site with all granulation tissue removed

The particulate bone which has been soaked with the patient's blood or sterile water is now condensed into the defect utilizing an amalgam plugger dedicated only for use in bone grafting (Fig. 24).

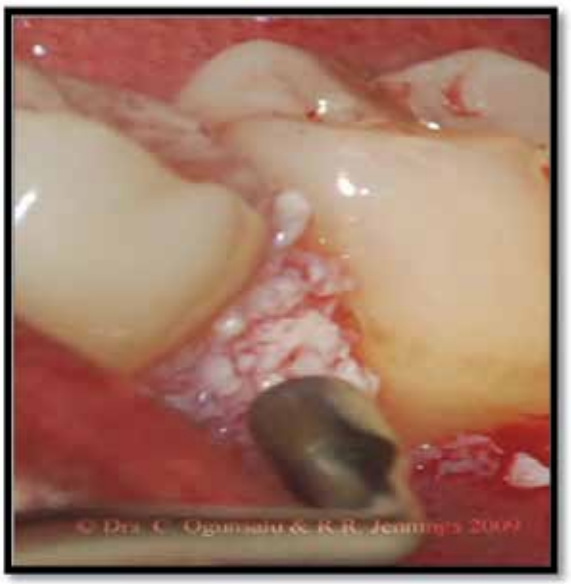
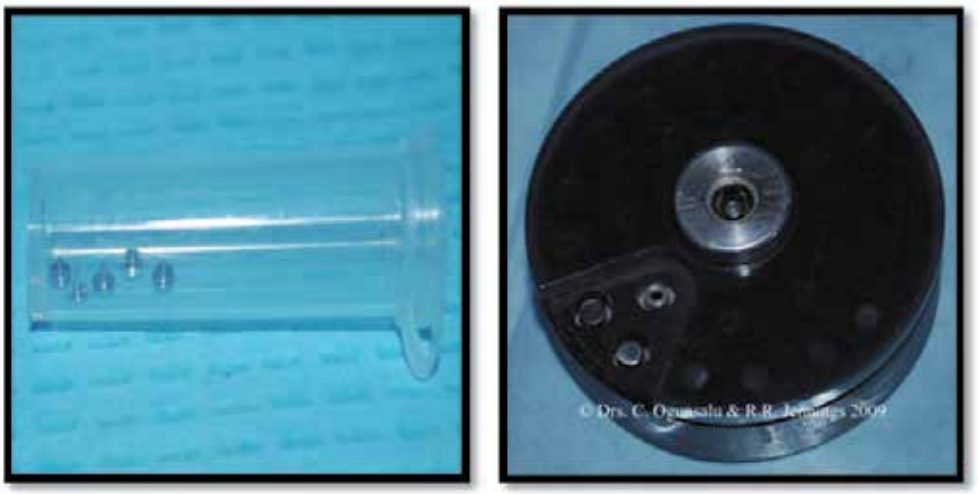


Fig. 24. Condensation of bone substitute with amalgam plugger

Once the particulate bone has been packed into the bony defect and appropriately condensed, the autotac pins and kit (Fig. 25) is now used to secure the GTR membrane which has been used to cover the grafted site (Fig. 26).



A B

C

Fig. 25. Autotac kit with extra pins in sterile bottle

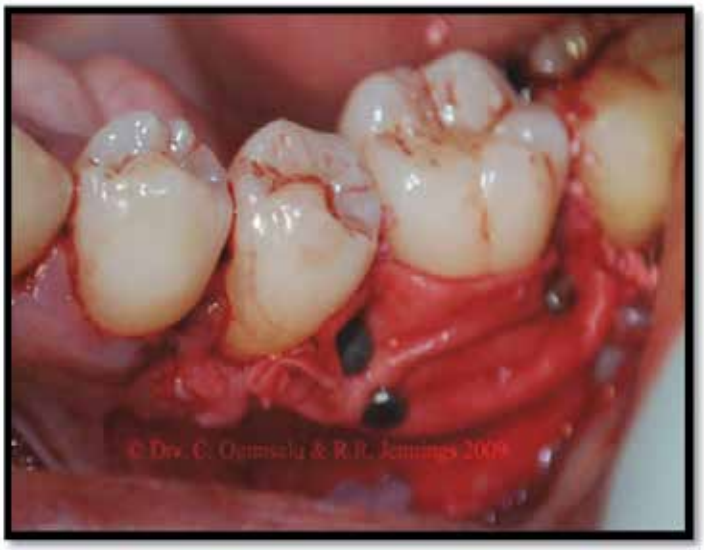


Fig. 26. Grafted site covered with resorbable membrane which is secured with stainless steel pins

The surgical procedure is then completed by suturing preferably with a non-resorbable suture material in such a manner that it is tension free (Fig. 27)

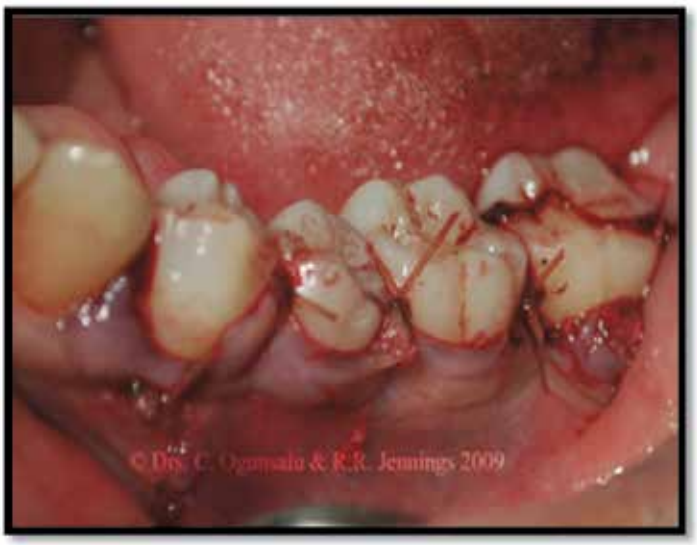


Fig. 27. Tension free suturing with resorbable suture

Most manufacturers of bone regenerative materials proudly explain the stage by stage use of their products in the appropriate section of their website. These information for use should be considered as very reliable and the clinician intending to use these products must adhere strictly to the suggested surgical sequence. For example Straumann, the manufacturers of a bone substitute, Emdogain proudly describe the use of this product which come in gel form variably by audiovisual on its website, *www.straumann.us.index/.../products-emdogain.htm*.

Additionally they also mention the functional advantage, cellular process involved in bone regeneration when Emdogain is used . The various scientific research to back-up this product is also mentioned.

9. Osteobstruction and bone substitutes

The osteobstruction mechanism in bone regeneration was coincidentally discovered during a sequential SPECT, histological and histomorphometric analysis on animal model in the validation of the Ogunsalu Sandwich Bone Regeneration Technique (Ogunsalu 2009).
This osteobstructive mechanism was demonstrated by episodes of overtaking and re-overtaking on SPECT following evaluation of osteoblastic activities in a sequential animal experiment to validate both the Ogunsalu Sandwich Bone Regeneration Technique (a double guided tissue technique; D-GTR) and the interceed membrane technique (a single guided tissue regeneration technique-;S-GTR) utilizing SPECT, histological and histomorphometric evaluation (Ogunsalu et al. 2008).
This new phenomenon of overtaking and re-overtaking and the newly discovered osteobstructive phenomenon, in bone regeneration are integral finding of my experiment, of which much discussion will follow in the next paragraph which will focus primarily on discussing the findings of sequential histological and histomorphometric findings, against the background of the SPECT findings at 8, 14,11,17,13 and 24 weeks. The implications of this sequential finding will also be discussed.
During the 8th week the total bone area was slightly more in the interceed side than the sandwich side and both side had vital bone with no non-vital bone. Also the marrow and fibrous tissue was more on the interceed side. This is in keeping with the superior osteoblastic activity on the interceed side on SPECT when compared with the sandwich side (Fig. 28)

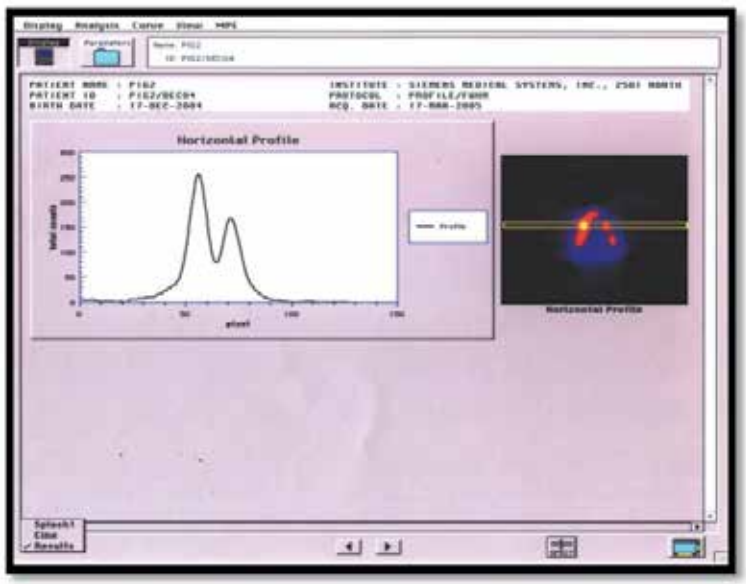


Fig. 28. Showing graphically the profile of osteoblastic activity of the sandwich side (SS) versus the interceed side (IS), together with the activity ratio. The IS obviously has more activity than the SS at 8 weeks. Note that the actual left side of the pig is represented by the right side of the profile.

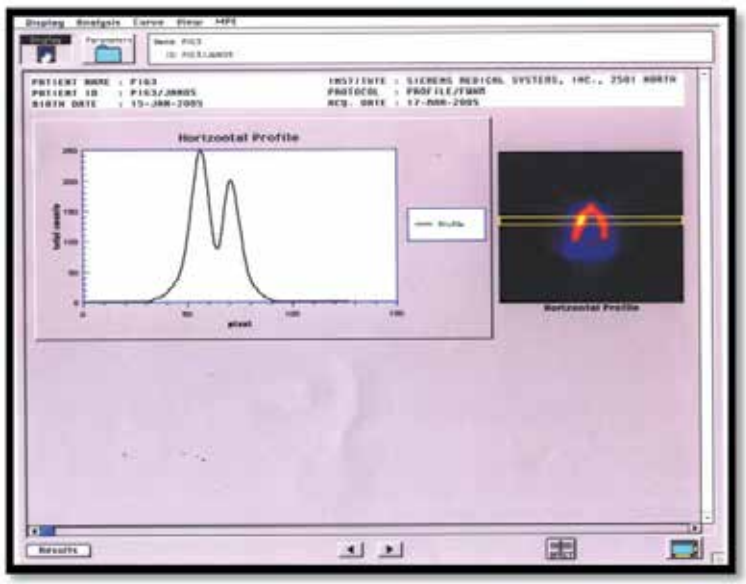


Fig. 29. Showing graphically the profile of osteoblastic activity of the sandwich side (SS) versus the interceed (IS) together with the activity ratio at 11 weeks. The interceed side still leads the SS at Fig 29: 11 weeks. Note that the actual left side of the pigs jaw is represented by the right side of the profile. The osteoblastic activity is still superior on the interceed side.

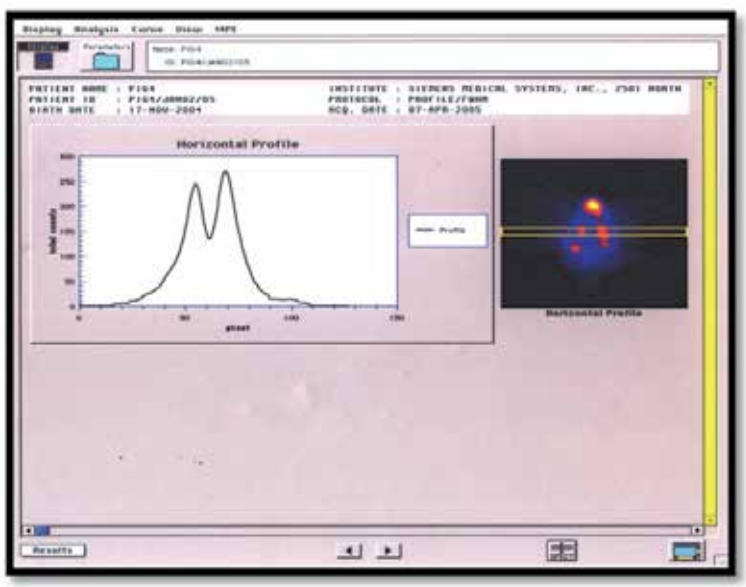


Fig. 30. Showing graphically the profile of osteoblastic activity of the sandwich side (SS) versus the interceed side (IS) together with the activity ratio at 13 weeks. The osteoblastic activity in the sandwich side has now overtaken the interceed side (slightly) at 13 weeks. Note that the actual left side of the pigs jaw is represented by the right side of the profile.

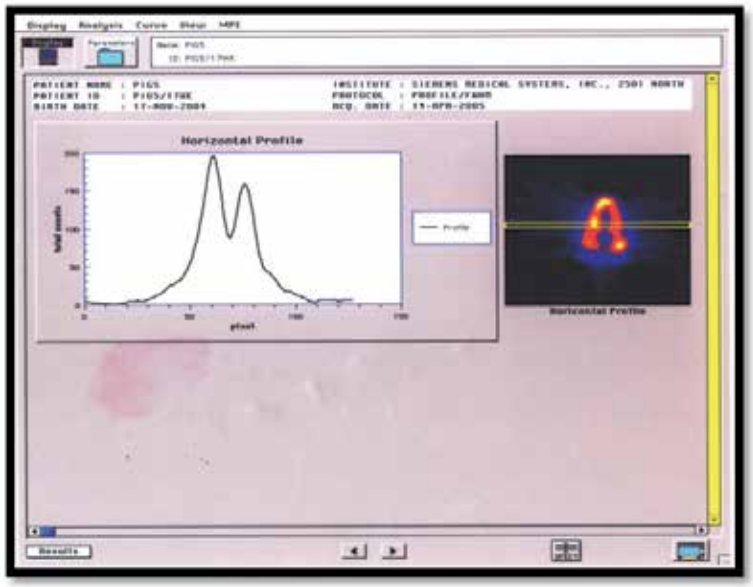


Fig. 31. Showing graphically the profile of osteoblastic activity of Sandwich side (SS) versus interceed side (IS) together with the activity ratio. The IS still leads the SS in terms of osteoblastic activity at 17 weeks as a result of an overtake. Note that the actual left side of the pigs jaw is represented by the right side of the profile

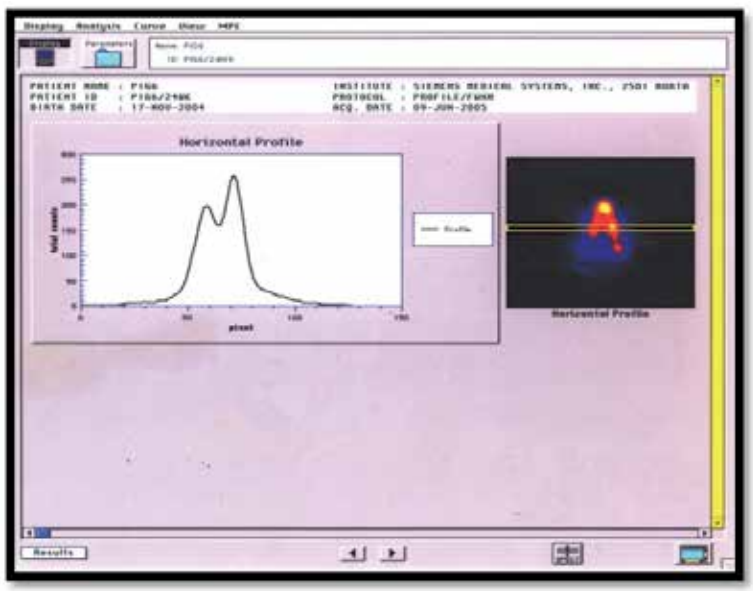
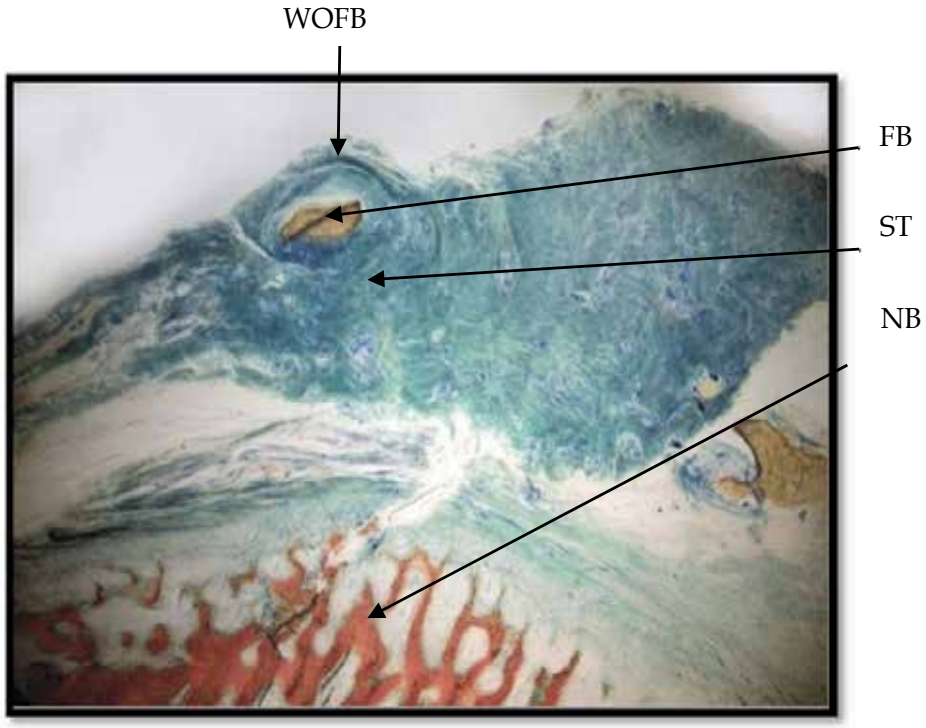


Fig. 32. Showing graphically the profile of osteoblastic activity of the sandwich side (SS) versus interceed side (IS), together with the activity ratio at 24 weeks. The sandwich side has finally exceeded the interceed side at 24 weeks.

The superiority of the interceed side was short lived at week 13 due to an overtake by the sandwich side (Fig. 29 to Fig. 32). This overtake as we found was due to the presence of foreign body reaction on the interceed side at week 11 as shown in Figure 33. The foreign body reaction is what can cause osteobstruction during bone regeneration. We as such include this as part of the mechanism of bone regeneration depite it being a negative mechanism when compared with osteogenesis, osteoinduction and osteoconduction which are all positive mechanisms.



NB = New Bone, ST = Soft Tissue, FB = Foreign Body, WOFB = Walling off of foreign body

Fig. 33. Medium power photomicrograph showing Bio-Oss in soft tissue representative if foreign body reaction. (Slide 22-06-49M; Stevenel's blue and van Gieson's picro fuchsin)

10. Conclusion

Various bone grafting substitutes continue to emerge into the market to assist with bone regeneration prior to, during and after implant therapy. These bone grafting substitutes preferably called second hand bones should be classified as shown in Figure 3. They can only be validated quantitatively and qualitatively by the monitoring of the triggered osteoblastic activity over a time period utilizing single photon emission computerized tomography(SPECT) as demonstrated in the work of Ogunsalu and co-workers.It is this validation that will inform the clinician which bone substitute performs better in terms of bone regeneration and osteoblastic activity/ index. Further more, the osteo-obstructive phenomenon in bone regeneration discovered by the above mentioned workers is new and warrants more investigation.

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7

Immediate Dental Implants and Bone Graft

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1. Introduction

Dental implants were initiated in 1922 by Branemark, who and associates described the relationship between titanium implant and bone which termed osteointegration, defined as the direct structural and functional connection between living bone and the surface of an implant (Albrektsson et al., 1981). Immediate implant placement, defined as the placement of dental implant immediately into fresh extraction socket site after tooth extraction , has been considered a predictable and acceptable procedure (Schwartz et al., 2000). In addition, with immediate implant placement there is minimal use of surgical drills because the socket is already found except for slight increase of the socket length in an attempt to improve primary stability (Barzilay et al., 1991). The decreased surgical trauma of immediate placement type will decrease the risk of bone necrosis and permit bone remodeling process to occur, i.e. the healing period is rapid and allows the woven bone to be transformed into lamellar bone (Hansson et al., 1983, Ericsson, 2000). Moreover, the natural socket is rich in periodontal cells and matrix, which makes the healing faster and more predictable.

Based on the review article by Penarrocha et al., 2004 and the time elapsed between tooth extraction and implantation, the following classification has been established relating the receptor zone to the required therapeutic approach. (a) Immediate implantation, when the remnant bone sufficient to ensure primary stability of the implant, which is inserted in the course of surgical extraction of the tooth to be, replaced (primary immediate implants). (b) Recent implantation, when approximately 6-8 weeks have elapsed from extraction to implantation – a time during which the soft tissues heal, allowing adequate mucogingival covering of the alveolus (secondary immediate implants). (c) Delayed implantation, when the receptor zone is not optimum for either immediate or recent implantation. Bone promotion first carried out with bone grafts and/or barrier membranes, followed approximately six months later by implant positioning (delayed implants). (d) Mature implantation, when over nine months have elapsed from extraction to implantation. Mature bone is found in such situations.

Block and Kent, 1991 described indications and contraindications for immediate dental implants placement into the extraction sites. Summarized in the following indications; 1) traumatic loss of teeth with a small amount of bone loss;2) tooth lost because of gross decay without purulent exudates or cellulites; 3) inability to complete endodontic therapy;4) presence of severe periodontal bone loss without purulent exudates; 5) adequate soft tissue health to obtain primary wound closure. Contraindications, include; 1) presence of purulent exudates at the time of extraction; 2) adjacent soft tissue cellulites and granulation tissue;3) lack of an adequate bone apical to the socket; 4) adverse location of the mandibular neurovascular bundle, maxillary sinus and nasal cavity; 5) poor anatomical configuration of remaining bone.
In order to achieve good osseiointegrated dental implant with a high degree of predictability, the implant must be1) sterile; 2) made of a highly bio-compatible material, e.g. titanium; 3) inserted with/an a traumatic surgical procedure that avoids overheating; 4) placed with initial stability; and 5) not functionally loaded during healing period of 4-6 months (Branemark et al., 1989).
It is important to note that, after tooth extraction there are four stages of healing. In the initial angiogenic stage, blood clotting occurs with capillary formation within the first five days after tooth extraction. In the second stage (new-bone formation stage) the entire socket transforming into granulation tissues. In the third stage (bone-growth stage) immature woven bone forms within four to five weeks. In the last stage (bone-reorganization stage), which occurs after six weeks and continues for six months. In addition, bone can take up to 52 weeks to fully mature (Block et al., 2002). Moreover**,** the periosteum has an important role to alveolar bone formation. Cells from the inner layer of the periosteum are responsible for bone remodeling, and the cortical bone receives 80% of its arterial blood supply from the periosteum. Therefore, careful a traumatic tooth extraction and maintenance of the periosteum helps in preservation of alveolar bone (Misch, 1999).
Regarding bone remodeling after tooth extraction, in the anterior maxilla the facial bone will resorb up to 25% within the first year after tooth extraction and up to 40% to 60% over the next three years. In the posterior region, the rate of bone loss is greater reaching up to 50% within the first year after extraction, especially if a portion of the buccal plate is lost during extraction resulting in limited bucco-lingual dimension, which reducing the available bone for placement of implants (Misch and Judy, 2000).
Several clinical as well as animal studies (Babbush et al., 1986, Whhrle et al., 1992, Sagaral et al., 1993, Piattelli et al., 1997, Levine et al., 1998 and Piattelli et al., 1998) demonstrated that when the implants are loaded immediately after placement, the osseintegration could occur under specific circumstances. The clinical success is dependent on a number of factors summarized in the following: 1) bone quality in the insertion site; 2) surface pattern of the implant material; 3) design of the implant; 4) amount of loading, e.g. the occlusal condition; and 5) immobilization of the implants immediately after surgery.

2. Bone-grafting materials and immediate dental implant

Following an extraction, there is a 25% decrease in the width of the alveolar bone during the first year, and an average 4mm decrease in height during the first year following multiple extractions (Carlson & Persson,1967). Misch (1999) have observed a 40%-60% decrease in alveolar bone width after the first two to three years post extraction, and Christensen (1996) reports an annual resorption rate of at least 0.5% to 1% during the remainder for the rest of a Patient's life. Preservation of bone contour for dental implants, pontic design, denture

stability, soft tissue aesthetics, and maintaining the periodontal status of adjacent teeth are important considerations following an extraction.
The natural pattern of resorption of the alveolar ridge after tooth extraction would result in a different ridge that could be problematic for future of implant placement. To achieve both functional and aesthetic requirements, it might be necessary to plan implant placement after tooth extraction and socket grafting/ridge preservation. Bone graft materials have played on important role in periodontal regeneration for many years (Hoexter, 2002). To preserve alveolar bone width and height for implant placement or for prosthetic concerns, allografts, xenografts and alloplasts have been used to graft extraction sites.
Additionally,in an effort to preserve and create sufficient bone for implant placement after tooth extraction, Michael et al., 2002 used human mineralized cancellous bone as a graft material. Their results indicate that restoration of extraction sites using human mineralized bone has potential. It can preserve or recreate an extraction site's bone bulk in preparation for implant placement.

2.1 Autografts

The gold standard of bone grafting materials is autografts. Autografts are obtained from the same patient, taken from one site and placed in another site and forms bone by the process of osteogenesis and osteoinduction. Osteogenesis is defined as bone growth from viable bone cells known as osteoblasts, osteoinduction is the process that involves materials that are capable of inducing cells to differentiate into osteoblasts. Autograft materials are obtained intraorally form edentulous areas, tuberosity mandibular symphysis and mandibualr ramus. Extra oral autografts are obtained from iliac crest, rib, tibia and calvarium. The advantages of autograft bone material is that it maintains bone structures such as minerals, collagen and viable osteoblasts and bone morphogenic proteins (BMPs), while the main disadvantage is the morbidity of a second surgical site (Hoextor, 2002). Authors such as Brugnami et al., 1996 and Dealemans et al., 1997 recommended the use of autografts instead of allografts, due to the absence of immune reactions associated with the former. In this sense, the general impression appears to be that autologous grafts are the best choice for osseoinductive purposes. Recently, Hassan et al., 2008 demonstrated a comparative evaluation of immediate dental implant with autogenous versus synthetic guided bone regeneration. The major aim of this clinical and radiographic study was to compare autogenous and synthetic bone grafting with dental implant placement. The results showed that the autogenous bone graft appeared to be superior and the graft of choice because it maintained bone structure and has activated the osteogenesis process (Fig.1, 2&Table1).
One region that has not been previously described as a potential donor site is the mandibular torus, defined as hyperostosis or enlargement of the lingual aspect of the mandible. The mandibular tori generally occur bilaterally in the premolar region, but the hyperostoses may extend distally to the third molar and mesially to the lateral incisor. The lingual tori are unnecessary bony extensions, which may limit tongue space and create phonetic difficulties. When considering any type of prosthetic reconstruction, the tori can become an obstacle. In the study of Scott D and Ganz DMD,1997 utilizing mandibular tori as a source for onlay bone augmentation with immediate implant placement. The author postulates that an ideal site to harvest bone for augmentation procedures would be a local area of excess bone (exostosis) that offers no structural or aesthetic benefits to the patient. In addition, When local bone augmentation is required, and the mandibular tori are present, they can be considered as excellent potential donor sites. However, these hyperostoses will

be exhibited by only a minute segment of the patient population. When the donor site and recipient site are located within the same half of the mandibular arch, the surgical procedure is reduced to one mutually inclusive incision, which gains access to the donor bone on the lingual aspect and the recipient site on the buccal aspect. The removal of the tori can be accomplished with minimal trauma and low postoperative morbidity. Therefore, the cortical and cancellous nature of the bone, with a thickened outer cortical plate of haversian bone, makes it an excellent choice as a donor site for onlay grafting procedures.

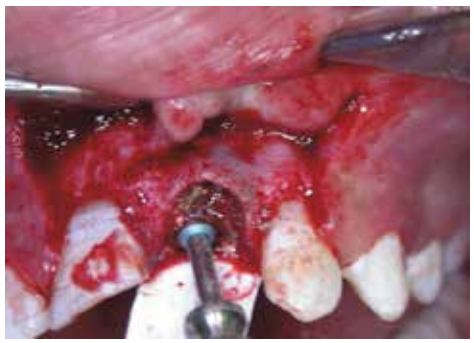

Fig. 1. Immediate dental implant placement into fresh socket augmented with autogenous bone graft.

Fig. 2. Autogenous bone graft collected from the same surgical site by safe scraper.

	Bone density			Marginal bone loss		
	GI	GII	P-value	GI	GII	P-value
3M	102.49 ±10.20	99.89 ±14.32	0.470	3.56 ±0.21	3.87 ±0.43	0.067
6M	110.06 ±7.67	102.54 ±13.44	0.185	3.20 ±0.23	3.70 ±0.30	0.001 **
9M	120.93 ±8.66	105.11 ±11.81	0.013	2.73 ±0.21	3.41 ±032	0.0001 **
12M	134.56 ±11.29	109.66 ±12.13	0.0009 **	2.50 ±0.21	3.27 ±0.24	0.000 **

Table 1. Bone density (in pixels) and marginal bone loss (in mm) in the Autogenous grafted group (I) and the synthetic grafted group (II) during different observation periods.

2.2 Allografts

Allograft bone is obtained from individuals of the same species, derived from human-cadaver bone that has been selected and tested to be free of HIV and transmitted diseases. The most common allograft used is dematerialized freeze-dried bone allograft (DFDBA), provide type I collagen, which comprises most of the organic component of bone (Scahalhorn, 1972). In addition, allograft contains BMPs, which stimulate osteoinduction. There are thirteen proteins have been identified (BMP1-BMP13) which are osteoinductive compounds and stimulate new bone formation (Hoexter, 2002). Fugazzatto (2004) demonstrated that, a combination of osseous coagulum collected during preparation and freeze-dried bone allograft placed at immediate implant insertion and loading. After six months from surgery, there was a clinically immobile implant and healthy surrounding soft tissues; no postoperative gingival recession. Moreover; there was no probing depth exceed than 3mm an any aspect of the implant; no bleeding on probing; no sensitivity to pressure; and does not exculpate around the implant.

2.3 Xenografts

Xenografts are graft materials derived from the inorganic portion of animal bones; the most common source is bovine the removal of the organic component are processed to remove their antigenicity, while the remaining inorganic components provide a natural matrix as well as an excellent source of calcium. The disadvantage of xenografts is that they are only osteoconductive and the resorption rate of bovine cortical bone is slow. In addition, patients may have anxiety to mad cow disease or bovine spongiform encephalitis (Berlungh and Lindhe, 1997). Cornelini et al.,evaluated the use of a porous bone mineral matrix xenograft (Bio-Oss) as an adjunct to a biodegradable barrier membrane (Bio-Gide) to support healing following the immediate placement of transmucosal implants into extraction sockets. Their results revealed that, the radiographic bone level remained unchanged compared to baseline in the test and control groups. No differences were observed between test and control groups in terms of mean probing attachment level. At proximal sites, the soft tissue margin was located 2.6 mm more coronal than the shoulder of the implant in the test group, compared to 1.3 mm in the control group. They concluded that the use of deproteinized bovine bone mineral as a membrane support at immediately placed transmucosal implants may offer an advantage in areas with high esthetic demands in terms of soft tissue support.

2.4 Alloplasts

Alloplastic bone grafts are synthetic materials that have developed to replace human bone to avoid transmitted diseases such as HIV, bovine spongiform encephalitis (BSE), or hepatitis. They are biocompatible and osteoconductive materials. The most common types of alloplasts used are calcium phosphates, bioactive glasses and biocompatible composite polymers. Moreover, the main disadvantage of alloplasts is that they are unpredictable in allowing bone formation; therefore, particles can be uncounted within the grafted site (Knapp et al., 2003). Furthermore, the natural biocorals are calcium carbonate materials, with similar to the natural bone hydroxyapatite structure.

Advanced synthetic bioactive resorbable bone graft materials having similar chemical and mechanical properties as the host bone, can provide the means to modify existing bone topography. In the study performed by Scott and Maurice, 2002 using a synthetic bioactive restorable bone graft of low-temperature HA material mixed with autogenous bone graft for implant reconstruction. The implants were allowed to osseointegrate for four months, at this

time the site was re-entered and examined. The results were showed that, the underlying implants were found to be covered with a thick layer of mature bone. In addition, the histological section revealed that mature bone was seen surrounding the remaining crystals of bioactive restorable particulates.
Paulino Castellon et al., 2004 demonstrated that immediate implant placement in sockets augmented with HTR synthetic bone. The restorative phase was initiated after six months from implant placement. The results of this study recorded that, the gingival contour was excellent in 61% and good in 35% of cares. They concluded that, immediate implant placement in combination with HTR synthetic bone graft is a predictable procedure and provides a good bone for successful prosthetic reconstruction.
In a study performed by Stanley et al., 1997 using bioglass cones as endosseous ridge maintenance implants to prevent ridge resorption. A bioactive glass can be regarded as a three-dimensional silica network, which is modified by incorporating oxides such as sodium oxide, calcium oxide, phosphorus pentoxide, aluminum oxides and barium oxide or halides such as calcium fluoride. The results of this study showed that a loss of 14.3% of the implants was seen and 7.7% of the implants requiring re-contouring (dehiscence) when the cones are placed into fresh extraction sockets during a 5-years, suggest that favorable clinical results can be obtained with this materials.

3. Combined bone-grafts and immediate dental implant

Bone defects can originate from infections, periodontal diseases or during the placement of implants, which can provoke a vestibular or lingual dehiscence due to the reduced buccal lingual dimension of the crest associated with the presence of fistula, root fractures, endodontic complications or from atrophies that developed after previous extractions.
A variety of regenerative techniques using combinations of bone grafts and barrier membranes have been suggested promoting bone regeneration in localized defects at implants placed into extraction sockets (Schwartz and Chaushu, 1997, Mayfield, 1999).
It is important to note that autogenous bone graft appeared to be the graft of choice in treatment of intrabony defects and defects around dental implants. The ability of bone replacement graft to bind with bone and enhance both osteoinduction and osteoconduction are important. In the study of Hassan, 2009 used a combined technique of using autogenous bone graft and polyglaycolic polylactic acid polymer in an attempt to obtain osteoinductive as well as the osteoconductive actions. The grafting materials used in this study included both autogenous bone graft and synthetic graft. They are safe materials because there is no disease transmission. The synthetic co-polymer used in these cases proved to be extremely easy to handle because of the various forms available (sponge, powder, and gel) which easily adapt to the various cases requiring use of a filler with subsequent formation of new bone. In addition, due to its synthetic origin, there is a complete absence of biologic risk. The histological findings showed that the co-polymer remained in the site of the graft until completion of the natural healing processes. At the same time, it is penetrated and gradually and totally replaced by trabecular bone.
In this study, the Safe-Scraper was used for autogenous bone collection from the intact area with good crestal bone in the same surgical field to avoid patient morbidity. Moreover, this work attempted also to avoid the common donor sites that are usually submitted to autogenous bone grafting, such as maxillary tuberosity and mental and mandibular retromolar areas, to preserve the tissues and to avoid morbidity of the donor area.

In this study using, a full-thickness mucoperiosteal flap with vertical releasing incisions where necessary. Teeth carefully luxated and removed with forceps. Extraction sockets were debrided with hand instruments to remove granulation tissue and prepared for implantation. Various sizes of titanium plasma spray implants (diameters 3.25 and 3.75 mm, lengths 14-16 mm) then placed according to standard protocol. Implants were then placed with the collar of the implant at the level of the bone crest on the labial aspect. Where the labial plate was damaged or absent, the level of the labial crest before loss was estimated in the following way. A periodontal probe was placed horizontally at the bone crest of intact portions of the labial plate on either side of the dehiscence defect. Then, the implant was placed with the collar positioned at this level. All implants were placed with primary stability and were completely housed within the extraction socket. After implant placement, the following measurements (bone sounding) were taken using the same William's color-coded periodontal probe (Hu-Friedy, USA). 1) Vertical height of the defect, measured from the most apical extent of the defect to the coronal aspect of the implant collar. 2) Horizontal depth of the defect, estimated by measuring the horizontal distance from the implant collar to a periodontal probe placed against the intact portions of the labial plate at the level of the implant collar. After local anesthesia, these measurements were taken again after six and nine months. Group I patients received autogenous bone grafts collected during preparation using a Safe-Scraper (Micross, Reggio Emilia, Italy) for cortical bone collectionand mixed with a synthetic bioabsorbable polylactic polyglycolic acid polymer (Fisiograft). Group II patients received autogenous bone grafts alone.

Furthermore, the results of this study showed a significant gain in clinical attachment levels, as well as reduction in PPD after the composite graft. There was a statistical significant difference between both groups regarding horizontal bone sounding suggesting that the use of Fisiograft combined with autogenous bone graft in this study was found to give optimum regenerative results (Fig.3, 4&Table.2).

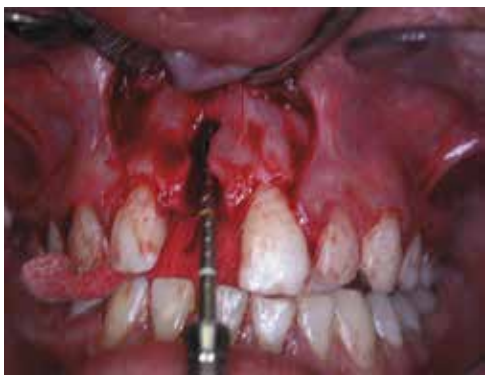

Fig. 3. Labial dehiscence at maxillary central incisor.

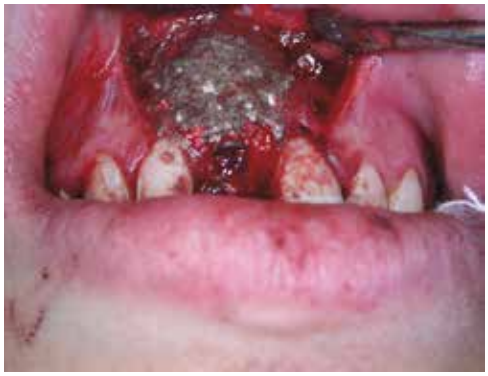

Fig. 4. Immediate dental implant placement into fresh socket and augmented with combined bone graft.

	GI n=10		GII n=10	
	VDH	HDD	VDH	HDD
Baseline	11.0±0.05	3.1±0.2	11.1±0.04	2.7±0.2
6m	5.3±0.04	1.9±0.3	5.6±0.04	1.7±0.2
9m	5.2±0.04	1.8±0.3	5.4±0.04	1.7±0.2
p-value	0.001	0.01	0.001	0.01

VDH= Vertical height of the defect HDD= Horizontal depth of the defect

Table 2. Vertical and horizontal bone sounding (Probing bone level measurements) at the base line and different postoperative periods in the tested groups.

4. Conclusion

1. To achieve a good osseointegrated implant with a high degree of predictability, the immediate implant might be placed with bone graft and without immediate loading. For aesthetic needs, can be used provisional restoration and free from occlusion.
2. The immediate dental implant placement with autogenous bone graft was significantly superior to synthetic bone graft. In addition, the immediate placement– delayed loaded dental implant remains the procedure of choice for predictably achieving osseointegration.
3. The combination of autogenous bone and synthetic grafts showed a slight superiority to autogenous bone graft alone, suggesting that it could be an optimum bone substitute for treatment of dehiscence around immediate dental implant.

5. Acknowledgment

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Part 2

Bone-Implant Interface: Biomechanics and Surrounding Tissues

8

Biomechanics of Cantilevered Implant-Supported Prosthesis (Biomechanics in Implant Prosthodontics)

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1. Introduction

The peculiar characteristic of an implant-supported prosthesis is the fact that its fixation is given by the connection of an alloplastic material (the implant) to a living tissue (the bone). This fixation has been defined as rigid and clinically asymptomatic and must be maintained during functional loading (Albrektsson & Zarb, 1993). Under load, bone tissue will undergo a remodeling process, which ultimately influences the long-term function of a dental implant system (Meijer et al., 1993). Bone remodelling is a complex process that involves a sequence of chemical- and mechanical-mediated biologic events known as mechanotransduction (Duncan & Turner, 1995).

Because the occlusal load will be transferred to the implants and subsequently to the bone, it is believed that the biomechanics of the implant-supported prosthesis play an important role in the longevity of the bone around dental implants (Skalak, 1985). It is commonly found in the literature that, for cantilevered implant supported mandibular prosthesis, stresses tend to be concentrated at the cortical bone on the disto-lingual aspect of the implant closest to the load (Meijer et al., 1993; Murphy et al., 1995; Rubo & Souza, 2008; Sertgoz & Guvener, 1996; Weinberg & Kruger, 1995). Many researchers have focused on the steps of force transfer to gain insight into the biomechanical effect of force directions, force magnitudes, prosthesis type, prosthesis material, implant design, number and distribution of supporting implants, bone density, and the mechanical properties of the bone-implant interface (Sahin & Cehreli, 2002). The resultant stresses should be kept below the failure stress limit of the materials involved (Frost, 1994; Skalak, 1985; Wiskott & Belser, 1999).

Failures in dental implant prostheses have been correlated with biomechanical complications. Overloading factors may negatively influence implant longevity (Kim et al., 2005). According to Isidor (2006), although it has been stated that occlusal forces may be associated with the loss of oral implants, a causative relationship has never been convincingly demonstrated. The related mechanisms of these displacements are still not completely understood and studies about the influences of the several biomechanical factors are inconclusive (Rangert et al., 1989). Implant failure caused by overload has been classified as a dogma and as such should be abandoned (Carlsson, 2009). There are contradictory studies regarding bone loss in overload conditions. From those studies it can be inferred that

bone loss around implants could happen with large occlusal interferences or in the coexistence of small interferences associated with inflammation (Oh et al., 2002).
According to Brunski, (1991), three tasks need to be accomplished in implant biomechanical studies: 1) identify and quantify the biomechanical variables that can affect bone biology; 2) measure the effects of these variables quantitatively and with precision; and 3) to distinguish the effects resultant of one variable from the effects resultant of other variable. Failure prevention demands testing and stress analysis of the implants and tissues in vitro as well in vivo.
The first attempt to solve the biomechanical problem was presented by Skalak (1983), who created a mathematical model to determine force components in implants. Skalak's model considered bone and prosthesis as being infinitely rigid, while implants were considered elastic. Although this model had some limitations, part of the results are still valid and have called the attention of researchers to the biomechanical problems of implant prosthesis.
However, stress transmission to the bone must be studied taking into account bone biology aspects. During function, bone around implants is subjected to repetitive loading. Like other rigid materials, bone is subjected to fatigue. Repetitive load may result in micro fractures that, if left to build up, can lead to structural failure. The effect of tension on bone tissue has been object of discussion. The concept that bone architecture is determined by induction of tension/deformation remounts to the 19th century (Chamay & Tschantz, 1972; Duncan & Turner, 1995; Rieger et al., 1990). Today it is believed that changes in bone structure are the result of a system where local mechanic signals induce bone cell activity. Frost (1994) proposed a theory according to which there is a minimal bone tension level, the "minimal effective strain" (MES), above which adaptative response would take place, while below it bone would remain stable (Duncan & Turner, 1995).
How much load implants can withstand without jeopardizing the surrounding bone is the question that remains unanswered (Brunski, 1991, 1992; Clelland et al., 1991; Murphy et al., 1995; Sertgoz & Guvener, 1996). Although Frost's theory establishes a minimal effective strain for each loading condition, certainly there is not a single answer to the question, since individual particularities are determinants in this quantification. There is a general agreement that a well-planned and executed prosthesis is essential to avoid excessive forces on bone and implant components. Implant dentistry would greatly profit if it were provided the means to predict how bone and implant components would behave considering each patient's unique jaw anatomy, quality of bone, amount of occlusal force exerted on the prosthesis, etc.
To shed light on this issue, the influence of factors such as the curvature of the mandible, the density of cancellous bone, the length of implants and abutments, the length of cantilever, the number of implants, and the framework stiffness have been objects of study (Bidez & Misch, 1992; Meijer et al., 1993; Pierrisnard et al., 2003; van Oosterwick 1998).

2. FEA studies

The creation of a computerized model of an implant-supported fixed prosthesis allows an analysis of the possible variation in stress distribution that is likely to occur with diverse prosthetic designs and occlusal load variables. A good understanding of how each component of the implant prosthesis behaves under load could facilitate optimal prosthesis design and fabrication. This could decrease the likelihood of mechanical failures as well as improve implant prosthesis longevity.

Finite-element analysis (FEA), with all its inherent limitations (Brunski, 1988; Murphy et al., 1995), is a valuable instrument in pursuing that goal. When associated with clinical findings and accumulation of reliable data on implant loading, bone-implant contact area and other factors, FEA models could help us understand the problems encountered in daily practice (Brunski, 1991). For the reasons above mentioned, FEA studies results have to be seen with a critical eye, and the values should not be taken as absolute but rather be used to compare the possible magnitudes of stress that bone and implant components undergo during function (Meijer et al., 1993; Murphy et al., 1995; Weinberg & Kruger, 1995).

To study some of the variables that affect the biomechanics of implant prosthodontics, a computerized 3-dimensional (3-D) finite-element model of the anterior segment of a human mandible provided with an implant-supported bridge was created. The basic model consisted of a curved beam with radius 15.0 mm and dimensions 69.0 mm long, 14.0 mm high, and 6.0 mm wide. This beam was covered with a 1.0-mm-thick layer on the buccal, occlusal, and lingual surfaces and a 3.0-mm layer at the base to simulate cortical bone. The final external dimensions were therefore 71.0 3 18.0 3 8.0 mm, respectively. Five 10.0-mm cylinders 3.75 mm in diameter were placed at the center of the beam, their centers 7.0 mm apart from each other. The cylinders were provided with 3.0-mm-high extensions to simulate the abutments. A second beam (71.0 3 4.0 36.0 mm) was added in connection to the abutments to simulate a framework (Figure 1).

The model was fixed at both ends for the sake of the stress analysis. All materials, bone included, were assumed to be linearly elastic and isotropic. An FEA program (I-DEAS Structural Dynamics Research, Milford, Ohio) installed in a desktop computer was used to analyze the many possible variations in prosthetic design and occlusal load.

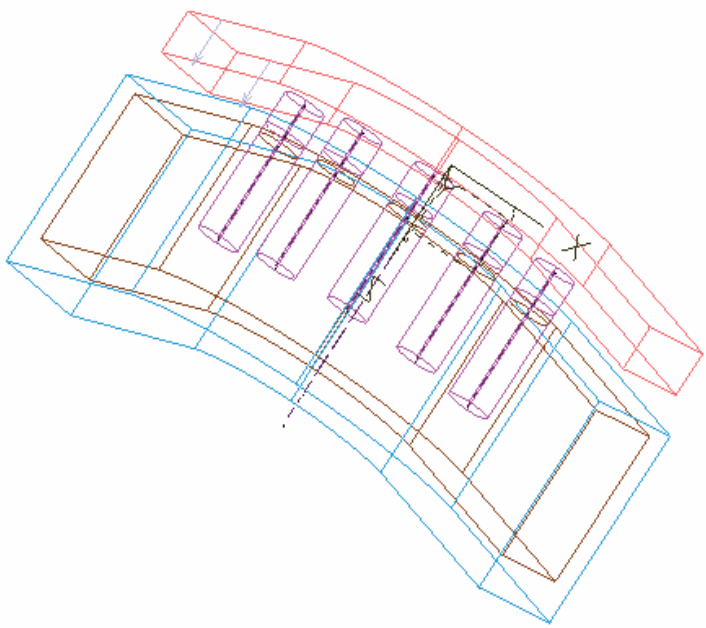

Fig. 1. 3-D model with its internal layers

The model was fed with elastic properties of the materials as derived from the literature. Elastic modulus for cortical bone was assumed to be 13.7 GPa, while for cancellous bone it was assumed to be 1.5 GPa based on a density of 25%. A 100-N vertical load was applied at 15.0 mm distally to the terminal abutment to simulate the occlusal force. Images with the fringes of stress were obtained for each of the components of the model, and maximum Von Mises stresses (σEmax) at each site were analyzed for comparison.
Seven clinical variables were chosen to be evaluated and are summarized in Table 1 for easy reference. Each variable was introduced alternately on the basic model, all other conditions being equal. Cantilever length was defined as the position where load was applied relative to the center of the terminal abutment. Quality of cancellous bone was expressed in terms of its modulus of elasticity (E) as derived from the literature. Abutment and implant lengths were based on the Brånemark system (Nobel Biocare AB, Goteborg, Sweden). Framework alloy was selected according to the modulus of elasticity of AgPd (E = 95 GPa) and CoCr (E = 218 GPa) alloys.

1) curvature of mandible	*r = 15 mm	r = 10mm	r = 18mm	
2) cantilever length	*15 mm	10mm	20mm	
3) cancellous bone quality	*E = 1.5Gpa	E = 4.0GPa	E = 7.9GPa	
4) abutment length	*3.0mm	5.5mm	7.0mm	
5) implant length	*10mm	13mm	15mm	
6) framework alloy	*E = 95GPa	E = 218GPa		
7) number of implants	*a)5	b)4	c)4	d)3

E – elastic modulus **r** – radius ***** - Features of the basic model

Table 1. Clinical variables selected for analysis.

The curvature of the mandible in the anterior region is related to its shape and ultimately influences the distribution of the implants in a more curved or straight line. Apparently there is an increase in stress concentration with the wider model since the implants tend to be located in a straight line creating an unfavorable mechanical situation. For conditions like that it is advisable to keep shorter cantilever arms to avoid torsion moments that could increase stress in bone and also pushes components, particularly the screws, to risk of fracture.
From the studies we conducted with FE analysis (Rubo & Souza, 2008, 2010) (Figures 2 to 7) it was possible to observe that the relative physical properties of the materials substantially affect the way stresses are distributed: 1. At each increment of 5 mm in cantilever length, stress increased by approximately 30% to 37% on the cortical bone around implants. 2. The stiffer the cancellous bone, the more stress it takes and the less stress the cortical bone appears to undergo. 3. A slight decrease in stress was observed with longer implants and abutments. 4. The use of a CoCr alloy contributes to a better stress distribution.
The conventional design of an implant supported fixed bridge on the edentulous mandible results in bilaterally cantilevered framework extensions, which under load create torque and moment on the implants. Many suggestions have been made in the literature regarding the extension of the cantilever, but in general the various authors agree that according to the quality of bone, a range of 10 to 20 mm of cantilever extension is acceptable. Perhaps one of the most compelling findings of this and other studies is the fact that the increase in cantilever length has a remarkable impact upon the stress concentration around the implants. The longer the cantilever arm, the more stress was observed.

Fortunately, this is one of the implant-supported prosthesis features most easily controlled by the dentist. The applications of the shortened dental arch concept and the express recommendation to the dental technician to keep the length of the cantilever arm to a minimum are procedures that cannot be neglected. Previous studies have demonstrated that the increase in cantilever length is directly proportional to the increase in stress concentration around the implants. Kunavisarut et al. (2002) observed that the presence of a cantilever arm significantly increased the stress in the prosthesis, implant, and surrounding bone. Besides, when no proper fit is achieved, the stress is magnified by the cantilever.

The effect of increasing the cantilever arm in this study was remarkable. Stress in the abutment/implant almost doubled when the force was moved from 10 to 20 mm along the cantilever. When analyzing the effect on the framework, a different pattern is observed. The stress increases from 10 to 15 mm but then decreases at the 15 to 20 mm change. According to Benzing et al. (1995) the load application in a framework for implant prosthesis produces deformation energy in the system that causes flexion. If a great amount of deformation energy is consumed by the framework, reduction of the transmitted energy happens, decreasing the stress in this structure.

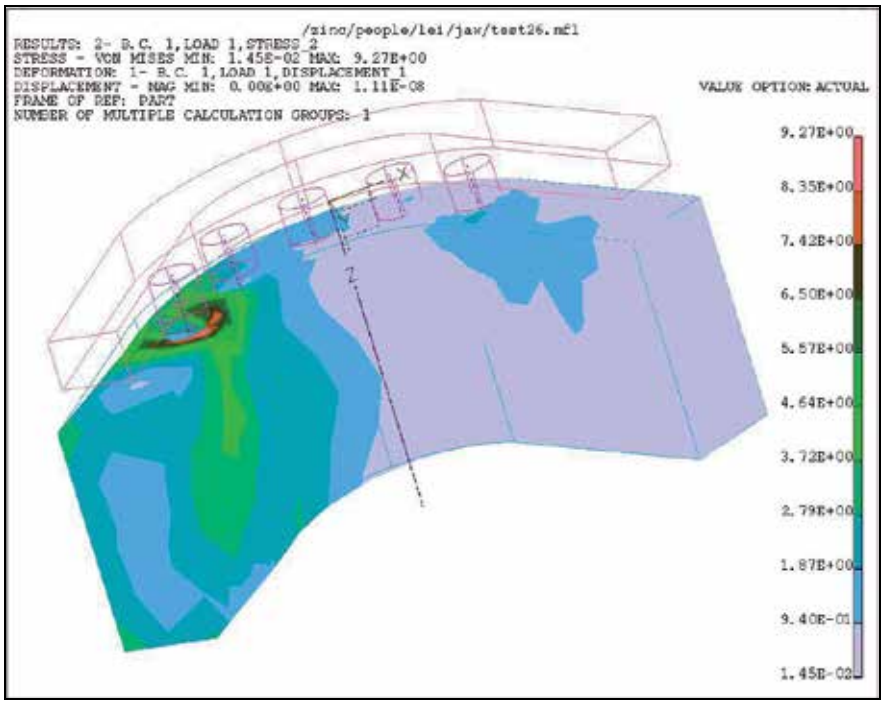

Fig. 2. Fringes of stress in cortical bone (MPa).

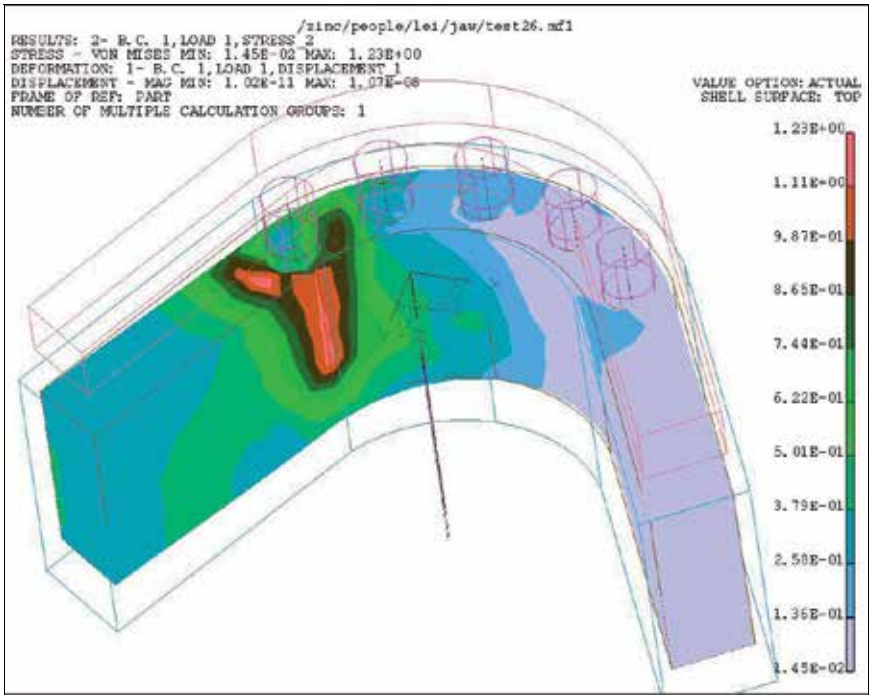

Fig. 3. Fringes of stress in cancellous bone (MPa)

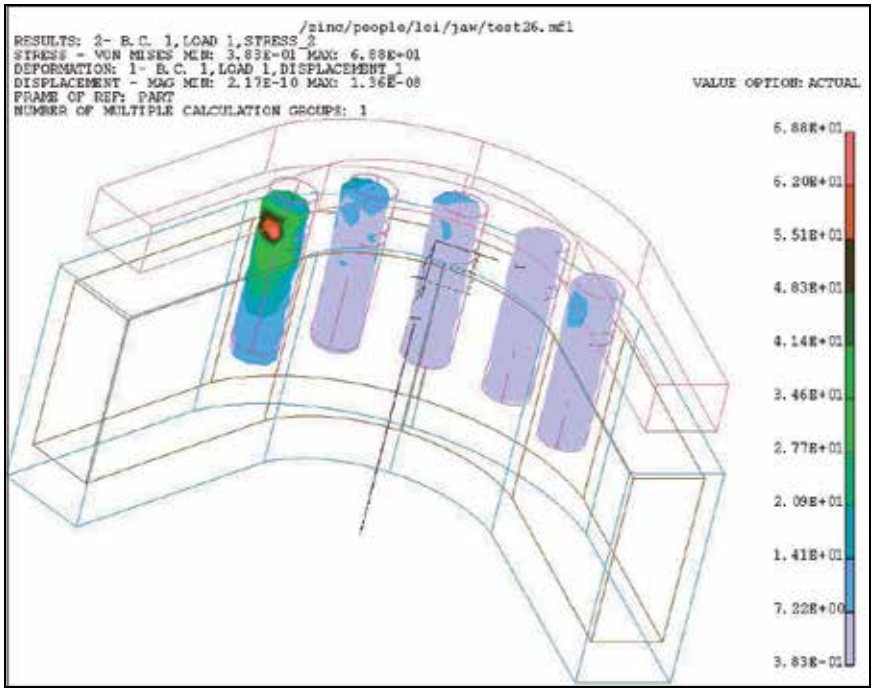

Fig. 4. Stresses on implants/abutments (lingual view).

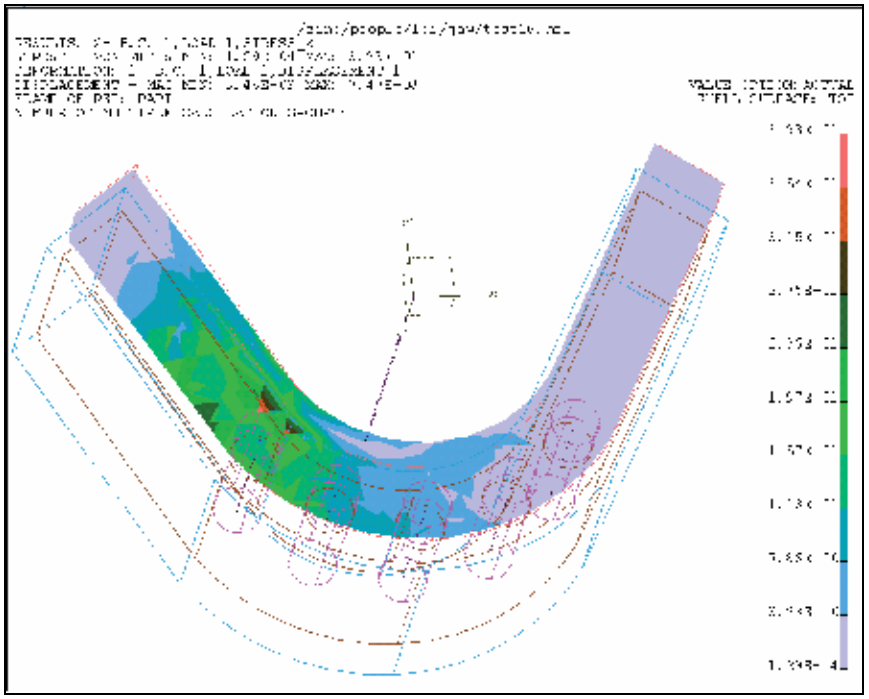

Fig. 5. Stresses on the framework (bottom view).

Structural strength is dependent on the elastic modulus, shape, and length of the prosthesis, which may affect the load distribution among the implants. The lower the elastic modulus, the greater the force applied to the abutment/implant closest to the load. Therefore, if an imaginary rubbery framework was used, nearly the totality of the load would be concentrated at the implant/abutment closest to the point of load application (Skalak, 1983). Likewise, an infinitely rigid framework would distribute the load equally among the implants. Brunski, (1992) compared two implant prosthesis frameworks of considerably different elastic moduli, one made out of acrylic resin and one made out of AgPd alloy, confirming the theory that most of the load concentrates on the terminal implant closest to the load. This finding opposes the original theory proposed by Skalak (1993) who underestimated the load on the implant closest to the load and super estimated the load on the implants on the opposite side, exactly because disregarded the mechanical properties of the structures.

The use of a CoCr alloy allowed a better stress distribution. There was an increase of the stress on the terminal abutment opposite to the load of the order of 135%, with a decrease of the stress in the remaining positions. This finding is corroborated by others in the literature (Benzing et al., 1995; Hulterstrom & Nilsson, 1994; Sertgoz & Guvener, 1996; Skalak, 1985), who found that a stiffer framework provides a more even distribution of forces among the abutments, decreasing the stress within the retaining screws, as a result of the reduced bending of the framework. Left aside the difficulties in the use of CoCr alloys, like casting shrinkage (around 2.3%) and melting point differences relative to gold cylinders, its use has been clinically tested with no complications that could be regarded to the alloy itself (Hulterstrom & Nilsson, 1994).

The analysis of the stress in the framework with different cantilever length revealed an interesting fact relative to the AgPd alloy: the increase in cantilever length is not followed by a proportional increase in stress levels of the framework. The stress in the framework increases when the cantilever changes from 10 to 15 mm, but this effect does not repeat at the 15 to 20 mm change. In fact, there is a decrease in the stress levels, denoting that, with longer cantilevers, the framework begins to bend, yielding to the load. Benzing et al. (1995) observed that the resistance to the deformation of a golden framework was two-thirds of that of a nonprecious alloy framework. Therefore, it can be suggested that, in cases of long cantilevers, there would be a benefit in the use of a more rigid framework. Nevertheless, excessive long cantilevers must be avoided even with frameworks of high elastic modulus.

When the osseointegration technique was introduced the standard procedure was the placement of six implants between the mental foramina to support a fixed prosthesis. Because of space limitations, this number was subsequently decreased to five and then to four implants without greatly compromising the distribution of stress. However, when an implant is lost, not only the number of remaining implants must be considered, but also their distribution in order to guarantee that the stresses are going to be properly dissipated (Figures 4, 6 and 7). Reducing the number of implants from five to four and then three implants resulted in an increase of stresses on the abutment nearest to the point of load application (6.88MPa with 5 implants, 71.2MPa with 4 implants and 7.72MPa with 3 implants). Also, the position of the implant that eventually is lost, for example the terminal implant, results in an even longer cantilever arm what could cause unpredictable consequences to the bone and components.

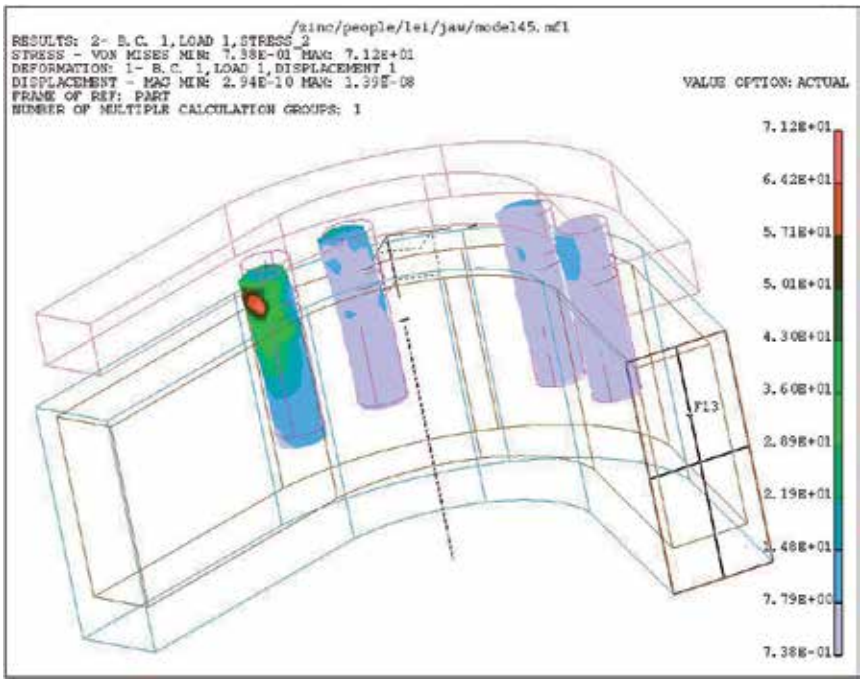

Fig. 6. Stresses on implants/abutments with four implants.

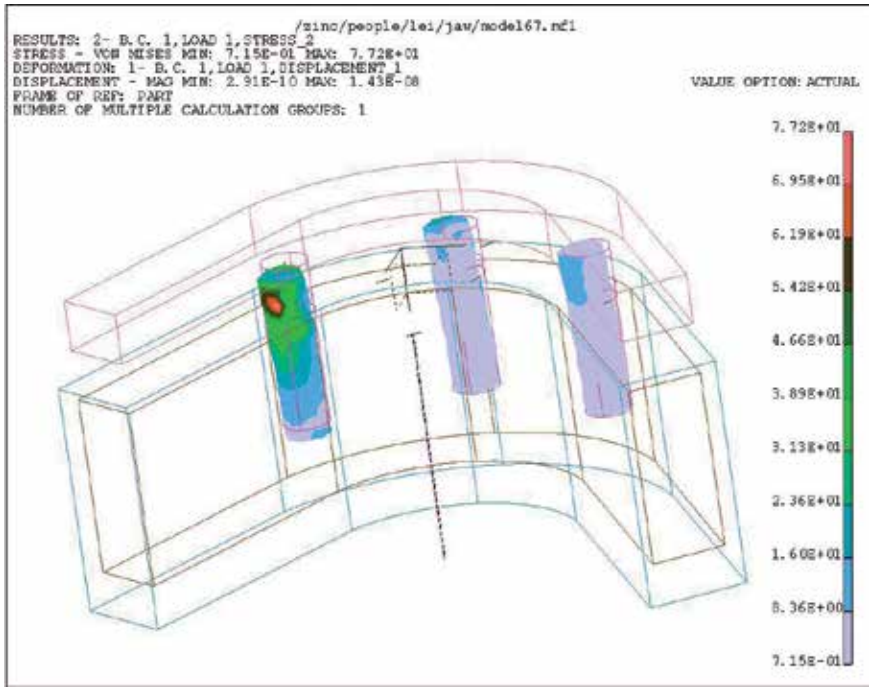

Fig. 7. Stresses on implants/abutments with three implants.

3. Strain gauge analysis

The relevance of osseointegration in today's prosthodontic practice is well recognized. Failure rates are low and easily surmounted by the improvements in function, esthetics, and quality of life. Nevertheless, failures do occur and are considered to be related to a range of biologic and mechanical factors. Among the most commonly reported failures are: resorption of the peri-implant bone crest, peri-implantitis, loss of osseointegration, screw loosening and fracture, and fracture of the prosthesis, prosthetic components, and even the implant itself (Jemt 1991; Kallus & Bessing, 1994; Worthington, 1987; Zarb & Schimitt, 1990). Many times the cause of failure is attributed to a non passive framework, which leads to implant overload (Adell et al., 1981; Carlson B, Carlsson, 1994; Patterson et al., 1995; Sahin & Cehreli, 2001; Zarb & Schimitt, 1990; Zitzmann & Marinello, 2002).

Framework passivity has become a concern in implant prosthodontics and all efforts have been made to pursue it. Restorative dentists have the task of obtaining a passive fit without any accepted clinical parameters for horizontal, vertical, or angular discrepancies. Thus, the goal is to create a fit as accurate as it is clinically possible to avoid strains in the components. Therefore, the way passivity of fit is understood leads to the belief that no stresses or strains must exist at all. But that may not be true, since when torque is applied to bring the joints together a tensile force appears on the screw, which is then elongated. This leads to the development of a compressive axial force between the cylinder and abutment, which maintains the union between the components (Isa & Hobkirk, 1995; Duyck & Naert 2002; Geng et al., 2001).

Compressive forces deform the components to a measurable level. If the compressive force is homogeneous along the periphery of the abutment-cylinder interface or, in other words, if

the cylinder fits properly to the abutment, deformation of the abutment should also be homogeneous and measurable. Usually, machined components are preferred to cast ones because casting may produce a rough surface, making it even more difficult to achieve a proper fit. Machined cylinders need to be overcast to create multiple element frameworks, which is another complicating factor in itself.

Hitherto, no method has been described to establish a reliable parameter for passive fit. Photoelasticity, finite element analysis, and strain gauge measurements have been proposed as tools to determine stresses/strains in implant prostheses. With the purpose of determining a measurable parameter to what constitutes a passive fit framework, a study (Moretti-Neto et al., 2009) was conducted aiming at the evaluation of the deformation to which abutments are submitted when free-standing cylinders are screwed onto them. Strain gauges were used to define the mean level of abutment deformation that can be expected with no interference of factors, such as laboratory procedures, in passive fit. The magnitude of the values found was minimal. The highest mean value of deformation was –625.35 με for abutment screw tightening. Conversion to the percentage of deformation yields the value of 0.062% deformation. It should also be observed that low levels of abutment deformation do not necessarily represent a desirable condition. In fact, deformation levels close to 0 may indicate that the components are completely apart and consequently, not transmitting any force to one another. In this instance, the prosthetic screw and the framework would be overloaded, and the framework adaptation would be forced by the torque applied to the screws (Duyck et al., 2001).

The results of this study corroborate the findings of Isa & Hobkirk (1995) who demonstrated that screw tightening generated compression and tension stresses on the abutments, even when using a framework with a misfit as small as 10 μm. Considering a system formed by a screwed joint, only compression forces should affect the abutments in an ideal condition of passive fit. However, tension forces were observed on the machined cylinders. These results may have occurred because the prosthesis does not establish a homogeneous and uniform contact with the surfaces of the abutments and may have presented adequate fit only at one side (Duyck et al., 2001; Clelland et al., 1993).

Horizontal and angular internal misfits, whose detection is difficult, may also contribute to the instability of the screws and generate tension forces on the implant components (Sahin & Cehreli, 2001; Millington & Leung, 1995; Helldén & Dérand, 1998; Tan et al., 1993). Such distortions, which are difficult to detect, may be responsible for the variability of the results observed in this and other studies. The method employed for evaluating passive fit of prosthetic cylinders to the abutments as a function of abutment deformation could be regarded as useful, since it revealed that despite the small magnitude of the outcomes, some amount of deformation is always present and is measurable, varying from 173.29 με (Pd-Ag machined cylinders) to 200.47 με (Co-Cr cast cylinders).

There is still a difficulty to overcome to apply these parameters to the clinical situation: It is technically difficult to connect strain gauges to patients' mouths to take measurements, which would give more reliable results. Therefore, the proposed method must be regarded as a source of information for what can be expected in an ideal condition. Variations on the amount of abutment deformation and the presence of tension forces should be related to poor quality fit of frameworks.

The clinical relevance of this study relies on the importance of understanding the mechanisms of force transmission from the framework/prosthetic cylinders to the

abutments, from these to the implants, and consequently, to the surrounding bone. In this study, the abutments were rigidly fixed to a metallic master cast simulating the bone. Since bone is the living tissue that ultimately responds to the stresses in the system, a model that takes into account the mechanical properties of bone needs to be developed to quantify bone stress after implant prosthesis connection.

Therefore, studies have been conducted on simulated bone models. Polyurethane was found to present elastic modulus similar to human bone (Miyashiro et al., 2011; Moretti-Neto et al., 2011) and was chosen to verify the tension levels that are developed around implants during function (Figure 8) When prosthetic restorations were connected to the abutments, the tension levels on the bone located at the cervical area of the implant were very low, around 58 με to 100 με for single crowns and reaching 350 με to 400 με for multiple element restorations. What can be regarded as relevant in these studies is that the tension levels fall within physiologic limits, as proposed by Frost's minimal effective strain theory, between 50-200 με to 1500-2500 με. Such tension levels would not be capable of causing bone resorption around implants (Rubo, 2010).

Fig. 8. A bone simulated model was used to quantify the tension around the cervical area of the implant when the restoration is connected.

The influence of cantilever length, abutment's height and framework alloy was also studied by means of strain gauge analysis (Jacques et al., 2009; Suedam et al., 2009) (Figure 9). Frameworks for implant-supported prostheses have been evolved from soldering a gold alloy framework to gold cylinders, to gold alloy frameworks cast directly onto the cylinders, to Ag–Pd alloy frameworks (Cox & Zarb, 1985; Jemt, 1991). The clinical results showed that the latter option gives consistently good results with respect to accuracy of fit, load bearing capacity, reduced cost and design versatility (Rangert et al., 1989). However, the use of an alloy with higher modulus of elasticity, such as Co–Cr, would allow for a more

evenly distributed load among implants with a less bulky framework, which would be an advantage if intraoral space is limited (Hulterstrom & Nilsson, 1994; Chao et al., 1988). For these reasons, the Ag–Pd alloy framework was chosen for comparison purposes.

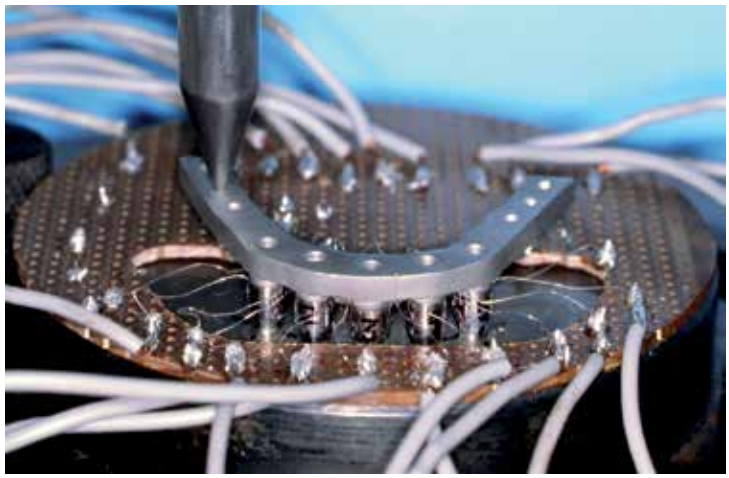

Fig. 9. A framework was screwed to the abutments with strain gages on the proximal aspects; 100N load applied at 5mm, 10mm and 20mm distal to terminal implant.

Cantilevered ends of fixed implant-supported prostheses increase the load on the first implant nearest the cantilever arm. In this study it could be noticed that the abutment closest to the point of load application on the cantilever extension registered deformation by compression, but higher levels of deformation by tension were observed in intermediary abutments. These results are according to data from other experiments (Benzing et al., 1995; Chao et al., 1988; Rubo & Souza, 2008; Skalak 1983; Suedam et al., 2009) reporting that in cantilevered prostheses, the most distal implants represent the fulcrum and, therefore, are subjected to compression forces while intermediary abutments suffer tension. For the alloy with lower elastic modulus, as the distance between the point of load application and the distal abutment increases, energy is consumed in framework deflection and low deformation levels are observed in abutments.

The results of this study showed that the pattern of deformation generated by the application of the static force varied according to the position of the strain gauges in the abutment's aspects, according to the implant position relative to the load application, to the abutment's height and also to the type of alloy used for the framework. The increase of the abutment's height promoted an increase of the deformation by compression in the strain gauges located in the abutment adjacent to the cantilever. This could be explained by the increase of the lever arm that takes place with the increase of the abutment's height leading to the deformation in the abutment adjacent to the point of load application.

The strains generated in the abutments did not present a uniform pattern among the abutment's aspects and not even in different abutments. However, deformations by compression are present in a larger intensity in the strain gauges located in abutment no. 1 closest to the point of load application in all specimens, evidencing that the abutment's proximity to the load influences the results. This brings about the importance of evaluating the implant adjacent to the lever arm, concerning its bony support, for planning the extension of the cantilever.

In Figs 10 and 11, a tendency line was added to help visualize the effect of the difference in elastic modulus of the alloy in the pattern of deformation of the framework. It clearly shows that the stiffer CoCr alloy framework is less affected by the load, resulting in compression on both ends and tension in the middle. On the other hand, the PdAg alloy framework showed a tendency to a more complex deformation pattern, resultant of its lower elastic modulus compared with the CoCr alloy framework.

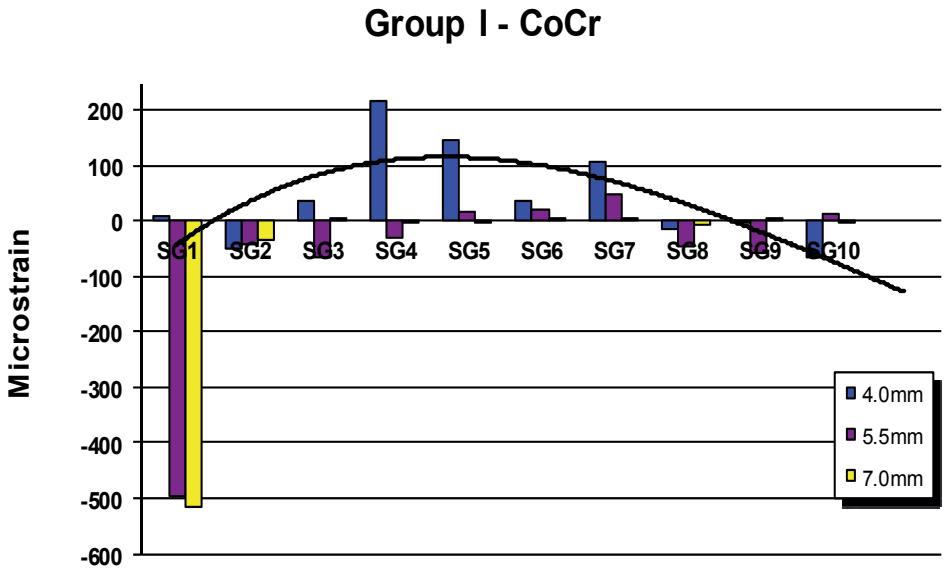

Fig. 10. Graphic of the deformation means captured by the abutment's strain gauges for CoCr frameworks.

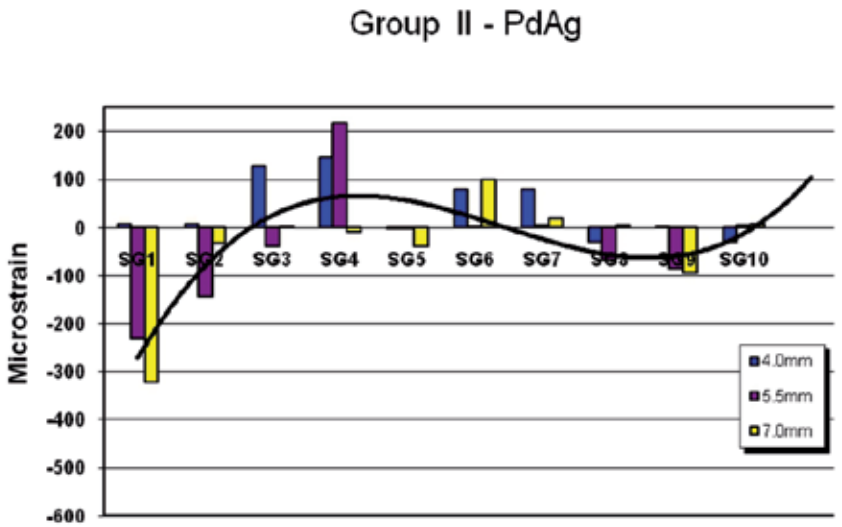

Fig. 11. Graphic of the deformation means captured by the abutment's strain gauges for PdAg frameworks.

On the other hand, the alloy with high modulus of elasticity undergoes few or no deflection with the increase of the cantilever, resulting in more abutment deformation. In both situations, the screws can be overloaded what underscores the need to keep a reduced cantilever arm, as this has been demonstrated to be the major cause of mechanical problems (Shackleton, 1992). The results observed with both alloys are inconclusive as to what material performs better.

Clearly, there is a difference in the mechanical behavior of the two frameworks and the resultant pattern of abutment deformation, but the relevance of this finding would only be determined by clinical comparative studies. (1) Abutment deformation was always higher with longer cantilever extensions; (2) The Co–Cr alloy framework resulted in higher levels of abutment deformation; (3) The pattern of abutment deformation was different for both alloy frameworks with a tendency to tension forces to appear closer to the load when Pd–Ag alloy was used. In this study it was concluded that (1) the abutment adjacent to the cantilever presented the largest deformation by compression captured by the strain gauges, (2) the type of alloy used for fabricating the framework influenced the deformation of the abutments and (3) the increase of the abutment's height increased the deformation on the terminal abutment.

4. Conclusion

Many studies have been made to present solutions or explanations to the biomechanical problems in implant prosthodontics. A large bulk of knowledge can be extracted from those studies, but it seams clear that most of the loading problems are not capable of generating bone resorption, which seems to be related to other biological events.

However, overload may be responsible for many events of screw loosening and fractures, resulting in the need for more frequent maintenance. Solving these problems may be time consuming and creates additional costs.

The professional must comply with sound concepts of good prosthodontic practice to improve prostheses longevity. Obviously, most of these principles are familiar to the prosthodontist. Those are basic oral rehabilitation concepts applied in daily practice, what underscores the notion that implants are one more element added to the prosthodontist armamentarium that must be naturally incorporated to the patient's treatment plan.

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9

Changes in Bone Metabolisim Around Osseointegrated Implants Under Loading

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1. Introduction

The use of osseointgrated dental implants to replace missing teeth is a highly predictable procedure. The scientific lierature is replete with reports of high success rates over long periods of time. Since the phenomenon of osseointegration was first introduced by Brånemark, this procedure has gained great popularity.

One of the measurements for success of an osseointegrated implant is that it be load-bearing and transmit these occlusal forces directly to the adjacent bone. Controlling this load is considered a determinant factor in the long term success of the implant. A related consideration is how this load or mechanical stress influences bone metabolism around the osseointegated implant.

Mechanical stress may lead to an alteration in bone quality and architecture and a distinct reaction within the bone cells at the bone-implant interface. However, there is little published data to support this theory. A few studies have suggested that occlusal overload may contribute to bone loss around an implant and/or loss of integration of a successfully integrated implant. (Rangert et al.,1995; Miyata et al., 2000; Piattelli et al., 2003). Isidor reported implant mobility caused by progressive peri-implant bone loss after the implant were expose to mechanical occlusal trauma for 18 month (Isidor, 1997, 1998). Others report that peri-implant bone loss and/or loss of osseointegration is associated with biological complications such as peri-implant infection (Lang at al., 2000).

A certain level of mechanical loading is required for normal, healthy bone remodeling (Frost, 1989). Misch observed that the change in bone strength from loading and mineralization after one year alters the stress-strain relationship and reduces the risk of microfracture during following years. Mechanical stress might induce a metabolic turnover of the bone based on the changes in osteocyte responses around the implant, resulting in bone remodeling (Misch, 1999).

This chapter is to investigate the dynamic changes in bone metabolism around osseointegrated implants under mechanical loading.

2. Basic information

2.1 Bone formation

Bone forms by either endochondral or intramembranous ossification. In endochondral ossification or long bone formation, there is an intermediate cartilage phase. All craniofacial bones are formed by intramembranous ossification. Wong and Rabie showed that demineralized intramembranous bone matrix induces bone without an intermediate cartilage stage, mesenchymal stem cells differentiate directly into bone cells (Wong & Rabie, 1999). The mesenchymal stem cells differentiate into osteoblasts and form osteoid in a collagen matrix (Palacci et al., 2001). Mineralization of osteoid occurs and the osteoblasts become trapped in mineralized bone and become osteocytes.

Appositional bone formation occurs when osteoblasts produce bone on existing bone surfaces. Examples of appositional bone formation occur in the periosteal enlargement of bones during growth and remodeling. Histological studies show woven bone formation by appositional growth may only begin to form the second week after implant insertion, at a rate of 30 to 50 microns per day. The bone to implant contact is weakest and at the highest risk of overload at approximately 3 to 5 weeks after implant placement (Strid, 1985).

2.2 Osseointegration process

Osseointegration is defined as direct bone deposition on an implant surfaces at the light microscopic level (Brånemark et al., 1997). It generally follows three stages: (1) incorporation by woven bone formation, (2) adaptation of bone mass to load (lamellar and parallel-fibered deposition) and (3) adaptation of bone structure to load (bone remodeling) (Schenk & Buser, 1998). During the third stage, when functional loading has been initiated, the bony structures adapt to the load by improving the quality of the bone; replacing pre-existing, necrotic and/or initially formed more primitive woven bone with mature, viable lamellar bone. This leads to functional adaptation of the bony structures to the load. The dimensions and orientation of the supporting elements change. *In vitro* studies have illustrated the importance of loading forces on the nature of the interface between an implant and the surrounding tissues (Brunski, 1988). Even if implants were initially integrated, the application of excessive loading can create microfractures and mobility which may promote bone resorption around the implant and may promote repair by the undesirable growth of fibrous tissue (Roberts et al., 1989). An undisturbed healing period along with adequate quality and quantity of bone available at the implant site are essential for proper osseointegration. (Wood, 2004). In addition, primary implant stability is determined by bone quality and quantity, implant design, and surgical technique (Sennerby et al., 1998).

2.3 Implant mechano-biology

Factors that influence mechano-implant studies are mechanical loading and detachment of cells. Previous *in vivo* data have demonstrated that optimal mechanical loading led to more favorable bone quality and quantity than situations with no loading. The testing equipment used for most of these studies were stretching devices borrowed from orthopedic stress-strain research measuring elastic stress relative to joint prostheses. Their application in dental implant research is not optimal as there is mostly shear stress and little elastic stress.

More recently, *in vitro* studies of osteoblastic behavior on titanium following loading have been developed. Movement seems to generate negative effects on osteointegration in terms of decreased Alkaline Phosphatase (ALP) activity and osteocalcin, which is consistent with

the results in mechano-cell studies. The supressed ALP activity may be attributed to PGE2 production (Bannister et al., 2002). With respect to osteoblast maturation following stimuli, biphasic ALP activity and triphasi osteocalcin level in a 3D study differed from 2D cultures characterized by typical gene expression pattern with time (early up-regulation of collagen and ALP) and osteocalcin production (Akhouayri et al., 1999). It was assumed that actin cables and collagen fibers would be aligned to amplify the mechanical forces and to supply bone maximum strength with little amount of material. This research has demonstrated that number of complex elements exist in the human body environment and it is still necessary to improve experimental designs in order to provide reliable *in vivo* implant data.

2.4 Implant under loading

It is clear that successful osseointegration depends on unfavorable loading. Other factors to consider are the timing of initiation, magnitude, and duration of the load or stress. Excessive occlusal loading will lead to disintegration while adequate loading leads to adaptive remodeling of the bone around the implant (Quirynen, 1992). However, there are some differences in data reported among rearchers.

Gotfredsen et al. demonstrated that implants subjected to a static lateral expansion load showed increased bone density and mineralized bone-to-implant contact compared with control implants (Gotfredsen et al., 2001a, 2001b, 2001c, 2002). Melsen and Lang reported that there was significantly higher bone apposition around loaded implants than unloaded implants but the dimensions of the applied load did not affect the turnover characteristics of the peri-implant alveolar bone (Melsen & Lang, 2001). Vandamme et al. also indicated significantly more osteoid in contact with the implant was found for the loaded conditions compared with no loading. Well-controlled micromovement favorably influenced bone formation at the interface of an implant (Vandamme et al., 2007). In an animal model, Berglundth et al. described osteoclastic activity as early as four days following implant placement and new bone was noted at one week post placement (Berglundth et al., 2003). These results suggest that bone metabolic activity is changed by mechanical stress and that it depends on the loading conditions.

These reports indicate that functinal loading does promote osseointegatein and that overloading or favorable loading may contribute to implant failure. Occlusal overload could result in progressive marginal bone loss or loss of osseointegration. Long ago, Adell recognized that early implant failure may be associated with overload (Adell et al., 1981). In a more recent study, Miyata et al. reported the outcome of occlusal overloading at three different occlusal heights (100μm, 180μm, 250μm) on implant prostheses for four weeks. Bone destruction was observed in the 180μm and 250μm excess occlusal height groups (Miyata et al., 2000).

Bruxism, a non-physiological parafunctional habit is more significant than the forces associated with normal mastication. Excessive micromovement creates stress or occlusal overload and leads to soft tissue encapsulation and prevents osseointegration, thus causing implant failure (Brunski et al., 1979). The occlusal scheme may jeopardize the success rates of immediately loaded implants; they found that 75% of failures in immediately loaded implants occurred in patients with bruxism (Balshi &Wolfinger, 1997). To avoid fibrous encapsulation and subsequent implant failure, implants must withstand functional load with less than 150 microns (Schincalglia et al., 2007). It is the excess of micromotion caused by excessive loading during the healing phase that interferes with bone repair. A threshold

of tolerated micromotion exists, that is somewhere between 50 μm and 150 μm in human case. Therefore, keeping the amount of micromotion beneath the threshold of deleterious micromotion might enable the loading protocols to be shortened (Szmukler-Moncler et al., 2010).

2.5 Immediate and early loading

Immediate and early loading of dental implants are concepts introduced to shorten treatment time and further improve a patient's quality of life (Barone, 2003). Linked to this is the fact that appropriate mechanical stimulation is a positive factor for bone formation. In fact, several studies have demonstrated that early functional loading is helpful in preventing marginal bone resorption and enhancement of osteointegration at the implant surface. In a human clinical case report, Ganeles et al. demonstrated that Straumann® implants placed in the posterior mandible and maxilla are safe and predictable when used with immediate and early loading procedures (Ganeles et al., 2008; Esposito et al., 2009). In poor quality bone, survival rates were comparable with those from conventional or delayed loading. The mean bone level change was not deemed to be clinically significant and compared well with the typical bone resorption observed in conventional implant loading (Kinsel & Liss, 2007; Fischer et al., 2008). Several animal studies also reported favorable results when using immediate and early loading principles.

In a study of radiographic evaluation in dogs, Corso et al. showed that immediate masticatory loading of single-standing dental implants did not jeopardize tissue integration, provided the implants had excellent primary stability (Corso et al., 1999). Cha et al. asserted that immediate loading of mini-implants in the dog model is possible for orthodontic applications with a high bone-implant contact and 100% survival rate (Cha et al., 2009). Some immediate loading studies involving the biting stress of mastication showed the potential to increase bone density and prevent crestal bone loss. With respect to histological bone implant contacts, no significant difference between immediately loaded implants and those of delayed loaded or those of unloaded was observed. It was postulated that mechanical stimulation quite possibly enhanced bone formation (Romanos et al., 2002, 2003)

Kawahara et al. studied the effect of immediate loading at the implant interface in dogs and concluded that micromotion of less than 30μm did not impede bone ingrowth (Kawahara et al., 2003). For an immediate loading model with varying degrees of implant displacement, micromotion had a positive effect on bone formation around a roughened implant surface and a negative effect on a turned implant surface (Vandamme et al., 2007). De Smet reported that early loading enhanced bone reaction around implant and contributed to stability of the implant (De Smet et al., 2005, 2006). At the same time, it has been reported that excessive loading leads to creater-like bone defects around implants, indicating bone resorption (Duyck et al., 2001).

The benefit of immediate loading was futher borne out by clinical and histological studies indicating that immediately loaded implants had a higher bone-to-implant contact (BIC) value than non-immediately loaded implants. In one case report, the BIC was 64.2% greater with immediately loaded Osseotite® implants (Testori et al., 2002). In another study, histological comparison of non-submerged unloaded and early–loaded implants in a monkey model found a tight contact with new bone to implant surfaces in both study groups (Piattelli et al., 1997, 1998). But the authors found that the bone of the loaded implants had a more compact appearance than non-loaded controls and the mean BIC of immediate-loaded implants was 67.2% in the maxilla and 80.7% mandible.

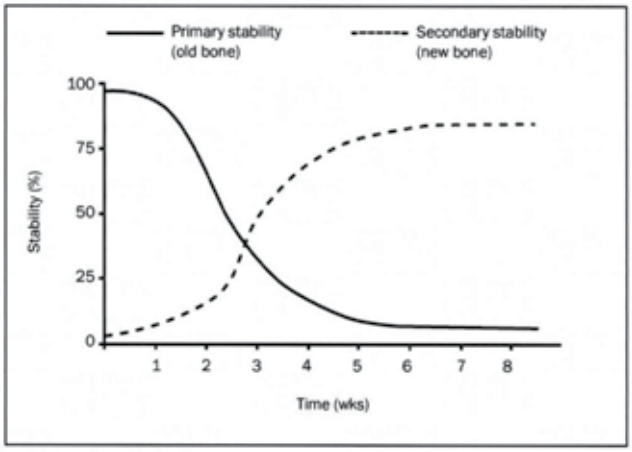

Fig. 1. Changeover from priomary stability created at the time of implant placement to secondary stability created by deposition of new bone (osseointegration) in humans. (Raghavendra et al., 2005)

They felt that the most critical factor in successful osseointegration of an implant is the stability in the bone at the time of placement since a static condition was thought to be a prerequisite during the early healing period. This initial mechanical stability is later replaced by biologic stability as the bone remodels and osseointegartion occurs (Raghavendra et al., 2005). This reportedly occurs during a 2 to 3 week transition phase following implant placement. Ostman listed requirements for long-term success with immediate-loaded implants as high primary implant stability, moderately rough implant surface, prolonged implant stabilization by splinting, controlled occlusion, and biocompatible prosthetic material (Ostman et al., 2008).

The actual type of bone healing around unloaded and loaded implants was investigated by Slaets et al. (Slaets et al., 2006, 2007). These studies showed that the manifestation and duration of the biological processes including the osseointegration of the implant were dependent on the type of bone, cortical or trabecular. Furthermore, the immediate loading protocol caused no differences in the sequential events leading to osseointegration in cortical bone (Slaets et al., 2009). In an unpublished study using bone scintigraphy, the current authors reproted on both immediate and early of loading of implants. The bone metabolic activity increased for the firts seven days after load application and then decreased gradually until returning to the baseline level despite continuous load-application with same magnitude. These results suggest that this change may be attributed to adaptive bone remodeling and immediate and early loading might not prolong the period until achievement of osseointegration.

3. Nuclear medicine approach with bone scintigraphy

Regarding bone metabolic activity, such as bone remodeling and adaptation, histological studies can merely depict static and cross-sectional aspects of the bone activity and phenomena in the remodeling process. In contrast, a nuclear medicine approach with radionuclide bone scanning, including scintigraphy, is widely used to evaluate the dynamic and longitudinal processes in biological response and to help in comprehending the condition of osseointegration (Bambini et al., 2004). Areas with an observed accumulation of

radiopharmaceutical isotopes show an increased level of bone metabolic activity, suggesting that this method enables measurement of the changes in bone metabolic activity around implants *in vivo (*Fleisch, 1998; Chisin et al., 1988). *In vivo* scintigraphic imaging using Tc99m-MDP enables the same region to be observed numerous times without sacrificing the host animal. Although the specific binding location of Tc99m-MDP remains unknown, it is associated with areas of bone growth and osteoblast activity (Lysell & Rohlin 1985; Kanishi,1993; Schwartz et al., 1993; Okamoto,1997). Furthermore, bone scintigraphy makes it possible to observe the accumulations with more sensitivity and higher reactivity over time than with conventional radiology (Bijvoet et al., 1995).

3.1 Materials and methods

3.1.1 Animals and insertion of Implants

Thirty-two 12-week-postnatal male Wistar rats were used. Nine rats were used to investigate the biological process of the osseointegration after implant insertion; and twenty-three rats addition to those nine rats were used to investigate the bone metabolic activity under loading. The rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally with supplemental ether inhalation. Two titanium implants (Orthoanchor®, Dentsply-Sankin, Japan), 1.2 mm in diameter and 9.25 mm in length, were installed in the tibiae perpendicular to the bone surface; the implant heads were exposed about 5 mm. In each rat, one implant was installed 10 mm from the knee joint. The other was installed 13 mm from the first and in the distal aspect.

3.1.2 Loading with coil spring

Healing and osseointegration had gained at eight weeks after insertion. To clarify the biological responses around the implants under continuous loading, closed coil springs (Sentalloy®, Tomy International, Japan) with 0.5 N were attached to the implant heads of nine rats for seven weeks to apply a continuous mechanical stress (Fig 2a). Closed coil springs with 1.0, 2.0, or 4.0 N were also attached to the implant heads of eight, seven, and eight, respectively. The group of rats with two 2.0-N springs is defined as the 4.0-N loading group (Fig 2b). It comprised a tension coil and a hook attachment. The effective length of the tension coil was 12 mm. The springs thus applied the same magnitude of loading continuously within their effective length (10 to 22 mm).

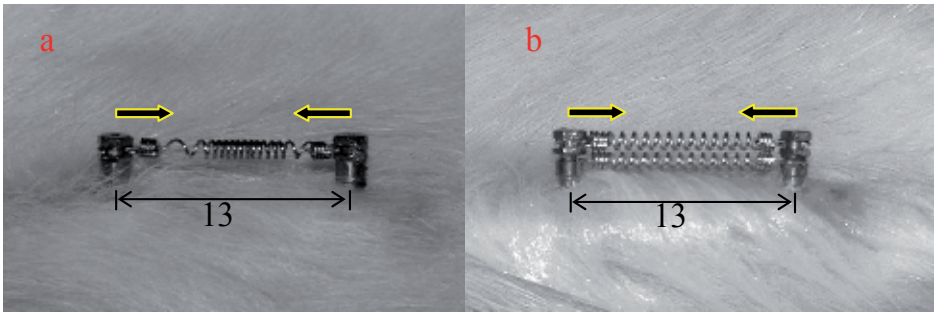

Fig. 2. Attached closed coil springs. Eight weeks after implant insertion, closed coil springs were attached to implant heads. With loading of 0.5, 1.0, or 2.0 N, closed coil spring was set prospectively (a). Two closed coil springs with loading of 2.0 N each were set in parallel for total loading of 4.0 N (b). Arrows show loading directions.

3.1.3 Scintigraphic imaging

Scintigraphic images of the bone were taken using a gamma camera (ZLC7500, Siemens, Japan) with a modified high-resolution pinhole collimator (pinhole diameter: 2 mm). Technetium-99m methylene diphosphonate was used as a radioisotope tracer. Sodium pertechnetate ($Na^{99m}Tc\text{-}O_4$) was eluted from a generator (Ultra-Techne Kow®, Daiichi Radioisotope Laboratories, Japan) and mixed with methylene diphosphonate (Techne® MDP injection solution, Daiichi Radioisotope Laboratories, Japan). The Tc99m-MDP was injected into a vein of a tail of each rat (74 MBq/rat). Static-planar acquisition was initiated two hours after the injection and finished at 50,000 counts with a 512×512 matrix size. The rats were fixed on an exclusive table in the dorsal position, with the implant heads turned to vertical. The images were taken from the backside direction of the rat.

To clarify the bone metabolic activity around the implants during osseointegration, images were taken at 1, 4, 7, and 10 days and 2, 3, 4, 7, and 8 weeks post-implantation. To clarify the bone metabolic activity around the implants under continuous loading, images were taken at three days and every week up to seven weeks after loading with the coil springs.

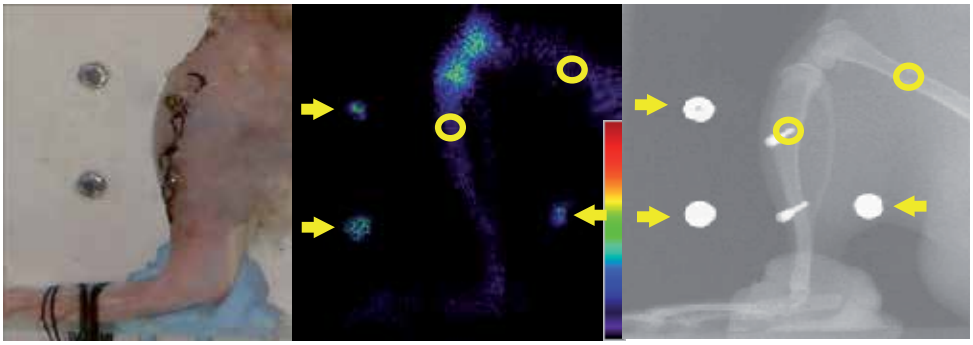

Fig. 3. Image analysis. Photograph on left shows tibia fixation on exclusive table. Device fixes tibia horizontally to equalize distance of implants from pinhole collimator. Scintigram in center and x-ray image on right show planar image. Guide tubes (arrows) were used to overlap two images and to define region of interest (open circles).

3.1.4 X-rayimaging

To identify the region of the reference site and the installed implants, an x-ray image was taken of each rat on the table using an imaging plate (IP; BAS-SR2505, Fuji Film, Japan). Each IP was then scanned with an imaging analyzer (BAS5000, Fuji Film, Japan). The exclusive table had three markers. Before the scintigraphic imaging, Tc99m-MDP was placed in each tube, resulting in an accumulation of Tc99m-MDP in the tube regions on the scintigrams for each rat. These markers indicate the points of overlap with the lead regions in the x-ray images, enabling identification of the implant and reference sites (Fig 3).

3.1.5 Data processing

The scintigrams were translated into TIFF format (16 bits) with a data processing unit (Scintipac 700, Shimazu, Japan) and conversion software (picMAO, Shimazu, Japan). Analysis processing was conducted with image analysis software (Osiris, Geneva University Hospital, Italy). After identification of the implant and reference sites by the overlapping the x-ray images and scintigrams, a round ROI (region of interest, 161 pixels) was defined

around both sites and the accumulations of Tc99m-MDP in both regions were measured. As a metric, the ratio of the metabolic activity around the implants to that around the reference site (uptake ratio) was used. The collected data were analyzed using Friedman, Steel, and Tukey tests with statistical software (SPSS 11.0, SPSS Inc., Chicago, IL, USA.). P-values <0.05 were deemed statistically significant.

3.2 Results

3.2.1 Metabolic changes after insertion of Implants

The uptake ratio increased during the first week after implant insertion and then decreased gradually. It was significantly higher than baseline on days 4, 7, and 10 and during the second and third weeks. However, it was not significantly higher on 4 weeks and 7 weeks, in other words, metabolic activity had returned to the baseline level (Fig 4). No clinical mobility of the implants was observed during the healing period. These results suggest that osseointegration is obtained about four weeks after implant insertion. In addition, the timing of the peak level of and subsequent decrease in bone metabolic activity found in this study correspond very well to those of a previous report on Tc99m-MDP activity around implants using bone scintigraphy (McCracken et al., 2001). Therefore, it should be possible to observe in real time the osseointegration process and the degrees and stages of bone metabolism using this method longitudinally.

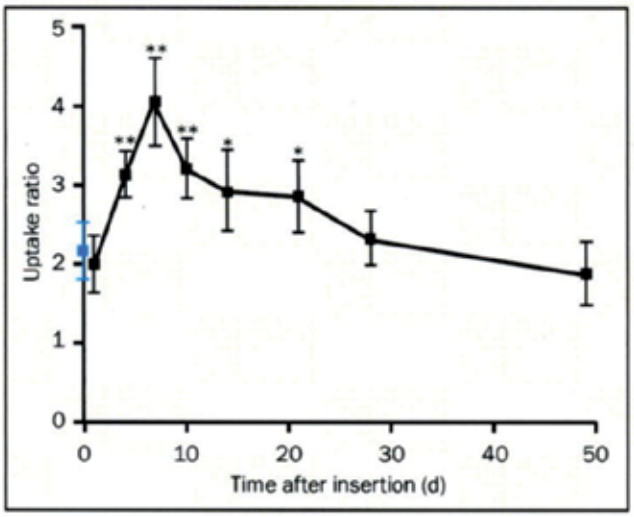

Fig. 4. Change in uptake ratio after insertion of implants using 9 rats.There were significant differences at days 4, 7, and 10 and second and third weeks after insertion. Blue = control. (* $p<0.05$, ** $p<0.01$) (Sasaki et al., 2008).

3.2.2 Effect of loading period

The uptake ratio changed with the loading. With 2.0- and 4.0-N loading, both changes of activities over the seven-week experimental period were almost the same in terms of magnitude and timing. The ratio reached a maximum during the first week (more than twice that without loading) and then decreased a little. Metabolic activity had returned to the baseline level. The ratio then returned to baseline level of about two on seven weeks

after loading. The ratio from three days to six weeks after loading was significantly higher than without loading. There was no significant difference seven weeks after loading.
The results for the 0.5- and 1.0-N loading groups were similar but differed from those for the 2.0- and 4.0-N loading groups. With the smaller loadings, the uptake ratio gradually increased after loading and returned to the baseline level at seven days. It then decreased, reaching about two on seven weeks after loading. With 1.0-N loading, the uptake ratio did not differ among measurement points.

3.2.3 Effect of loading magnitude

The uptake ratios with the 2.0- and 4.0-N loadings were significantly higher than those with the 0.5- and 1.0-N loadings (Tukey test, $p < 0.05$) (Fig 5). This indicates that the metabolic activities are affected by the magnitude of the mechanical loading on the implant. The uptake ratio showed dynamic changes, and the peak levels were similar in the heavy loading group, i.e., there was no difference between 2.0- and 4.0-N loading. It is conceivable that the bone metabolic activity may have an upper limit as far as the loading does not exceed the physiologic threshold of bone adaptation (Frost, 1994). On the other hand, it is possible that the bone metabolic activity increases remarkably when excessive loading is applied to the implant, causing implant disintegration.

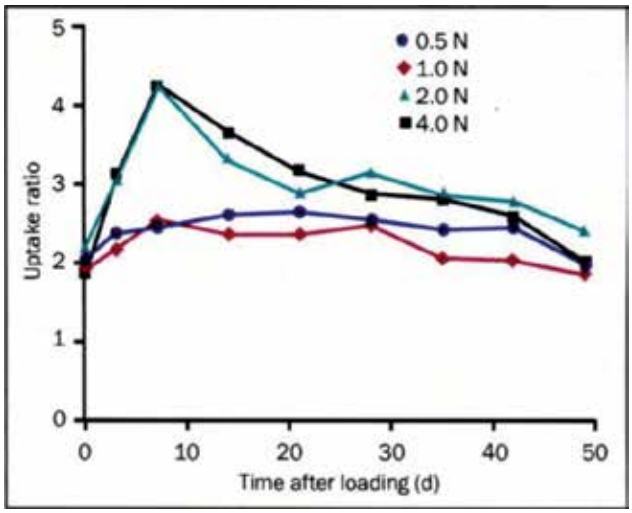

Fig. 5. Change in uptake ratio after loading with coil springs using 32 rats. There were significant differences between 2.0- and 4.0-N loadings and 0.5- and 1.0-N loadings. With 2.0- and 4.0-N loading, the ratio from 3 days to 6 weeks after loading was significantly higher than without loading (Sasaki et al., 2008).

3.3 Discussion

The bone metabolic activity gradually decreased from the peak level despite a static force being applied to the implants and it eventually returned to the pre-loading level. This change can be attributed to an adaptive bone remodeling process similar to those previously reported. Saxon et al. demonstrated that mechanical loading on the rat ulna greatly improved bone formation during the first five weeks of loading, while continual loading

reduced the osteogenic response. Moreover, restoring the same level of loading after a period of no loading increased bone formation again (Saxon et al., 2005). Warden et al. investigated the use of mechanical loading with rat ulna to induce bone adaptation and found that fatigue resistance was advanced than control because the structural properties changed due to loading (Warden et al., 2005). Mechanical loading is thus an important factor in the formation and maintenance of skeletal architecture. Bone morphology adapts to the functional loading patterns by responding to the size and distribution of strains that loading engenders in the bone tissue (Lanyon, 1987, 1992). It is concluded that the bone around the implants adapted to the mechanical stress of long-term loading by structurally changing and that the responsiveness to the loading diminished over time.

Mobility normally occurs during the osseous remodeling process (Ganeles et al., 2002). Remodeling is a variable process with balanced osteoclastic and osteoblastic activity, so that a stable implant is preserved during osseointegration (Schnitman et al., 1997). However, clinically stable implants may exhibit mobility on the micro-level when loaded. Sennerby and Meredith revealed that all implants display varying degrees of stability or resistance to load (Sennerby & Meredith, 2008). That is, functional adaptation and maintenance of the bony structures around implants would be caused by cells (i.e., osteocyte, osteoblast, or osteoclast) in active response to environmental biophysical stimuli, in other word, mechanical stress. Microstrain levels 100 times less than the ultimate strength of bone may be responsible for remodeling rates within the structure, since the bone sell membrance are able to act as a mechanosensory system in bone (Cowin & Moss-Salentyin ,1991). In other words, the cellular behavior of bone cells is largely determined by the mechanical environment of strain or deformation of the bone cell (Jones et al., 1991).

At the interface of implant, osteoblasts and osteocytes play roles of transducers of received strain, leading bone modeling and remodeling phase. Verborgt et al. found that fatigue loading produced a large number of osteocytes in bone surrounding microcracks, and stated a strong association between microdamages, osteocyte apotosis, and subsequent bone remodeling (Verborgt et al., 2000). Noble et al. showed that mechanical loading of the bone can be used to regulate osteocyte apoptosis, which has a mechanism for the precise targeting of osteoclasts for bone adaptation (Noble et al., 2003). Miyata et al. speculated that long-term occlusal stress on implants within the physiologic tolerance might stimulate blood circulation, which has an intraosseous bone-inducing factor that promotes bone metabolism and, consequently, enhances bone remodeling to obtain the width needed to counter occlusal stress (Miyata et al., 2000). Futhermore, Isidor indicated that depending on the properties of the tissue, a given force may affect different bones or bone tissues differently, but mechanically loaded bones adapt to the load. If the strain in the bone surrounding an oral implant is in the mild overload range, apposition of bone seems to be the biological response. On the other hand, strain in the bone beyond this range will at some point result in fatigue fracture and bone resorption (Isidor, 2006).

4. Conclusion

Changes in bone metabolic activity around dental implants are dependent on mechanical stress relative to timing, direction, quality, and duration of loading conditions.

The mechanical stress induces metabolic bone remodeling and adptation around osseointegrated implants with structurally changing.

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Biological Sealing and Defense Mechanisms in Peri-Implant Mucosa of Dental Implants

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1. Introduction

Much attention during the early stages of basic and clinical research on dental implants has been focused on the bone (jaw)-to-titanium (implant) interface, because direct bone contact with the implant was thought to be a critical factor in implant therapeutics. However, since wide acceptance of the important concept of "osseointegration", developed by Branemark et al. (1977) in the late 1960's, biomaterial implants, usually titanium, have been shown to be able to bind directory and tightly to the bone surface at the engraftment sites, with no tissue intervention. This concept also avoids the risk of fibrous encapsulation, indicating that successful biological and clinical sealing can occur at the bone-to-implant interface (Kajiwara et al., 2005). The applications of titanium implant therapy have therefore increased for patients in medical and dental clinics.

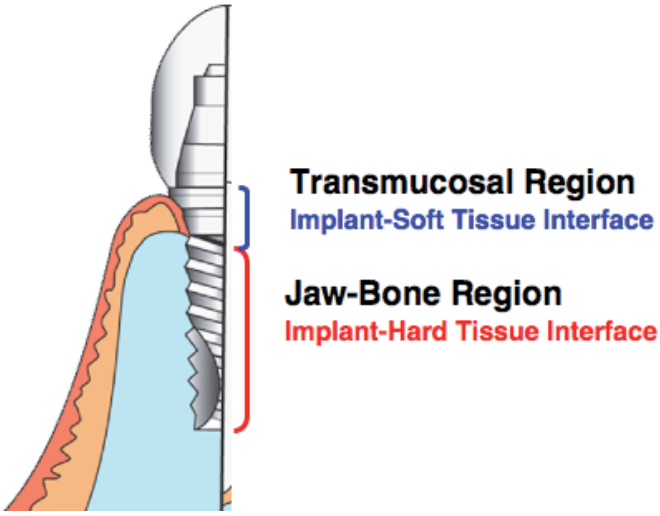

Fig. 1. Schema of biological region around dental implants. A unique part of dental implants is their pierce through oral mucosa. The dental implants are hided in jaw-bone to support the implant body. For functional and aesthetic matters, dental implants expose to the oral cavity. The surrounding tissue of dental implants is biologically divided into two regions; jaw-bone and transmucosal regions.

To date, several factors have been considered to improve the outcomes of clinical dental implants. The functional and aesthetic constraints on dental implants require their insertion through the oral mucosa/gingiva, thus inevitably establishing a transmucosal region between the oral cavity and the dental implant body (Figure 1). This environment exposes dental implants to several external stimuli from the oral cavity through the implant-soft tissue interface. Inflammation of the area surrounding the dental implant is one of most critical problems associated with the clinical failure and short- and long-term maintenance of dental implants, in a similar manner to the problems of tooth-loss caused by periodontal disease (Baillie et al., 2004; Esposito et al., 2010). Bacterial invasion of the transmucosal region leads to the progressive destruction of the peri-implant tissues and their subsequent failure (Mombelli, 1999), indicating that effective protection of the peri-implant mucosa is mandatory (Pontoriero et al., 1994; Tonetti & Schmid, 1994) (Figure 2). Regeneration of firm soft tissues surrounding dental implants, especially in the transmucosal region, is thus required for the long-term success of therapeutic dental implants (Grusovin et al., 2008; Canullo et al., 2011).

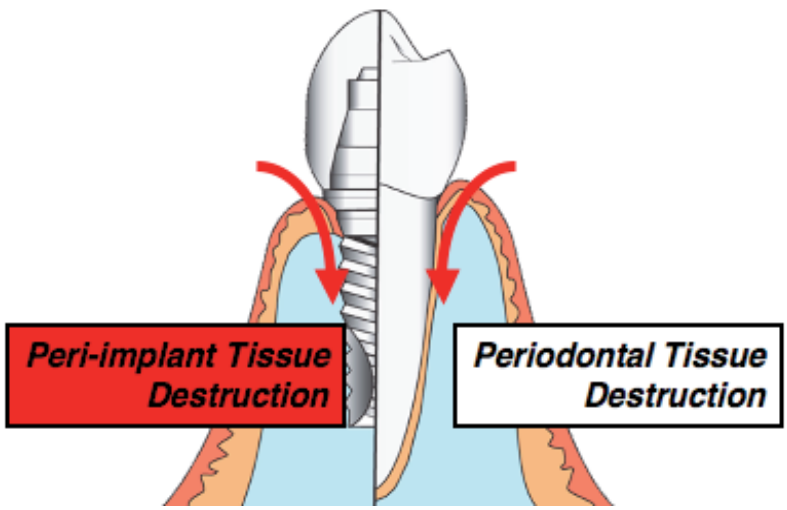

Fig. 2. Schema of a clinical importance of the transmucosal region around dental implants. A variety of oral pathogens (red arrows), likely bacteria and their products, are able to penetrate into the submucosal tissue around dental implants through the transmucosal region around dental implants, followed by causing the destruction of peri-implant tissue, similar to the destruction of periodontal tissue around tooth.

Dental implant research has focused on the interface between dental implants (titanium) and the surrounding soft tissues (Cairo et al., 2008; Grusovin et al., 2008). *In vivo* and *in vitro* investigations can help to understand the structural, functional and molecular properties of

the biological seal and defense mechanisms acting at the interface between the peri-implant mucosa and dental implants (Baschong et al., 2001; Chai et al., 2010). Histological analysis of *in vivo* models, including animal and human subjects, is one of the gold standard methods for investigating the mechanisms at the implant-soft tissue interface. However data on peri-implant tissue from human subjects are scarce because of the limited collecting opportunities and ethical issues (Piattelli et al., 1993, 1997a, 1997b; Arvidson et al., 1996; Corpe et al., 1999; Baschong et al., 2001), animal models have therefore been widely used (Albrektsson et al., 1985; Buser et al., 1992; Berglundh et al., 1994; Berglundh & Lindhe, 1996; Weber et al., 1996; Abrahamsson et al., 1998, 2001, Fujii et al., 1998, 2003; Kawahara et al., 1998; Moon et al., 1999; Hermann et al., 2000, 2001). Peri-implant tissue contains both hard (bone) and soft (mucosa) tissues, which presents a challenge in terms of the histochemical examination of the intact implant-soft tissue interface.

This chapter reviews the morphological and functional features of the soft tissue surrounding dental implants, with emphasis on the epithelial interface between the implant and the peri-implant mucosa. The evidence is based on recent animal studies, especially our investigations using a unique oral implant rat model (Ikeda et al., 2000, 202; Atsuta et al., 2005a, 2005b; Yamaza et al., 2009). This *in vivo* model uses a 4-week implantation system, immediately after tooth extraction (maxillary first molar). Screw-type pure titanium implants (4 mm long, 2 mm in diameter) were inserted, and a complete peri-implant mucosa developed around the titanium body. Furthermore, this model allows the preserved implant-soft tissue interface to be examined at the ultrastructural level, including cellular features (e.g., microvilli, cytoplasmic processes, cytoplasmic organelles of epithelial cells, nerve fibers and terminals, blood vessel components, immune cells) and the epithelial attachment apparatus (basal lamina and hemidesmosomes).

2. Topological features of peri-implant mucosa

A tissue surrounding tooth, known as periodontium, is characterized by four types of tissue: periodontal ligament, cementum, alveolar bone, and gingiva (Schroeder, 1986). These components play critical roles in the support, maintenance, and repair of the tooth and the supporting tissue under both physiological and pathological conditions. The gingiva is a special oral mucosa that surrounds the tooth surface enamel, and covers the alveolar bone that supports the tooth (Schroeder & Listgarten, 1997). This mucosa is composed of connective tissue and epithelial components (Figures 3a and 4a). The gingival connective tissue component, the lamina propria, attach directly and tightly to the alveolar bone, while the gingival epithelium, which overlies the lamina propria, faces the oral cavity and the tooth surface. A tiny slit between the tooth surface and the gingival epithelium, known as oral sulcus, is present even under healthy conditions. This narrow space forms a route for outward flow from the sub-epithelial tissue, as well as an inward pathway into the connective tissue.

To achieve their therapeutic function based on recent established clinical techniques, dental implants pierce the oral mucosa and are inserted into the alveolar bone (Branemark et al., 1977). A tissue surrounding dental implant, known as peri-implant tissue, exhibits various morphological and structural similarities to the natural periodontium; it contains implant-supporting jaw bone and surrounding oral mucosa (peri-implant mucosa), but no

intercalated tissue between the bone and implant surface equivalent to the periodontal ligament and cementum.

The peri-implant mucosa is specifically acquired after dental implant surgery, and resembles the natural gingiva, consisting of peri-implant connective tissue and peri-implant mucosal epithelium (Ikeda et al., 2000) (Figures 3b and 4b). The peri-implant connective tissue integrates with the surface of the jaw bone supporting the dental implant body, while the peri-implant mucosal epithelium faces the oral cavity and the implant surface. There is also an acquired fissure between the implant surface and the peri-implant mucosa, known as the peri-implant sulcus.

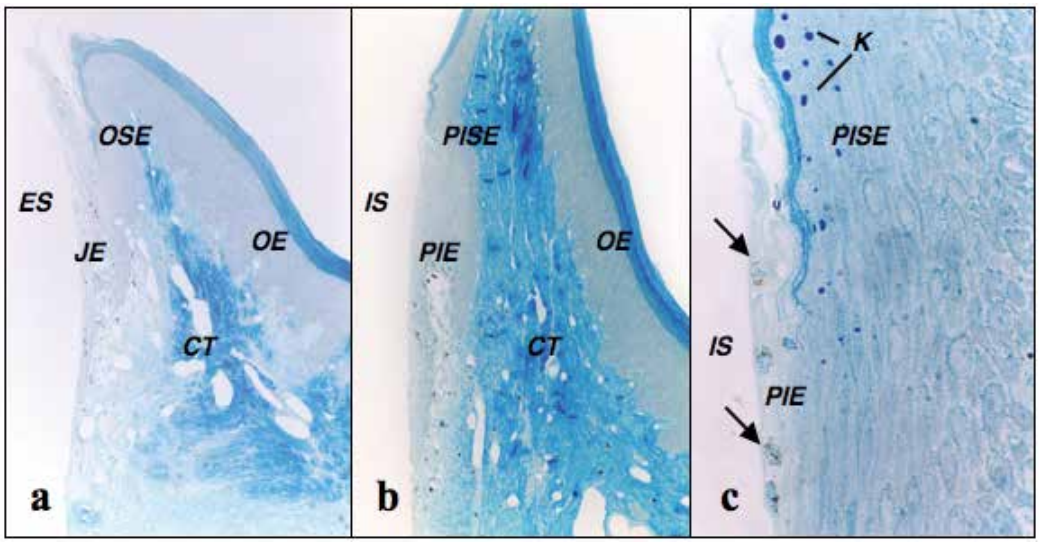

Fig. 3. Light micrographs of healthy gingiva and peri-implant mucosa in rats. (a) Healthy gingiva consists of an epithelial component and a connective tissue component (*CT*). The gingival epithelium can be divided into three parts: oral epithelium (*OE*), oral sulcular epithelium (*OSE*), and junctional epithelium (*JE*). *ES*: enamel space. (b, c) The components of peri-implant mucosa resemble those of the healthy gingiva. The peri-implant mucosa epithelium consists of oral epithelium, peri-implant sulcular epithelium (*PISE*), and peri-implant epithelium (*PIE*). The oral epithelium and peri-implant sulcular epithelium in the peri-implant mucosa, as well as the oral epithelium and oral sulcular epithelium in healthy gingiva, are keratinized, stratified squamous epithelia, while the PIE and junctional epithelium are non-keratinized epithelia with invading neutrophils (arrows). Blood vessels are developed under the PIE and junctional epithelium. *IS*: implant space. *K*: keratohyaline granules. Toluidine blue staining.

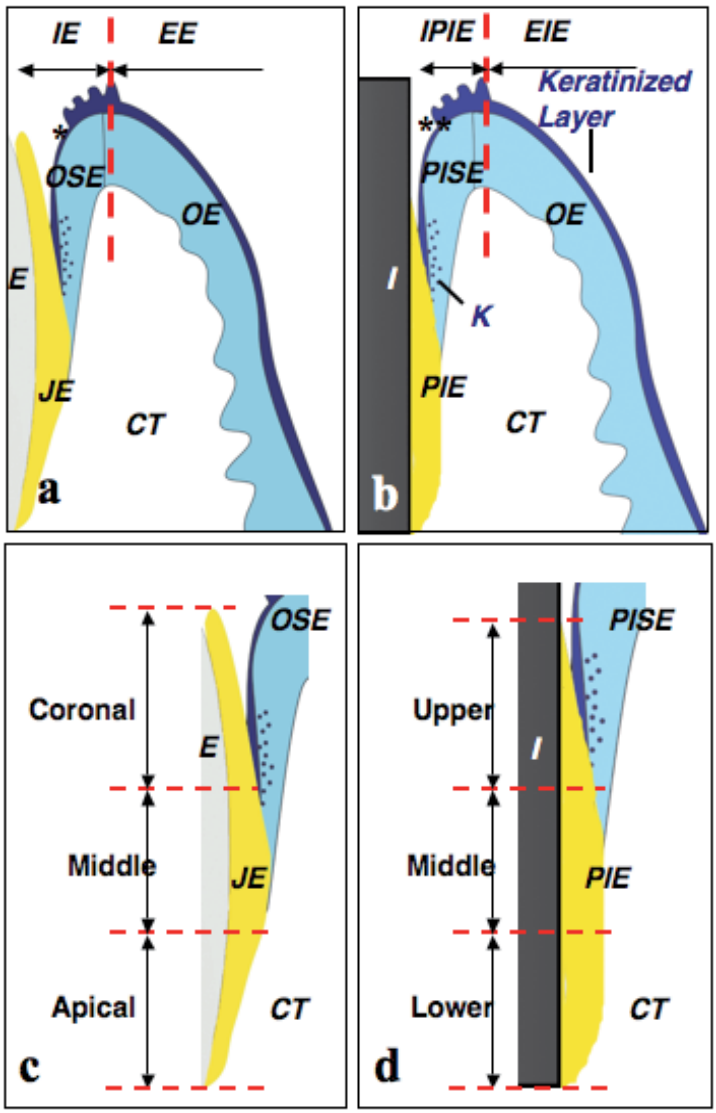

Fig. 4. Schemata of gingiva and peri-implant mucosa. (a) The gingival epithelium can be divided into oral epithelium (*OE*), oral sulcular epithelium (*OSE*), and junctional epithelium (*JE*). The external marginal epithelium (*EE*) covers the gingiva facing the oral cavity, while the inner marginal epithelium (*IE*) covers the gingiva facing the tooth. The space (asterisk) between the enamel (*E*) and inner marginal epithelium is the oral sulcus. *CT*: connective tissue. (b) Epithelia in the peri-implant mucosa consist of the oral epithelium, peri-implant sulcular epithelium (*PISE*), and peri-implant epithelium (*PIE*). The external peri-implant epithelium (*EPIE*) faces the oral cavity, while the inner peri-implant epithelium (*IPIE*) faces the implant (*I*). The peri-implant sulcus (double asterisk) comprises a narrow space between the inner peri-implant epithelium and the implant surface. (c) Natural junctional epithelium is divided into three regions: the coronal, middle and apical regions. (d) The PIE is also divided into three regions: the upper, middle and lower regions.

3. Peri-implant mucosal epithelium

The peri-implant mucosal epithelium exhibits histological and structural features similar to those of the natural gingival epithelium (Ikeda et al., 2000, 2002) (Figures 3 and 4). This epithelium is classified as a stratified squamous epithelium, and consists of three parts: oral epithelium, peri-implant sulcular epithelium, and peri-implant epithelium (PIE) (Figures 3b and 4b), equivalent to the parts of the natural gingival epithelium: oral epithelium, oral sulcular epithelium and junctional epithelium (Figures 3a and 4a). The junctional epithelium is considered to be unique among the three types of gingival epithelium, and participates in forming a fixed tooth-gingiva interface (Schroeder, 1986; Schroeder & Listgarten, 1997).

Topologically, the peri-implant mucosal epithelium is also divided into two parts in relation to the dental implant surface (Ikeda et al., 2000, 2002; Atsuta et al., 2005b) (Figures 4a and 4b). The external-implant marginal epithelium is the part of the epithelial component facing the oral cavity, and consists of the oral epithelium, and the inner-implant marginal epithelium lies directly against the implant surface and the peri-implant sulcus.

3.1 Oral epithelium

The oral epithelium of the peri-implant mucosal epithelium is directly exposed to the oral cavity, forming the external-implant marginal epithelium of the peri-implant mucosal epithelium (Ikeda et al., 2000, 2002) (Figures 3b and 4b). This epithelium is common to the natural gingival epithelium. Histologically, the oral epithelium forms a keratinized stratified squamous epithelium (Figure 3b). The most superficial layer of this epithelium contains keratin, which helps to protect the oral epithelium from foreign stimuli.

3.2 Peri-implant sulcular epithelium

The peri-implant sulcular epithelium shares histological and topological properties with the natural oral sulcular epithelium (Ikeda et al., 2000, 2002). This epithelium, which forms part of the inner-implant marginal epithelium, forms a collar around the peri-implant sulcus (Figures 3b and 4b). The peri-implant sulcular epithelium is keratinized, similar to the oral epithelium, but also contains keratohyaline granules, indicating a keratinized barrier (Figure 3c). The basal layers of both the oral and peri-implant sulcular epithelia form general epithelial-connective barriers, including basement membrane and hemidesmosomes, which join the epithelium to the sub-epithelial tissue. The peri-implant sulcus provides a direct connection to the oral cavity, and acts as a passage for foreign substances into the peri-implant tissue (Ikeda et al., 2002) (Figure 5a), in a similar manner to the oral sulcus (Schroeder & Listgarten, 1997).

3.3 PIE

The PIE, the other part of the inner-implant epithelium, demonstrates unique and specific characteristics, forming a solid transmucosal interface around the dental implant (Ikeda et al., 2000, 2002) (Figures 3 and 4). The topological and structural features of the PIE resemble those of the tooth-enamel interface epithelium, the junctional epithelium, suggesting an important function for the transmucosal region around dental implants in biological sealing and defense.

4. Biological characteristics of the PIE

4.1 Topological and ultrastructural features of the PIE and PIE cells

PIE is formed by epithelial cells derived from the oral epithelium and/or residues of the junctional epithelium after tooth extraction (Fujii et al., 1998; Atsuta et al., 2005a). The epithelial cells move and grow down along the implant surface whilst secreting laminin 5, resulting in reorganization of the intercalated epithelial tissue to form the PIE (Atsuta et al., 2005a).

PIE and PIE cells show some structural and cellular phenotypic similarities to natural junctional epithelium and junctional epithelial cells (Ikeda et al., 2000). The PIE is a non-keratinized and stratified squamous epithelium (Figures 3b, 3c), consisting of basal and supra-basal cell layers. It is located between the surface of the dental implant and the lamina propria of the peri-implant mucosa (Figure 5a, 5b). Blood vessels, especially post-capillary venules, mostly occur under the sub-PIE connective tissue, compared to other sub-peri-implant epithelial tissue (Figure 3b).

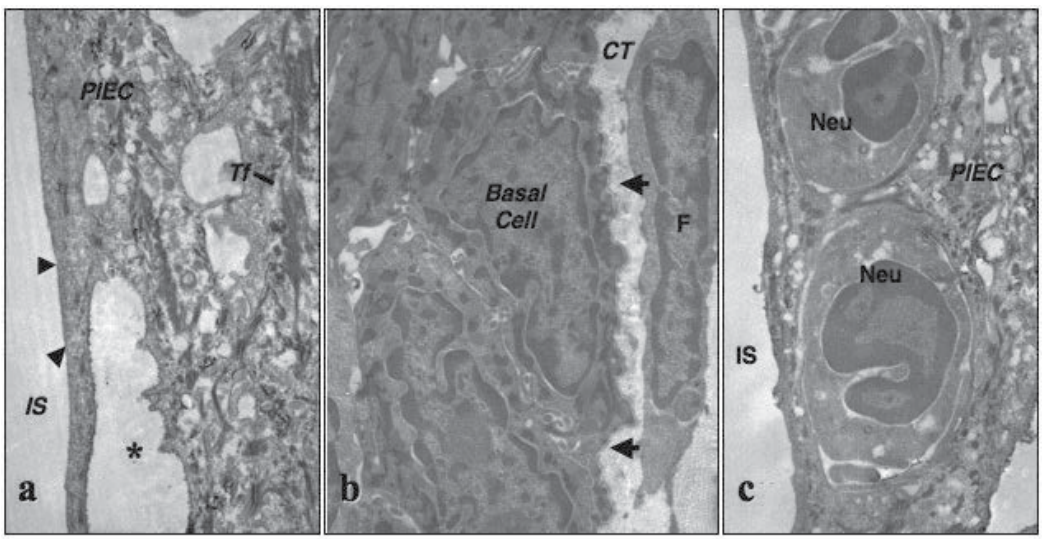

Fig. 5. Electron micrographs of the transmucosal region of the peri-implant mucosa. (a) Typical electron micrograph of the suprabasal peri-implant epithelium (*PIE*), especially the lower PIE. PIE cells (*PIEC*) are arranged parallel to the implant surface, are flattened, and contain a variety of vesicles and vacuoles. Tonofilaments (*Tf*) are localized within the PIE cells. Wide intercellular spaces (asterisk) are found between the cytoplasmic processes. Internal basement lamina (arrowheads) is laid on the innermost PIE cell. *IS*: implant space. (b) The basal part of the PIE consists of cuboidal cells (*Basal Cell*) lining the external basal lamina (arrows). *CT*: connective tissue, *F*: fibroblast. (c) Neutrophils (*Neu*) migrate into the intercellular spaces between PIE cells.

PIE cells are flattened, undifferentiated epithelial cells with few organelles, such as mitochondria and endoplasmic reticulum (Ikeda et al., 2000) (Figure 5a). The PIE cells are arranged parallel to the implant surface. They extend their cytoplasmic processes to other PIE cells, and connect via desmosomes to form wide intracellular spaces (Figure 5a). Neutrophilic granulocytes leak from the blood vessels of the sub-epithelial connective tissue

and invade into the PIE (Ikeda et al., 2000; Yamaza et al., 2009) (Figure 5c). These ultrastructural characteristics support the idea that the PIE acts as a pathway not only for foreign molecules penetrating into the sub-epithelial connective tissue of the peri-implant mucosa (Ikeda et al., 2002), but also for the flow of peri-implant cervicular fluid from the sup-epithelial tissue (Eley et al., 1991). Both inward (McDougall, 1971; Romanowsky et al., 1988; Yamaza et al., 1997) and outward (Golub et al., 1976; Tanaka 1984; Tanaka and Sakano, 1987) flow are also recognized in the junctional epithelium.
The PIE cells also contain tonofilaments (Figure 5a) that associate with desmosomes and hemidesmosomes (Schroeder, 1986). Many lysosomal vesicular and vacuolar structures are located in the cytoplasm (Ikeda et al., 2000; Yamaza et al., 2009) (Figures 5a and 10c).

4.2 Ultrastructural features of transmucosal epithelial attachment around dental implants

4.2.1 Epithelial attachment around natural teeth

Epithelial attachment components in the junctional epithelium are located throughout the tooth-gingiva interface. The attachment apparatus includes hemidesmosomes and basement lamina, which in turn comprises the external basement lamina (EBL) and the internal basement lamina (IBL) (Schroeder, 1986). The EBL, which is formed between the basal cells of the junctional epithelium and the connective tissue, shows a typical basement-membrane structure. The IBL, however, is a unique and specific adhesive structure at the interface between the innermost junctional epithelial cells and the enamel surface. Both the IBL and the EBL consist of two laminal structures, known as the lamina densa and the lamina lucida. These contain the major components of the basal lamina, laminin-1 and laminin-5 (Sawada et al., 1990; Hormia et al., 1998; Mullen et al., 1999). Hemidesmosomes are arranged in the cytoplasm under the plasma membrane of the junctional epithelial cells to anchor the IBL and EBL (Schroeder, 1986).

4.2.2 Epithelial attachment around dental implants

The PIE is divided to three regions: the upper, middle and lower regions (Ikeda et al., 2000) (Figure 4c), equivalent to the three regions of the junctional epithelium: the coronal, middle, and apical regions (Tanaka, 1984) (Figure 4d). The upper region of the PIE is closest to the peri-implant sulcus, while the lower region is connected to the sub-PIE tissue. The middle region is intercalated between the upper and lower regions. The PIE expresses a unique distribution of epithelial attachment, compared to the junctional epithelium (Ikeda et al., 2000; Atsuta et al., 2005b).
Several studies have demonstrated the formation of attachment structures at the titanium-epithelium interface both *in vivo* and *in vitro* (Gould et al., 1984; Mckinney et al., 1985; Donley & Gillette, 1991). At the internal interface between the implant and the PIE, the epithelial attachment apparatus, including the basal lamina and hemidesmosomes, are limited to the lower region of the PIE (Ikeda et al., 2000; Atsuta et al., 2005b) (Figures 5a and 6c). The hemidesmosomes lie beneath the plasma membrane of the innermost PIE cells. The basal lamina of the IBL shows structural similarity with the lamina densa and lamina lucida of the natural junctional epithelium. Laminin-1 and laminin-5 are strongly expressed and distributed heterogeneously in both the lamina densa and lamina lucida of the PIE IBL-like structure similar to the IBL of the junctional epithelium (Ikeda et al, 2000: Atsuta et al., 2005b) (Figure 6c). This indicates that the lower PIE provides epithelial attachment structures similar to those in the natural junctional epithelium. However, no comparative

studies have yet elucidated the distribution of hemidesmosomes at the internal interface of the PIE.

In the upper and middle regions of the innermost interface, the innermost PIE cells are close to the implant surface, but epithelial attachment structures are absent or rare (Ikeda et al., 2000; Atsuta et al., 2005b) (Figure 6b). The innermost PIE cells extend short cytoplasmic processes to the titanium surface, probably forming a loose attachment (Ikeda et al., 2000) (Figure 6b). Laminin-1 and laminin-5 are located in the innermost cells, but are not deposited on the interface (Ikeda et al., 2000; Atsuta et al., 2005b) (Figure 6b).

The external interface is formed between the basal PIE cells and the sub-epithelial connective tissue (Figure 5b). Basal lamina and hemidesmosomes are located throughout the external interface (Ikeda et al., 2000; Atsuta et al., 2005b). The basal lamina contains laminin-1 and laminin-5 and shows feature in common with the EBL of the natural junctional epithelium. Some part of the EBL of the PIE, especially in the lower region, is discontinuous, indicating epithelial migration of PIE/PIE cells (Atsuta et al., 2005b).

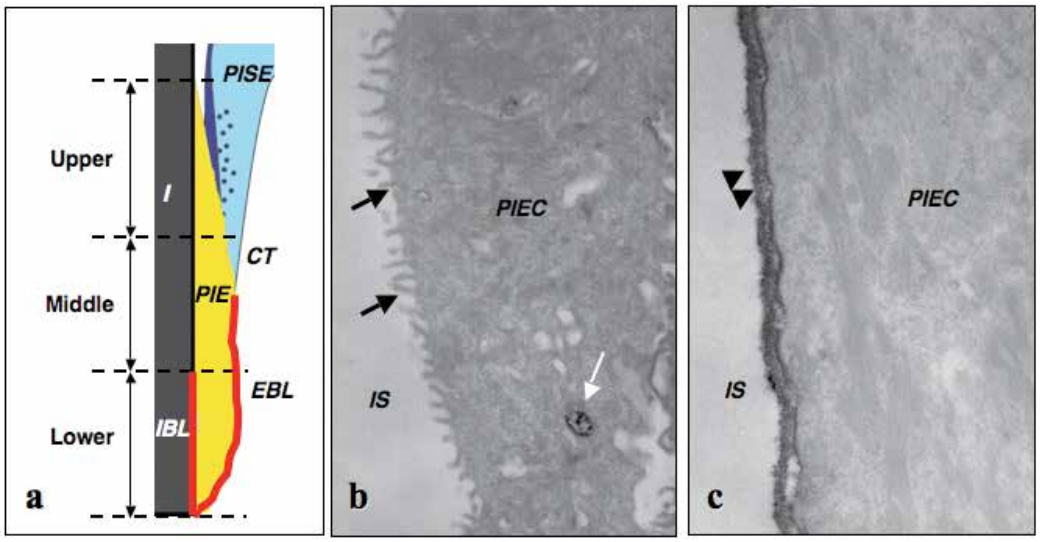

Fig. 6. Interface of the transmucosal region of the peri-implant mucosa (a) Schema of the transmucosal region of the peri-implant mucosa. The transmucosal region consists of the peri-implant epithelium (*PIE*). Only the inner interface of the lower portion of the PIE has an internal basement lamina (*IBL*), and IBL-like structure is lacking in the other portions. The external interface, the external basement lamina (*EBL*), is found through the PIE. *I*: implant, *PISE*: peri-implant sulcular epithelium. *CT*: connective tissue. (b, c) Immunoelectron micrographs showing the localization of laminin 5 at the inner interface (*IS*) of the PIE. (b) No specific epithelial attachment structures are found in the upper region of the PIE. An innermost PIE cell (*PIEC*) extends short cytoplasmic processes (arrows) to the implant surface. No laminin 5 is detected at the interface between the PIE cell and titanium, but laminin 5 is recognized in vesicles within PIE cells (white arrow). (c) In the lower region, IBL-like structure is found between the innermost PIE cell and the implant surface. Laminin 5-immunnoproducts (arrows) are deposited in the IBL structures, the lamina densa and lamina lucida.

4.3 Innervation of PIE by sensory nerve fibers

The natural junctional epithelium, as well as its sub-epithelial connective tissue, is abundantly supplied by nerve fibers derived from the trigeminal ganglion (Byers & Holland, 1977; Kondo et al, 1992; Sugaya et al., 1994). The sensory nerve fibers contain the neuropeptides, calcitonin gene-related peptide (Byers et al., 1987; Nagata et al., 1992, 1994) and substance P (Nagata et al., 1992, 1994; Tanaka et al., 1996; Kido et al., 1999), and terminate close to endothelial cells, neutrophils, and junctional epithelial cells (Kondo et al, 1992; Tanaka et al., 1996).

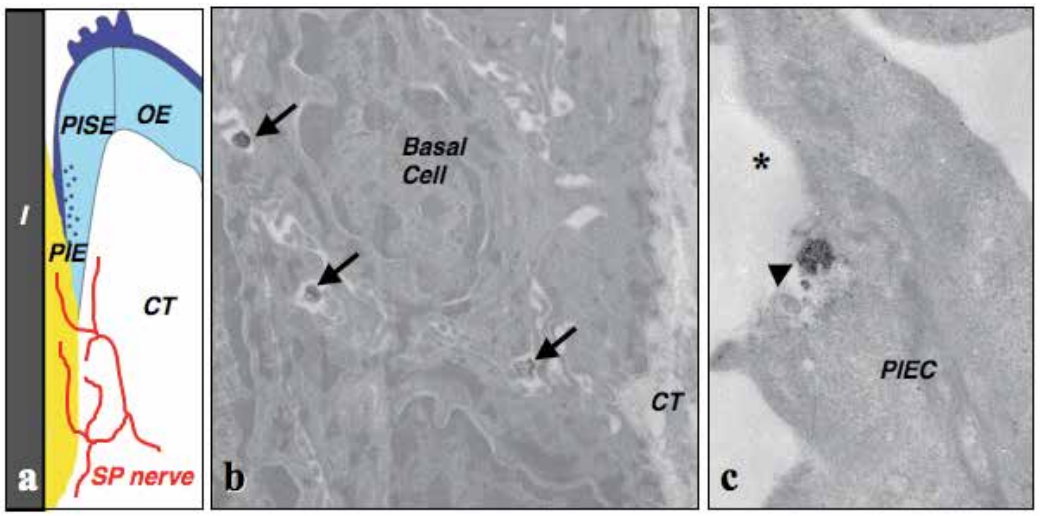

Fig. 7. Distribution of sensory nerve fibers in the transmucosal region of the peri-implant mucosa. (a) Schema of innervation by substance P-containing fibers (*SP nerve*) in peri-implant mucosa. The peri-implant epithelium (*PIE*) is rich in substance P-containing nerve fibers, compared to other epithelia. *CT*: connective tissue, *I*: implant, *OE*: oral epithelium, *PISE*: peri-implant sulcular epithelium. (b, c) Immunoelectron micrographs showing the localization of substance P-containing nerve fibers in the PIE. Nerve fibers with substance P immununoproducts (arrows) penetrate into the PIE via the intercellular spaces between basal PIE cells (*Basal Cell*) (b). The substance P-positive nerve ending (arrowhead) is closely localized to a PIE cell (*PIEC*) (c) Asterisk: intercellular space between PIE cells.

The peri-implant mucosa is also supplied with sensory nerves containing calcitonin gene-related peptide (Fujii et al., 2003) and substance P (Yamaza et al, 2009) (Figure 7a). The innervation of the PIE is denser than in other parts of the epithelium (peri-implant sulcular epithelium and oral epithelium) (Figure 7b). The free ends of the nerve fibers terminate close to PIE cells (Figure 7c), neutrophils and endothelial cells (Yamaza et al, 2009). In addition, neurokinin-1 receptors, which are receptors for substance P, are expressed on the extra- and intra-epithelial nerve fibers, endothelial cells and PIE cells (Yamaza et al, 2009) (Figures 8a, 8b, and 8d). These receptors are also localized in neutrophils invading into the intercellular spaces between PIE cells (Figure 8c). Overall, the PIE shows a similar distribution of substance P-containing sensory nerve fibers and their neurokinin-1 receptors, compared to junctional epithelium (Kondo et al, 1992; Tanaka et al., 1996; Kido et al., 1999).

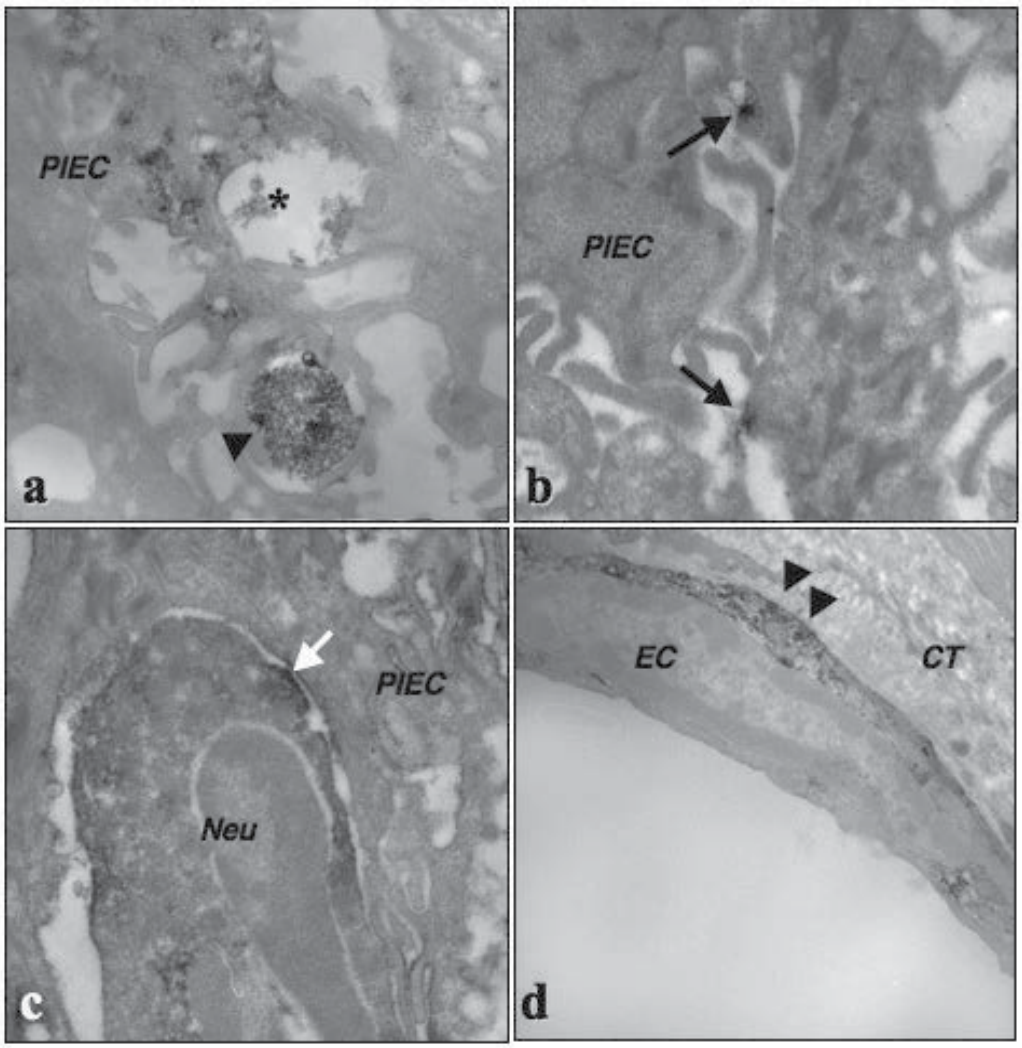

Fig. 8. Distribution of neurokinin 1 receptors in the peri-implant epithelium (*PIE*). (a) Neurokinin 1 receptor-positive products (arrowhead) are seen in a nerve ending close to a PIE cell (*PIEC*). Asterisk: intercellular space between PIE cells. (b) Neurokinin 1 receptor-positivity (arrows) can be detected on the plasma membrane of a PIE cell. (c) An invading neutrophil (*Neu*) was positive for neurokinin 1 receptors (white arrow). (d) A nerve fiber close to an endothelial cell (*EC*) was also positive for neurokinin 1 receptors (double arrowhead). *CT*: connective tissue.

5. Biological sealing and defense mechanisms in the transmucosal region around dental implants

The junctional epithelium is a critical transmucosal region for innate defense against periodontal inflammation. Various mechanisms of peripheral host defense have been demonstrated in this epithelium (Schroeder & Listgarten, 1997) (Figure 9): (1) phagocytosis

and anti-bacterial activity of neutrophils infiltrating into the junctional epithelium (Yamasaki et al., 1979, Tanaka et al., 1988), (2) outward flow of gingival sulcular fluid through the junctional epithelium (McDougall, 1970; Tanaka, 1984; Tanaka and Sakano, 1987), (3) fast turnover or apoptosis of junctional epithelial cells (Schroeder, 1986, Ekuni et al., 2005), (4) continuous epithelial attachment via the IBL throughout the enamel surface (Squier, 1991; Bartold, et al., 2000), (5) endocytotic capacity of junctional epithelial cells for external pathogens (Yamasaki et al., 1979; Tanaka 1984; Tanaka & Sakano, 1987; Ayasaka et al., 1989, Yamaza et al., 1997), and (6) neurotrophic modulation in the junctional epithelium (Kondo et al., 1995; Tanaka et al., 1996; Kido et al, 1999).

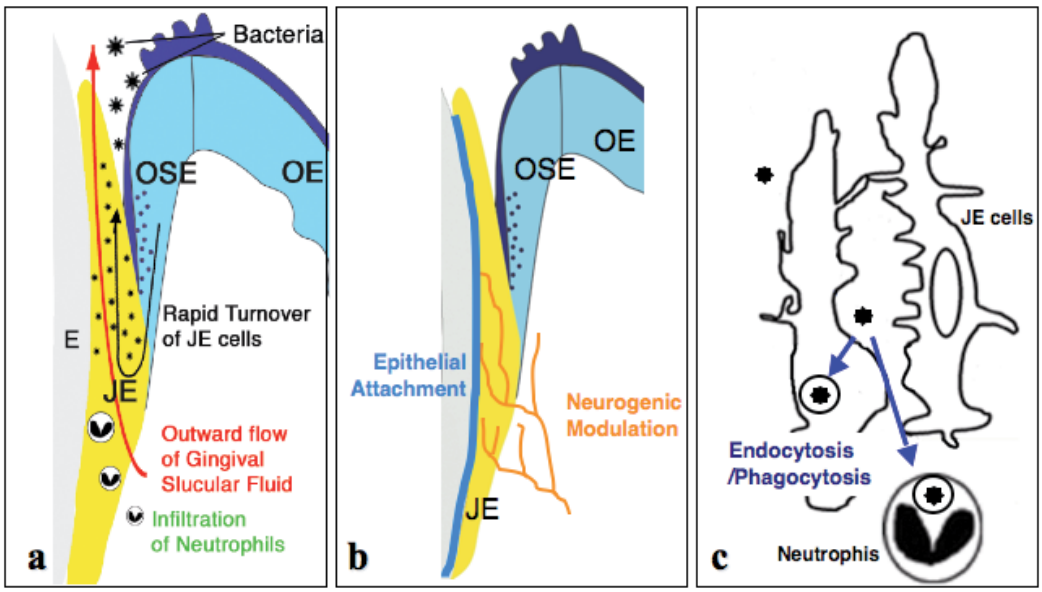

Fig. 9. Schemata of defense mechanisms in the junctional epithelium (JE) around natural teeth. To block invading pathogens, JE has defensive mechanism of (a) outward flow of gingival sulcular fluid through the junctional epithelium (red arrow), fast turnover or apoptosis of junctional epithelial cells (black arrow), and neutrophils infiltrating into the junctional epithelium, (b) continuous epithelial attachment via the internal basement lamina throughout the enamel surface (blue line, and neurotrophic modulation by sensory nerve innervation in the junctional epithelium (orange lines), and (c) endocytotic capacity of junctional epithelial cells and phagocytosis of neutrophils for external pathogens (blue arrows). E: enamel, OE: oral epithelium, OSE: oral sulcular epithelium.

The ultrastructural findings support the idea that the PIE can allow the penetration of foreign molecules into the sub-epithelial connective tissue, indicating that the PIE acts as a first line of defense in protecting against the invasion of several pathogens (Ikeda et al., 2000, 2002). Transmucosal defense around dental implants is suggested to involve the acquisition and maintenance of a similar defense system to that shown by the junctional epithelium. This review considers three aspects of the mucosal defense system around dental implants; (1) PIE-titanium barrier (2) endocytotic system in the PIE, and (3) neurogenic regulation in the PIE (Figure 13).

5.1 Epithelial attachment of PIE-titanium barrier

Laminin-1 and laminin-5 are major components of the basal lamina, and participate in the formation of a molecular network in the basal lamina (Tryggvason, 1993; Burgeson et al., 1994; Aumailley & Krieg, 1996). They also play important roles in cell differentiation, migration, and adhesion, as well being involved in determining cell phenotype and survival (Timpl, et al., 1979). Laminin 5 forms anchoring filaments in hemidesmosomes and promotes their assembly, indicating a strong binding function in the basal lamina (Green & Jones, 1996). This suggests that IBL- and hemidesmosome-like structures contribute, at least in part, to the formation of a tight attachment at the inner interface between the PIE and the dental implant (Ikeda et al., 2000; Atsuta et al., 2005b).

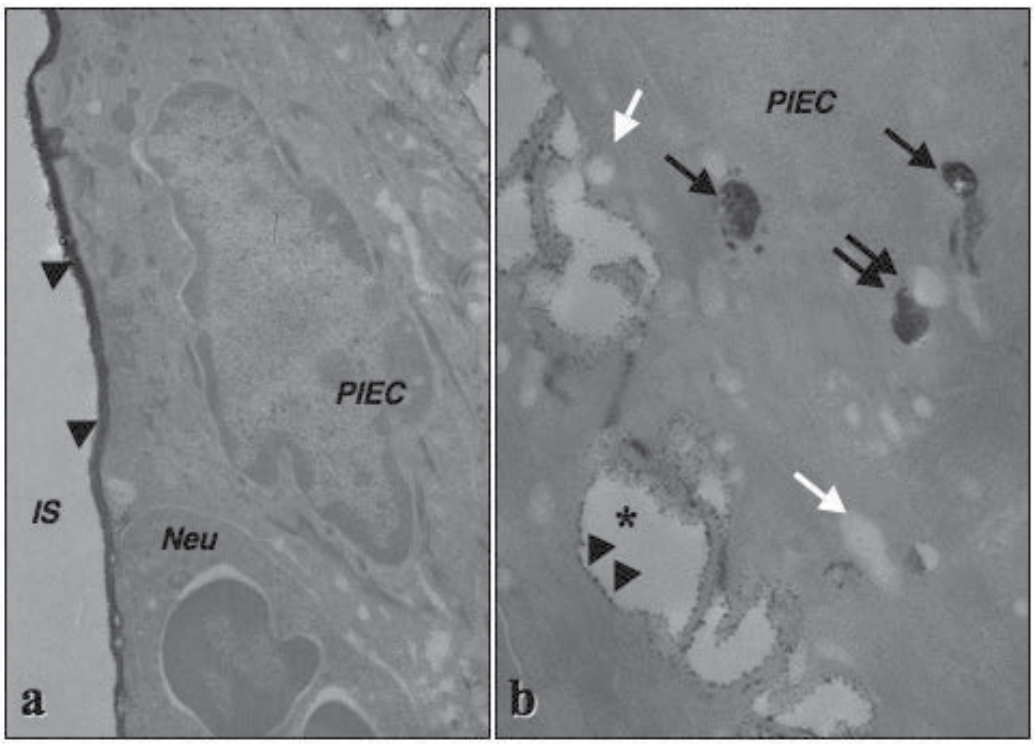

Fig. 10. Electron micrographs showing the localization of applied horseradish peroxidase in the periimplant epithelium. (a) Horseradish peroxidase-reactive products (arrowheads) were found in the internal basement lamina in the lower PIE. However, horseradish peroxidase was not found in the intercellular spaces between PIE cells (*PIEC*). *Neu*: neutrophil, *IS*: implant space. (b) Horseradish peroxidase-reactive organelles show several feature in PIE cells. Endosome-like structures in the cells contained various densities of horseradish peroxidase-reactive products (arrows). Horseradish peroxidase-negative endosomes (white arrows) were also found in PIE cells. Some endosomes were fused with other horseradish peroxidase-negative endosomes (double arrow). Horseradish peroxidase-reactive products (arrowheads) were deposited on the plasma membrane of PIE cells. Asterisk: intercellular space between PIE cells.

The application of horseradish peroxidase (HRP) as a tracer in the mucosa around dental implants or teeth shows a different distribution at each transmucosal interface (Ikeda et al., 2002) (Figures 10, 11). At the natural interface, abundant HRP is stopped at the coronal region of the junctional epithelium and IBL (Yamaza et al., 1997). In contrast, high levels of horseradish peroxidase are widely distributed from the upper to middle regions of the PIE around the dental implants (Ikeda et al., 2002) (Figures 10a, 11a). The lower region also contains HRP, but in smaller amounts (Figures 10b, 11a). The IBL of the lower PIE accumulates exogenous HRP, suggesting a functional role for the IBL in protecting against external invasion (Ikeda et al., 2002) (Figure 11). These findings indicate that the IBL in the PIE participates in local defense around dental implants (Figure 13a).

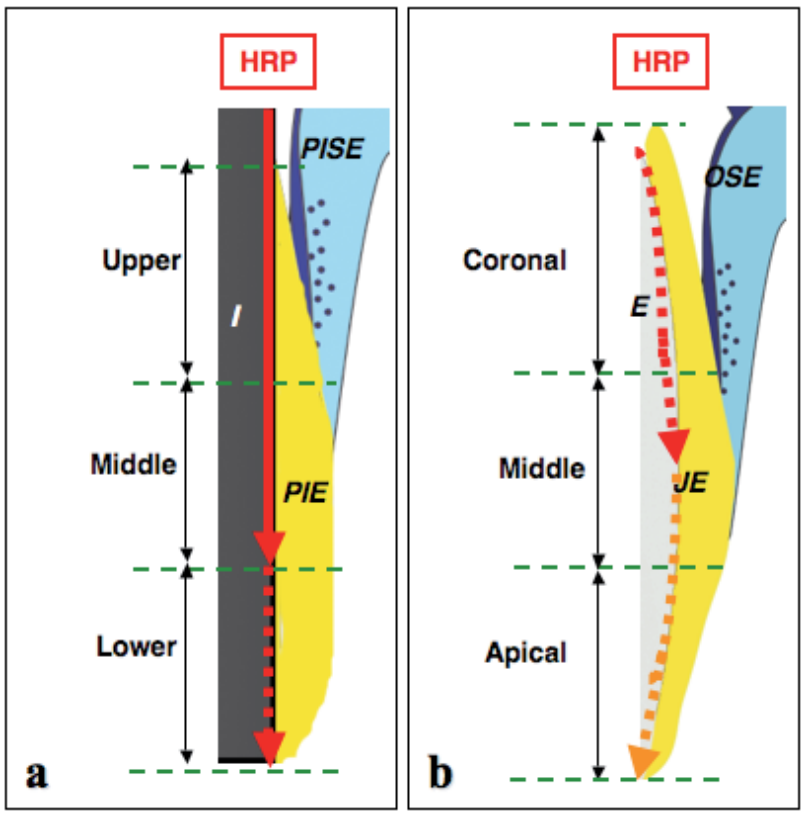

Fig. 11. Schema of the difference of barrier capability of internal basement lamina in the peri-implant epithelium (*PIE*) and junctional epithelium (*JE*). (a) In the PIE, only the lower region has a solid epithelial attachment structure, not in the middle and upper portions. Locally applied horseradish peroxidase (HRP) is easy to penetrate into the PIE thorouth the direct interface between the dental implant (*I*) and PIE (red arrow), but the lower interface showing basal lamina structure defend the penetration (red dot arrow). *PISE*: peri-implant sulcular epithelium. (b) In the natural teeth, basal lamina structure is present throughout the interface between enamel (*E*) and junctional epithelium (*JE*). Loccaly appile HRP is hard to invade along not only to the internal interface of the coronal to middle portion of JE (red dot arrow), but also to the middle to lowe interface (orange dot line).

5.2 Endocytotic system in PIE

Neutrophils generally play an important role in the front-line defense against foreign bodies, through their phagocytotic capacity. They infiltrate into the PIE (Ikeda et al., 2002), suggesting that they are resident or transient leukocytes in the PIE, as well as in the natural junctional epithelium (Yamaza et al., 1997), and function effectively to prevent peri-implant inflammation/disease by their phagocytotic capacity (Ikeda et al., 2002).

Junctional epithelial cells have the ability to endocytose foreign substances (Tanaka et al., 1984; Yamasaki et al., 1985; Ayasaka & Tanaka, 1989; Yamaza et al., 1997). These cells can also digest materials using the intracellular lysosomal system containing aspartic proteinase, cathepsin D (Ayasaka et al., 1993), and the cysteine proteinases, cathepsins B and H (Yamaza et al., 1997). Lysosomal compartments participate in intracellular degradation using lysosomal enzymes (Mellman et al., 1986). The cathepsins show high protein degradative activities (Barrett & Kirschke, 1981), especially in the case of cathepsin B and H (Nishimura et al., 1988). Periodontal pathogens can invade the gingival epithelial cells (Lamont et al., 1995; Rautemaa et al., 2004). PIE cells also contain a variety of intracellular endosome/lysosome systems, suggesting a capacity to take up and digest foreign materials in the vesicles and vacuoles (Ikeda et al., 2000, 2002). PIE cells also show the ability to endocytose HPR applied locally to the peri-implant mucosa, indicating their digestive capacity against foreign materials (Ikeda et al., 2002) (Figure 10b). These results suggest that the endocytotic system of PIE cells, as well as the presence of neutrophils, may play a role in the local defense of the transmucosal region around dental implants (Figure 13).

Cystatin C is an endogenous cysteine proteinase inhibitor (Abrahamson et al., 1986) expressed in junctional epithelial cells, and secreted extracellularly (Yamaza et al., 2005). This natural inhibitor also plays a role in the anti-bacterial activity against the periodontal microorganism, *Porphyromonas gingivalis* (Blankenvoorde et al., 1998), which releases a specific cysteine proteinase (Chen et al., 1992). Cystatin C is present in gingival cervicular fluid (Ulker et al., 2008), supporting the extracellular secretion of this inhibitor into gingival tissues, including the intercellular spaces of the junctional epithelium. Secreted cystatin C from junctional epithelial cells may participate in the inhibition of *P. gingivalis*-derived proteinase activity and suppression of *P. gingivalis* growth, suggesting that cystatin C also acts as a candidate molecule governed by the PIE cell-mediated defense system. Thus several functions of PIE cells may contribute to local defense in the transmucosal region around dental implants.

5.3 Neurogenic regulation in PIE

Substance P is a nociceptive neuropeptide responsible for transmitting pain stimuli, such as those due to chemical irritants, heat, cold or other noxious stimuli. It is expressed in sensory nerves of the peripheral nervous system and participates in the afferent transmission of pain impulses from the mucosa through sensory receptors at the nerve endings (Lundy & Linden, 2004) (Figure 12). In contrast, this transmitter is also released efferently from nerve endings on stimulation by noxious phenomena (Figure 12). Released substance P can affect the responses of the target cells through direct interaction with neurokinin-1 receptors (Scholzen et al., 1998) (Figure 12). The presence of substance P in gingival cervicular fluid supports the release of this neuropeptide from nerve terminals (Linden et al., 1997). Acting via neurokinin-1 receptors, substance P induces several cellular responses (Figure 12), In the endothelial cells, vasodilation, and modulates blood flow and plasma leakage are occurred

by the substance P-nurokin 1 receptor binding (Lembeck & Holzer 1979; Nicoll et al., 1980; Otsuka & Yoshida 1993). The substance P-neurokinin-1 receptor pathway can also enhance endocytosis in neutrophils (Bar-Shavit et al., 1980; Tanabe et al., 1996). Substance P also stimulates the chemotaxis of neutrophils and macrophages, the proliferation and migration of keratinocytes and fibroblasts, degranulation of mast cells, the expression of various adhesion proteins on endothelial cells, and the release of inflammatory cytokines from immune cells (Kähler et al., 1993; Ziche et al., 1994; Scholzen et al., 1998; Koon et al., 2006; Liu et al., 2007). Furthermore, substance P-binding neurokinin-1 receptors are known to regulate the innate immune system (Tuluc et al., 2009; Douglas & Leeman, 2011), and participate in immunomodulation in immune diseases (Koon & Pothoulakis, 2006).

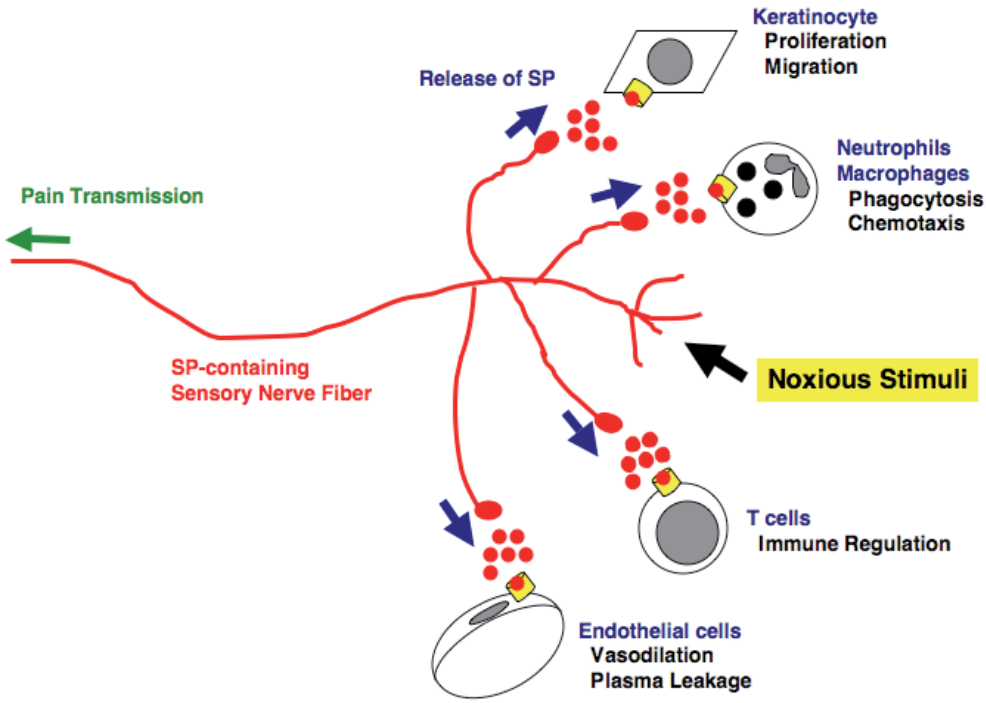

Fig. 12. Scheme of biological functions of substance P. Once the nerve ending catch noxious stimuli such as chemical irritants, heat, cold stimuli, (black arrow) to substance P-containing nerve fibers, substance P has a capable of transmitting pain stimuli (green arrow). On the other hand, substance P can be released from the free nerve endings by the noxious stimuli (blue arrows). Released substance P (red dots) are able to bind to their receptors neurokinin 1 recetors (yellow squeares) on the several types of cells, such as keratinocytes, neutrophils, macrophages, endothelial cells of blood vessels, and T cells to induce a variety of cellular functions, as shown on the scheme.

The distribution of substance P-containing sensory nerve terminals and neurokinin-1 receptors in the PIE (Yamaza et al., 2009) suggests that released substance P may bind to neurokinin-1 receptors on PIE cells, endothelial cells, and intraepithelial neutrophils, and induce a variety of innate defense mechanisms in the PIE (Figure 13). These mechanisms might include the induction of neutrophil infiltration from blood vessels into the PIE, the

promotion of plasma extravasation from blood vessels beneath the PIE subjected to peri-implant sulcular fluid, and up-regulation of the endocytotic capabilities of PIE cells and neutrophils.

Substance P is also known to have anti-microbial activity (Kowalska et al., 2002; El Karim et al., 2008; Douglas & Leeman, 2011). Substance P released from nerve terminals into the intercellular spaces between the nerve endings and neurokinin receptor-bearing cells may inhibit intraepithelial growth of peri-implant bacteria in the PIE. Thus substance P-neurokinin-1 receptor function could, at least partly, govern the local defense mechanisms in this transmucosal region around dental implants, as in the natural junctional epithelium (Kido et al., 1999; Brogden et al., 2005).

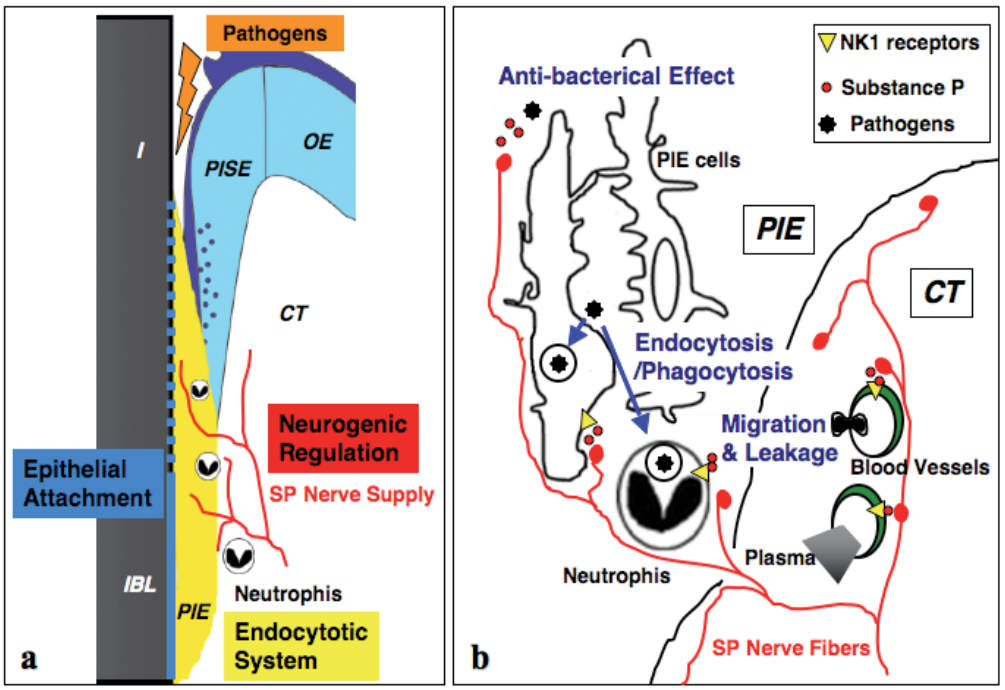

Fig. 13. Schemata of defense mechanisms in the transmucosal region around dental implants. (a) Epithelial attachment structure, internal basement lamina (*IBL*) and substance-P (SP) nerve supply in the peri-implant epithelium (*PIE*) and its subepithelial connective tissuesuggests the restoration of biological seals and defenses against several pathogens (bacteria or their products) from the oral cavity. *CT*: connective tissue, *I*: implant, *OE*: oral epithelium, *PISE*: peri-inplant sulcular epithelium. (b) Invaded pathogens stimulate the release of substance-P from the nerve endings of extra-and intra-epithelial nerve fibers. The released substance-P induces various cellular functions by binding to neurokinin-1 (NK1) receptors localized on endothelial cells, neutrophils, and peri-implant epithelial cells. This linkages result in migration of neutrophils from blood vessels into the peri-implant epithelium, and increased permeability of blood vessels to pass outwardly throughout the peri-implant epithelium. The cascades also promote the endocytosis or phagocytosis of peri-implant epithelial cells or neutrophils. The released substance P may also leads to act as a direct anti-bacterial agent.

6. Conclusion

The transmucosal region around dental implants demonstrates similar anatomical and biological features to the natural interface between the tooth enamel and the junctional epithelium. Regeneration of the PIE with its defense functions will produce a more secure implant-soft tissue interface, and thus improve the success of clinical dental implant therapy.

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11

Ultrastructure of Dentogingival Border of Normal and Replanted Tooth and Dental Implant

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1. Introduction

The interface between the gingiva and the tooth enamel is characterized by the presence of an attachment apparatus composed of well-developed hemidesmosomes at the basal surface of the junctional epithelium and internal basement membrane (Schroeder, 1986; Bosshardt & Lang, 2005). This apparatus plays an important role in the firm attachment of the epithelium to the tooth and in sealing the periodontal tissue from the oral environment. High resolution ultrastructural studies in our laboratory provided further evidence of this effective sealing (Sawada & Inoue, 1996, 2001a, 2003). In the first part of this review article, the ultrastructure of the dentogingival border in a normal tooth is described in detail.
The original attachment apparatus is mechanically broken down immediately after any surgical procedure such as tooth replantation or implantation. Whether the attachment apparatus is regenerated at the dento (implant)-gingival border in either case remains to be determined. In the latter half of this article, we will, therefore, describe the ultrastructure of the dentogingival border in replanted teeth and implants based upon our recent study (Shioya et al., 2009).

2. Materials and methods

The animals used in this study were as follows: Japanese monkeys (*Macaca fuscata*) and Rhesus monkeys (*Macaca mulatta*) provided by the Primate Research Institute of Kyoto University, Kyoto, Japan; a shark (*Cephaloscyllium umbratile*) freshly caught off the coast of Suruga, Shizuoka Prefecture, Japan; and Wistar rats purchased from CLEA JAPAN, Inc., Tokyo, Japan. All experiments were performed in accordance with the "Guidelines for the Use of Experimental Animals at Tokyo Dental College".

2.1 Monkeys

The head and neck regions of 3-5-year-old monkeys were perfused, under anesthesia, with a fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1M sodium cacodylate buffer, pH 7.4, through the carotid arteries for 60 min. Isolated upper and lower jaws were

further fixed by immersing them in the same fresh fixative for 24 hr at 4°C. Molars with associated gingiva were isolated and washed with 0.1 M sodium cacodylate buffer containing 0.2 M sucrose. An aliquot of teeth was demineralized in 10% EDTA for 6-8 weeks at 4°C. Both demineralized and non-demineralized tissues were postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1.5 hr at 4°C, dehydrated in a graded series of ethanol, and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue for light microscopy. Thin sections of tissue containing the internal basement membrane spanning from the cemento-enamel junction to the gingival groove were prepared for observation with either the H-7100 or H-7650 electron microscope (Hitachi Co., Tokyo, Japan), with or without counterstaining with uranyl acetate and lead citrate, and operating at 100 kV.

2.2 Shark

Tooth-bearing jaws of a shark were dissected out under anesthesia with MS222 and cut into small pieces. The pieces were fixed by placing them in a fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, for 24 hr at 4°C. An aliquot of the specimens was demineralized in 10% EDTA for 3-4 weeks at 4°C. Both demineralized and non-demineralized tissues were postfixed with 1% osmium tetroxide in 0.1 M sodium phosphate buffer for 1.5 hr at 4°C. They were further processed as described above for the observation of semi-thin and thin sections.

2.3 Rats

Experiment I: Tooth replantation was performed by a previously described method (Ihara et al., 2007). Briefly, under general anesthesia with ketamine hydrochloride, upper right molars were luxated with a dental excavator and carefully extracted with forceps in order to avoid damaging surrounding tissues. Then, they were immediately replaced in their original sockets. Gingiva around the maxillary left first molars was used as a control. All animals were allowed free access to water and a powdered diet. For ultrastructural examination as described below, the animals were sacrificed at 1, 2 or 4 weeks after the procedure.

Experiment II: After extraction of the tooth by the method described above, a screw-type implant was immediately placed in the socket. A custom-made pure titanium implant (Ti) (Fig. 1) 1.6 mm in diameter and 4 mm in length was used (Platon Japan Co., Tokyo, Japan). Sufficient space was left between the opposing lower first molar and the implant to avoid occlusal stimuli during mastication. After the operation, the animals were allowed access to water and a powdered diet *ad libitum*. They were sacrificed in groups at 1, 2, 4 or 8 weeks after the operation.

Under anesthesia with ketamine hydrochloride, the animals were perfused with a cold fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, for 20 min. Isolated upper jaws were further fixed by immersing them in the same fresh fixative for 5 hr at 4°C, after which they were washed with sodium phosphate buffer and demineralized in 10% EDTA for 4 weeks at 4°C. In experiment II, implants were mechanically separated from the surrounding tissue according to the method of Ikeda et al. (2000). Both replanted teeth with gingiva and peri-implant tissues were postfixed with 1% osmium tetroxide in 0.1 M sodium phosphate buffer for 1 hr, dehydrated in a graded series of ethanol, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate before observation by electron microscopy.

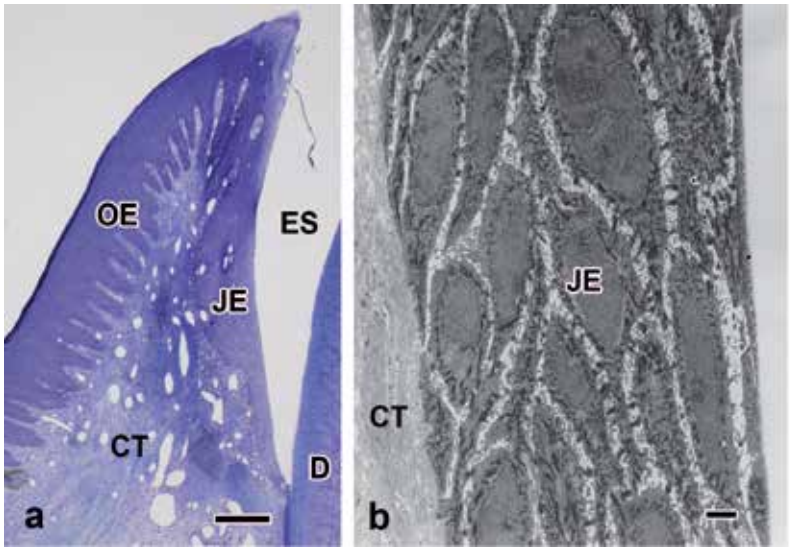
Fig. 1. Photograph showing rat upper jaw implanted with custom-made pure titanium implant

3. Ultrastructure of dentogingival border

3.1 Attachment apparatus in normal tooth

The surface (enamel) of the tooth is known to attach to the gingiva by means of an attachment apparatus. This attachment apparatus is composed of the hemidesmosomes of the junctional epithelium and the internal basement membrane (Listgarten, 1966, 1972; Schroeder, 1969; Schroeder & Listgarten, 1977; Stern, 1981). The internal basement membrane was initially described as an 80-120-nm wide homogeneous layer (Stern, 1981). It directly faced the enamel, and an intervening laminated or non-laminated layer of cuticles was found to be present in dog (Matsson et al., 1979), pig (Marks et al., 1994), monkey (Kobayashi et al., 1976; Sawada & Inoue, 2001b) and man (Listgarten, 1966; Schroeder & Listgarten, 1977). The internal basement membrane was either directly facing the surface of the enamel or doing so through intervening layers of cuticles (Kobayashi et al., 1976; Sawada & Inoue, 2001b) or afibrillar cementum in rhesus monkey (Kobayashi et al., 1976). The latter authors also reported that numerous fine strands crossed the lamina densa of the internal basement membrane at the hemidesmosomes. These strands may have been the anchoring filaments of hemidesmosomes, reported to be composed of kalinin and epiligrin (Eady, 1994; Garrod, 1993). In the cytoplasm of the cells of the junctional epithelium, the tonofibrils are associated with hemidesmosomes.

A more recent study investigated the internal basement membrane of the dentogingival border in monkey by transmission electron microscopy (Fig. 2) and found that it was uniquely specialized for mechanical strength, sealing off the periodontal tissues from the oral environment (Sawada & Inoue, 1996). Morphologically, basement membranes may be classified into three types: common, "thin" basement membranes; "double" basement membranes such as glomerular basement membrane; and often multilayered, "thick" basement membranes such as Reichert's membrane, the lens capsule, and the basement membrane matrix of mouse EHS tumor (Inoue, 1989).



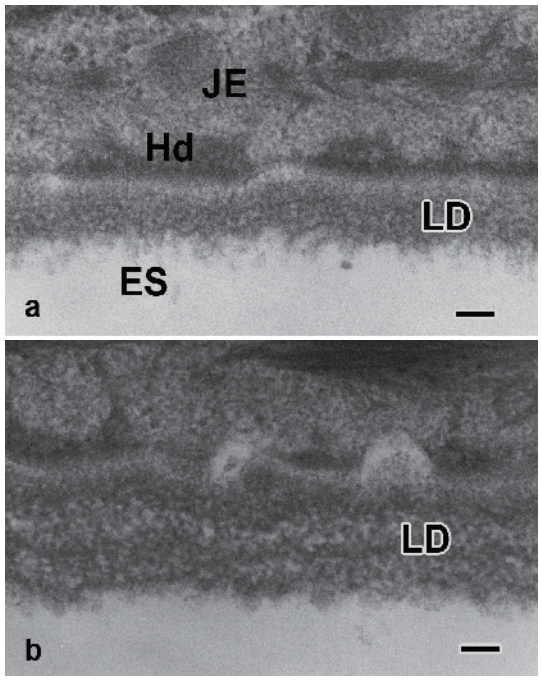
Junctional epithelium (JE) is bordered by enamel (enamel space, ES) and supporting gingival connective tissue (CT). OE, oral epithelium; D, dentin. Scale bars = 100 μm (a), 2 μm (b). Modified from Sawada & Inoue, 2003 © Calcified Tissue International.

Fig. 2. Light micrograph (a) of semi-thin section and electron micrograph (b) of thin section of an area of dentogingival border of monkey tooth

Firstly, in monkey, the internal basement membrane is unique in that it takes the form of both thin and multilayered thick basement membranes (Fig. 3). The thickening and multi-layering of the basement membrane may be directly related to the role of specific basement membranes such as the multilayered Reichert's membrane of the parietal yolk sac (Inoué et al., 1983). The capsular portion of Reichert's membrane provides reinforcement to the parietal wall of the embryonic yolk sac (Jollie, 1968). Similarly, multilayered internal basement membrane may provide mechanical strength for firm attachment of the tooth to the gingiva and the sealing off of the periodontal tissues from the oral environment. The monolayered, thin basement membrane portion of the internal basement membrane is also unique. This lamina densa, at 160 nm in width, is unusually thick compared with 30-80 nm in other types of basement membrane. Another example of unusually thick basement membrane is that of seminiferous tubules in rat (Inoue & Leblond, 1988). Again, the role of this particular basement membrane is mechanical strength to protect the integrity of the epithelium against the rhythmic contractions of the seminiferous tubules for the movement of sperm. Similarly, unusual thickening of the monolayered part of the internal basement membrane at the dentogingival border may provide mechanical strength for the tight sealing of this border.

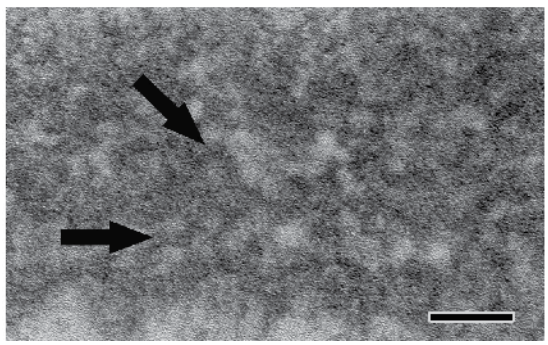
Secondly, the finer level structure of the internal basement membrane, that is, the "cord" network, is also unique (Fig. 4). In basement membrane, in general, the basic texture of the lamina densa is made up of a 3-dimensional network formed by anastomosing, irregular, thread-like structures referred to as "cords" (Inoue, 1989, 1994; Sawada & Inoue, 2001a). In most types of basement membrane, the thickness of the cord is 3 nm-5 nm, and the average size of the openings in the network ("intercordal space diameter index") is 14 nm. The average thickness of the cords and the size of the openings in the internal basement

membrane are 8.3 nm and 18.9 nm, respectively (Sawada & Inoue, 1996). These unusually wide cords and large openings are similar to those found in Reichert's membrane (5 nm and 15 nm, respectively) and the basement membrane of seminiferous tubules (4.5 nm and 14.1 nm, respectively). This indicates the role of the cord network in the mechanical strength of the internal basement membrane.



Internal basement membrane is composed of either single broad lamina densa (a) or multi-layers (b). JE, junctional epithelium; LD, lamina densa; Hd, hemidesmosomes; ES, enamel space. Scale bars = 0.1 μm. Modified from Sawada & Inoue, 1996 © The Anatomical Record.

Fig. 3. Electron micrograph of internal basement membrane of junctional epithelium



Lamina densa is composed of fine network of irregular anastomosing cords (indicated by arrows). Scale bar = 50 nm. Modified from Sawada & Inoue, 1996 © The Anatomical Record.

Fig. 4. High-magnification view of internal basement membrane of monkey tooth

3.2 Dental cuticle at dentogingival border

The dental cuticle is usually found at the dentogingival border in healthy teeth or at the surface of the roots of teeth with periodontal disease. Ultrastructurally, the dental cuticle has been described as an electron-dense, non-mineralized organic structure with an unlaminated amorphous appearance (Schroeder, 1986). In adult periodontitis, the dental cuticle covering the cementum showed a lobulated and layered structure with perforations (Friedman et al., 1993). Histochemical studies indicated that the structure may contain a protein-rich material (Kobayashi & Rose, 1978, 1979; López et al., 1990), or anionic polymers including glycoproteins (Friedman et al., 1993). Based on the results of morphological, as well as histochemical studies, the origin of the dental cuticle has been suggested to be a secretory product of the junctional epithelium (Ito et al., 1967; Listgarten, 1970; Nagatsuka, 1983; Sato, 1973; Schroeder & Listgarten, 1971), that is, either the accumulation of basement membrane components produced by the cells of the epithelium, or the formation of a layer of serum proteins originating from gingival exudates in the process of aging (Eide et al., 1983; Frank & Cimasoni, 1970; Friedman et al., 1993; Lie & Selvig, 1975; López et al., 1990); it has also been suggested to originate in hemoglobin resulting from the degradation of red blood cells (Hodson, 1966). Thus, its origin has yet to be conclusively determined.

In our previous study (Sawada & Inoue, 2001b), the detailed ultrastructural nature of the dental cuticle in monkey tooth was examined by high resolution electron microscopy. The dental cuticle, seen as a dense amorphous, usually unlaminated layer, was localized between the internal basement membrane and the enamel surface (Fig. 5a). High resolution electron microscopy showed that its basic structure was a fine network of irregular anastomosing strands identified as the cord network of the basement membrane of the junctional epithelium, as described above (Fig. 5b).

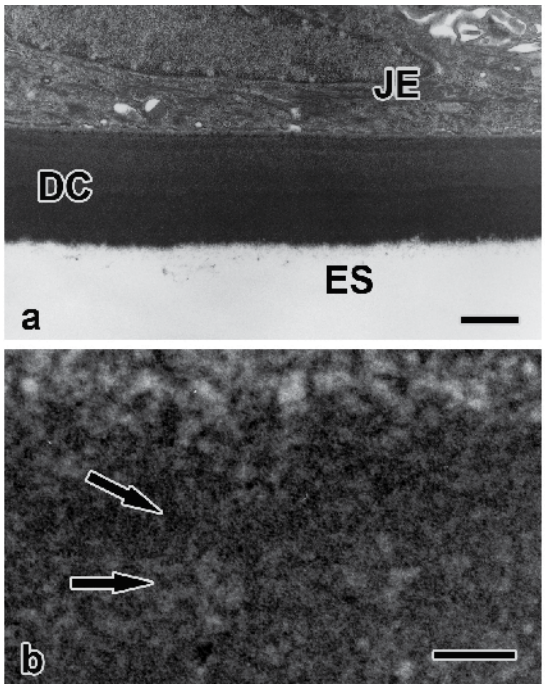
In the cuticle, openings of most of the network were filled with a dark amorphous material. Based upon the data, it was suggested that an additional layer of cord network formed from basement membrane components probably secreted by cells of the junctional epithelium during formation of the cuticle is added to the cord network of the lamina densa of the basement membrane. In addition, a dark amorphous material is deposited within the newly added cord network at the enamel side of the basement membrane. The origin of this dark material still remains to be clarified, but it possibly originates, as has previously been suggested, from either serum protein in gingival exudates or from hemoglobin produced by the degradation of red blood cells.

3.3 Mechanism of binding of normal tooth to gingiva

Detailed ultrastructural observation of the dentogingival border was carried out to elucidate how comparatively strong binding of the tooth to the gingiva is achieved in mammals (monkey) and non-mammalian vertebrates (shark) (Sawada & Inoue, 2003).

In monkey, this specialization of the lamina densa of the internal basement membrane is closely associated with an additional layer referred to as the supplementary lamina densa found on the enamel side of the tooth (Fig. 6a). Observation of non-demineralized tissue revealed that one part of the basement membrane, the supplementary lamina densa, was mineralized (Fig. 6b). This mineral deposit was continuous with that of the enamel of the tooth, and thus this deposit on the supplementary lamina densa formed an advancing edge of mineralization. Under this arrangement, two different phases, an organic and a mineral phase, overlap, with direct contact at this part of the basement membrane, ensuring intimate

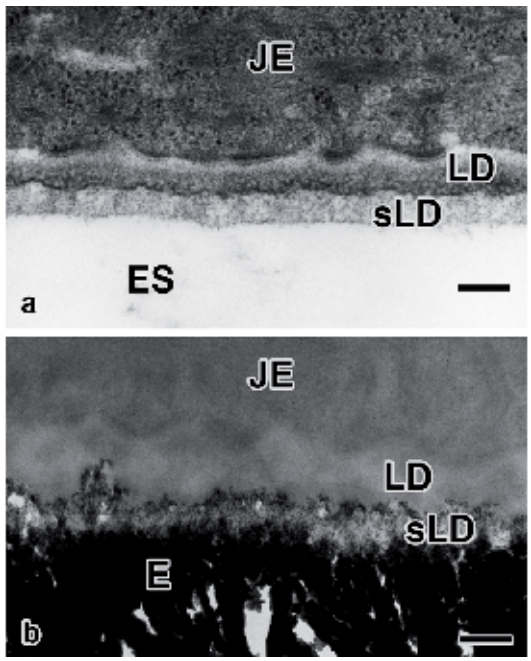
contact between and strong binding of these phases. Furthermore, detailed observation revealed that, in the mineralized portion of the lamina densa, mineral crystals were arranged in a network pattern which was comparable to the pattern of the cord network. This may facilitate more powerful gripping, and further demonstrates the elaborate mechanism by which firm binding of the mineral and organic phases is achieved.



Cuticle contains network anastomosing structure (arrows) resembling cord network of internal basement membrane. Intercordal space is filled with dark amorphous material. JE, junctional epithelium; ES, enamel space. Scale bars = 1 μm (a), 0.1 μm (b). Modified from Sawada & Inoue, 2001b © Journal of Periodontal Research.

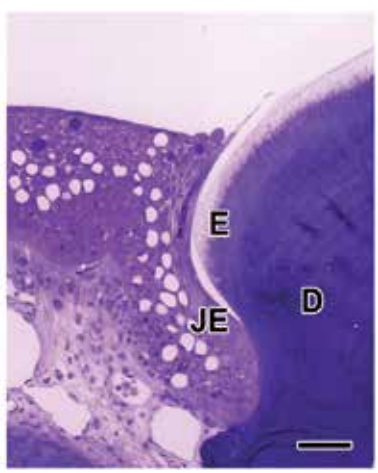
Fig. 5. (a) Dental cuticle (DC) found at dentogingival border in monkey (b) High magnification view of dental cuticle

The specialization of the lamina densa of the internal basement membrane in shark tooth was more complex (Fig. 7). Along the surface of the lamina densa of the internal basement membrane facing the oral epithelium (junctional epithelium), hemidesmosome-related, semicircular or rectangular bulges were intermittently present (Fig. 8a). In non-demineralized tissue, the entire lamina densa, apart from the specialized area of bulges, was mineralized, and this mineral deposit was continuous with that of enameloid/dentine (Fig. 8b). Furthermore, overlapping and binding of the organic and mineral phases was shown to occur throughout the internal basement membrane. Thus, in one comparative study (Sawada & Inoue, 2003), it was demonstrated that firm association of the tooth-gingiva occurs according to the same mechanism, that is, partial mineralization of the internal basement membrane, in both mammalian and non-mammalian vertebrates.



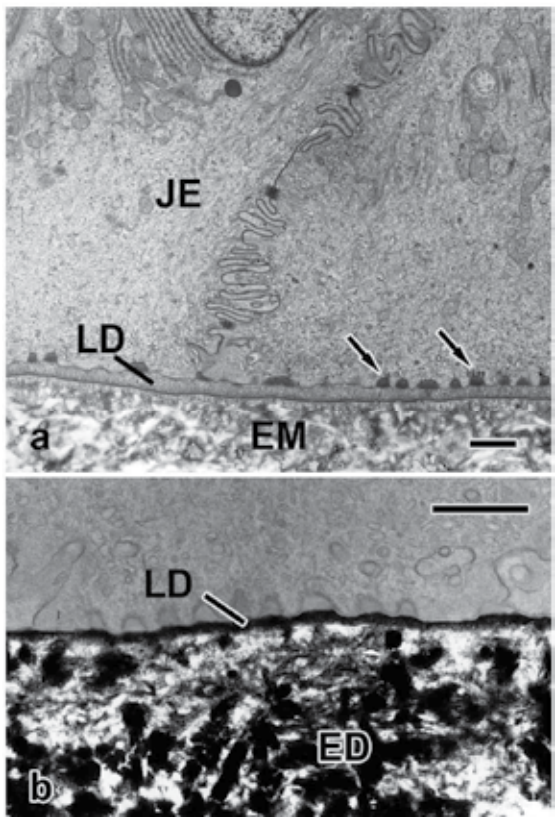
(a) Supplementary lamina densa (sLD) is well preserved after tissue demineralization with EDTA. It is composed of a network of cords similar to that of the internal basement membrane lamina densa (LD). (b) Supplementary lamina densa (sLD) is mineralized with deposit of fine mineral crystals which is continuous with mineral deposited in enamel (E). JE, junctional epithelium; ES, enamel space; LD, lamina densa. Scale bars = 0.2 μm. Modified from Sawada & Inoue, 2003 © Calcified Tissue International.

Fig. 6. Dentogingival border of monkey tooth from demineralized (a) and non-demineralized samples (b)



JE, junctional epithelium; E, enameloid; D, dentin. Scale bar = 100 μm. Modified from Sawada & Inoue, 2003 © Calcified Tissue International.

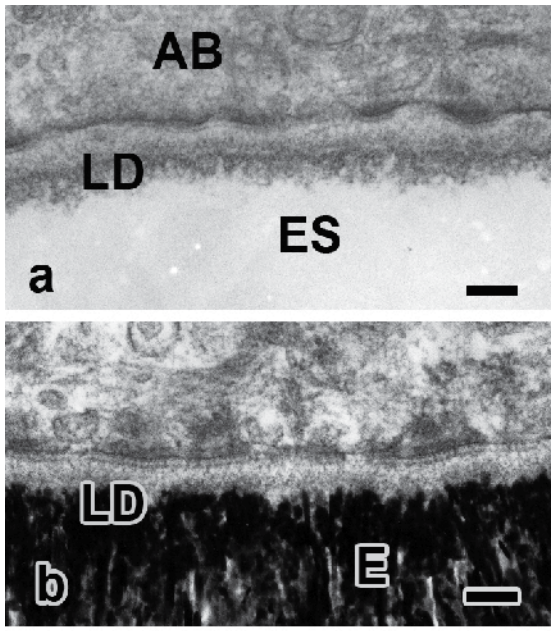
Fig. 7. Light micrograph of area of dentogingival border in shark tooth



(a) Internal basement membrane of junctional epithelium (JE) is composed of lamina densa (LD) with extremely narrow lamina lucida to which semicircular or rectangular structures (arrows) are associated on its epithelial side. (b) Internal basement membrane at dentogingival border in shark tooth (non-demineralized sample). Lamina densa (LD) of internal basement membrane is mineralized by deposition of fine mineral crystals whose orientation is distinct from that of enameloid (ED). EM, matrix of enameloid/dentin. Scale bars = 1 μm. Modified from Sawada & Inoue, 2003 © Calcified Tissue International.

Fig. 8. Electron micrograph of dentogingival border in cervical region of shark tooth

Similarly, in the maturation stage of amelogenesis in monkey (Sawada & Inoue, 2000), mineralization of the lamina densa-like layer and part of the lamina densa was reported to proceed along the cords, ensuring firm attachment of the organic and mineral phases. The basement membrane of a layer of maturation-stage ameloblasts was specialized, showing an association between the lamina densa at its enamel side and a wider layer of what appeared to be an additional lamina densa (Fig. 9a). Observation of non-demineralized tissue revealed that almost the entire layer of combined lamina densa and its closely associated lamina densa-like structure were associated with enamel crystals, forming with advance in mineralization (Fig. 9b). Again, these grain-like crystals, unlike the larger needle-like crystals of enamel, were arranged along the individual cords of the cord network of the lamina densa or lamina densa-like layer (Sawada & Inoue, 2000).



(a) Lamina densa (LD) is composed of cord network. (b) Part of lamina densa (LD) is mineralized and embedded in advancing edge of enamel (E). AB, maturation stage ameloblasts; ES, enamel space. Scale bars = 0.1 μm. Modified from Sawada & Inoue, 2000 © Calcified Tissue International.

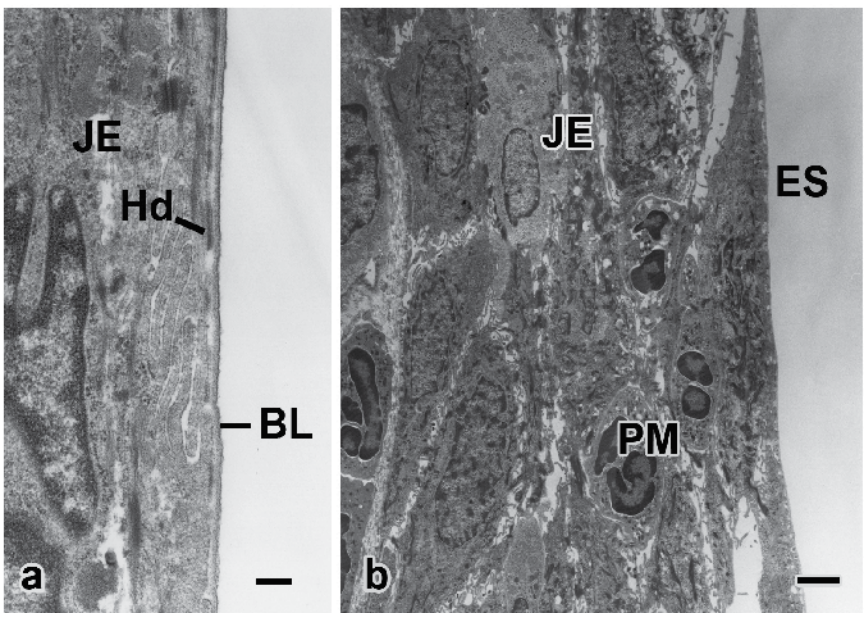
Fig. 9. Electron micrograph of basement membrane of maturation stage ameloblasts

3.4 Dentogingival border of replanted tooth

The technique of tooth replantation has successfully been used in endodontic therapy. The original attachment apparatus is likely to be mechanically disrupted immediately following surgery. Regeneration of the attachment apparatus after gingival surgery has been reported (Listgarten, 1967; Maríková, 1983; Masaoka et al., 2009; Taylor & Campbell, 1972). However, whether or not regeneration of the attachment apparatus at the dentogingival border occurs following tooth replantation remains to be clarified. It is known that when replantation of avulsed teeth is delayed, conditions such as desiccation, bacterial infection and added inflammation cause damage to the periodontal ligament and may lead to unfavorable prognoses such as ankylosis. However, if avulsed teeth are immediately replanted with minimum extra-oral dry time, "favorable healing" results, with repair of damaged root surface by cementum (Andreasen, 1981; Line et al., 1974).

In a recent study, tissues around replanted teeth in rat were examined morphologically and ultrastructurally in detail in order to determine whether the attachment apparatus at the dentogingival border was regenerated following replantation (Shioya et al., 2009). Rat molars, luxated and extracted with care to keep damage to the surrounding tissues to a minimum, were immediately replanted into their original sockets. Most of the junctional epithelium at the dentogingival border was lost in one week. The coronal side of the enamel was covered with oral sulcular epithelium, from the tip of which a thin layer of epithelium

formed and extended towards the apical side along the surface of the enamel. At the ultrastructural level, the cells composing this new epithelium closely resembled those of junctional epithelium. In addition, a basement membrane-like layer appeared along the surface of this new epithelium. Although many inflammatory cells were observed invading this area, two weeks later they had disappeared, and at this stage numerous hemidesmosomes appeared on the enamel side of the new epithelium closely attached to a newly formed internal basement membrane (Fig. 10a). Four weeks after replantation, the cells of the newly formed epithelium, which covered the enamel and extended towards the apical side, appeared almost identical to those of the junctional epithelium (Fig. 10b). No inflammation was present in the lamina propria at this stage.



(a) Note occurrence of basal lamina-like structure (BL) at enamel surface. Well differentiated hemidesmosomes (Hd) are evident in regenerated junctional epithelium (JE). (b) Cells of regenerated junctional epithelium (JE) 4 weeks after replantation. Epithelial cells covering enamel were morphologically almost identical to cells of junctional epithelium in control animals. Polymorphonuclear leukocytes (PM) were observed between epithelial cells. ES, enamel space. Scale bars = 0.2 μm (a), 2 μm (b). Modified from Shioya et al., 2009 © Clinical Oral Implants Research.

Fig. 10. Electron micrograph showing interface at 2 (a) and 4 (b) weeks after replantation in rat

3.5 Tissues surrounding dental implants

In addition to tooth replantation, the technique of replacing teeth with dental implants has also been successfully applied (Weber & Fiorellini, 1992). As in tooth replantation, the original attachment apparatus is broken down following surgery. Views in the literature regarding the subsequent regeneration of the attachment apparatus in the peri-implant

epithelium remain conflicting. The aim of the final part of this section is to describe the ultrastructure of the dentogingival border of the dental implant in detail.

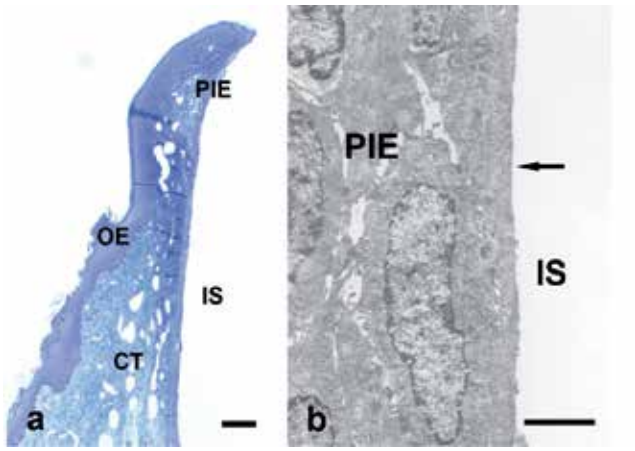
3.5.1 Interface between dental implant and epithelium

A number of authors observed eventual restoration of the junctional epithelium at the surface of implants using light microscopy (Abrahamsson et al., 1996, 1999, 2002; Berglundh et al., 1991, 2007; Fujii et al., 1998; Moon et al., 1999; Schüpbach et al., 1994). A few reports dealt with the *in vivo* reconstruction of the attachment apparatus at the electron microscopic level (Gould et al., 1984; Hashimoto et al., 1989; McKinney et al., 1985). Hashimoto et al. (1989) demonstrated that the innermost cells of the peri-implant epithelium in monkey gingiva attached to a single-crystal sapphire dental implant surface by means of basal lamina-like structures and hemidesmosomes at 3 months after implant insertion. In addition, they showed a lack of attachment apparatus at the apical portion of the peri-implant epithelium.

On the other hand, no attachment apparatus was formed between plasma-sprayed ITI implants and the peri-implant epithelium of dogs, and the nature of the epithelium was closer to that of oral mucosal epithelium than to that of junctional epithelium, based on immunohistochemical results (Fujiseki et al., 2003). In their electron micrograph, many microvilli were evident at the periphery of the cells at the implant sites in place of hemidesmosomes and basal lamina after 6 months implantation. In another recent group research, formation of both internal basement membrane and hemidesmosomes was observed only in the lower region of the boundary (Ikeda et al., 2000). The presence of laminin 5, known to be important in epithelial cell adhesion and reported to be localized in the basement membrane of the junctional epithelium in normal tooth (Hormina et al., 1998; Oksanen et al., 2001), was observed by immunoelectron microscopy in the cells of the innermost layer and basal layer of peri-impant mucosa (Atsuta et al., 2005). Internal basement membrane, which also contained laminin 5 and hemidesmosomes, formed an adhesive structure at the apical portion of the interface between implant and peri-implant epithelium (Atsuta et al., 2005). These observations indicate the importance of laminin 5 in the attachment of an implant to the peri-implant epithelium. This notion may be supported by a recent study showing that a laminin-5-derived peptide coating strongly favored *in vitro* formation of adhesion structures (Werner et al., 2009).

We have demonstrated that peri-implant (pure-titanium) epithelium was formed at 1 week after implantation in rat. At 8 weeks after implantation, the leading edge of the peri-implant epithelium receded in the direction of the gingival crest, and this epithelium showed the characteristics of oral sulcular epithelium at the light microscopic level (Fig. 11a). In detailed examination, we showed that binding of the pure-titanium implant and the peri-implant epithelium was imperfect at the ultrastructural level. That is, neither hemidesmosomes nor basal lamina were present at the interface between the epithelium and the implant (Fig. 11b). No cells with the morphology of junctional epithelium were observed in the peri-implant epithelium, unlike with tooth replantation.

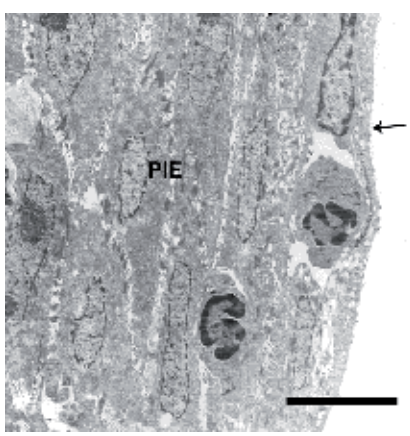
The discrepancies between these results were probably caused by a number of factors, including implant diameter, contact surface topology, surgical protocol, experimental period, and animal model used. A preliminary animal experiment was performed to determine whether regeneration of the attachment apparatus was influenced when $CaTiO_3$ implants were replaced with pure-titanium implants.



(a) Coronal side of pure titanium implant was covered by peri-implant epithelium (PIE). (b) Peri-implant epithelial-cells (PIE) resembled oral sulcular epithelial cells of control rat. Note no attachment apparatus at implant surface (arrow). CT, connective tissue; IS, implant space; OE, oral epithelium. Scale bars = 100 μm (a), 2 μm (b). Modified from Shioya et al., 2009 © Clinical Oral Implants Research.

Fig. 11. Light micrograph (a) and electron micrograph (b) of peri-implant epithelium

The results showed that no attachment apparatus was organized between the peri-implant epithelium and the $CaTiO_3$ implant (Fig. 12: unpublished data). This suggests that the surface topography of an implant does not, at least, influence the regeneration of the basal lamina or hemidesmosomes at the interface between the dental implant and the epithelium. Attachment of soft tissue to titanium implants was found not to be influenced by the roughness of the surface of the implant (Abrahamsson et al, 2002). These findings are consistent with a recent report by de Sanctis et al. (2009) showing that different implant designs and implant surfaces did not significantly influence bone healing at fresh extraction sockets.



Peri-implant epithelium (PIE) lacked attachment apparatus at interface with implant surface (arrow). Scale bar = 5 μm.

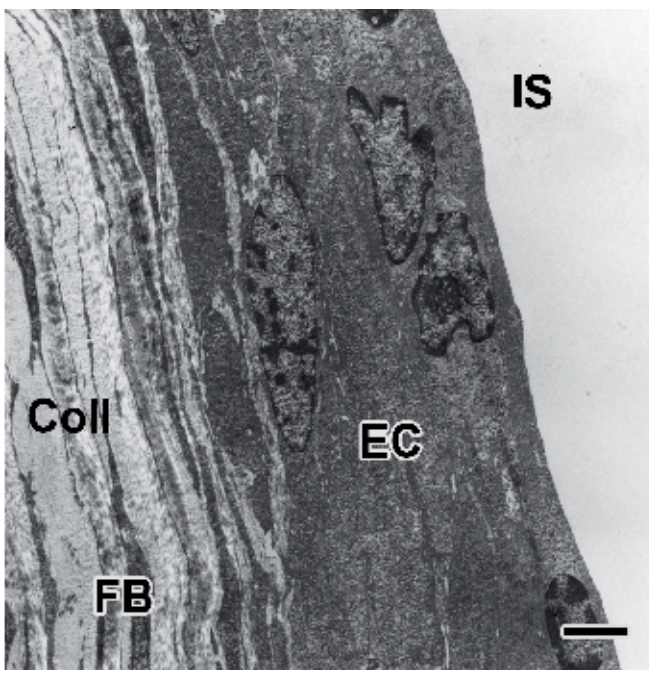
Fig. 12. Electron micrograph of peri-implant epithelium at 8 weeks after implantation ($CaTiO_3$ implant)

3.5.2 Interface between dental implant and connective tissue

Alternative structures of peri-implant tissues were reported (Abrahamsson et al., 2002; Berglundh et al., 2007; Moon et al., 1999; Shioya et al., 2009). A layer of aligned, epithelial-like cells emerged within peri-implant tissue, and this cell layer was unaccompanied by any structures related to attachment apparatus, including hemidesmosomes or basement membrane (Fig. 13). This cell layer was surrounded by bundles of collagen fibrils and elongated fibroblasts oriented parallel to the long axis of the implant. This structure was thought to seal the peri-implant tissue from the oral environment. The biological significance of the epithelium-like layer of cells is not clear, but it may, as Abrahamsson et al. (2002) suggested, cooperate with fibroblasts to help stabilize peri-implant tissues.

In a very recent study, Rinaldi & Arana-Chavez (2010) investigated the ultrastructure of the interface between periodontal tissues and orthodontic titanium mini-implants in rat mandibles. The results demonstrated that a thin cementum-like layer was formed at longer times after implantation at the interface between the surface of the implant and the periodontal ligament. The cementum-like layer contained some collagen fibrils, although it did not contain collagen fiber bundles such as Sharpey's fibers from the periodontal ligament.

Therefore, the next step of our study is to elucidate the origin of epithelial-like cells and their possible functional role in the formation of the cementum-like structure forming at the interface with the implant surface.



Cells (EC) showing epithelial-like alignment with narrow intercellular spaces were observed in peri-implant connective tissue, which was closely attached to implant. Cells had numerous ribosomes and a large amount of rough endoplasmic reticulum. Fibroblasts (FB) among bundles of collagen fibers (Coll) were oriented parallel to long axis of implant. IS, implant space. Scale bar = 2 μm. Modified from Shioya et al., 2009 © Clinical Oral Implants Research.

Fig. 13. Interface of peri-implant connective tissue 8 weeks after implantation

4. Conclusion

The internal basement membrane of the junctional epithelium at the dentogingival border in normal tooth is specialized for mechanical strength by means of its much wider lamina densa with unusually thick cords. In addition, strong gingival-tooth adhesion is established by partial mineralization of the internal basement membrane.
Newly formed internal basement membrane and numerous hemidesmosomes were observed between regenerated junctional epithelium and replanted teeth. On the other hand, peri-implant epithelium was preserved in the form of oral sulcular epithelium, and neither junctional epithelium nor attachment apparatus was restored after implantation. An alternative structure in peri-implant tissues was observed, comprising a layer of aligned, epithelial-like cells surrounded by collagen fiber bundles. The role of this epithelium-like layer may be stabilization of peri-implant tissues together with fibroblasts.

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Part 3

Implant Surfaces and Clinical Practice

12

Oral Bacterial Adhesion and Biocompatibility of Silver-Amorphous Carbon Films: A Surface Modification for Dental Implants

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1. Introduction

Bacterial adhesion and the subsequent biofilm formation on dental implants is a persistent problem that can cause implant failure. Once biofilm is formed, bacterial cells become highly resistant to antibiotics and host defences (Costerton et al., 1999, Patel, 2005), and clinical experience has shown that biofilms must be removed physically before the infection can be resolved (Costerton, 2005).

There is an apparent clinical and microbiological similarity between peri-implantitis and periodontitis (Listgarten and Lai, 1999, Papaioannou et al., 1996). The first indication of the specific role of bacteria in peri-implant infections was originated from microscopic analysis of samples taken from failing implants that shown an abundance of motile rods, fusiform bacteria and spirochetes, whereas samples from successful implants contained only a small number of coccoid cells and very few rods (Mombelli, 2002, Mombelli et al., 1987, Rams and Link, 1983). These findings revealed a site-specific disease process with microorganisms associated in patterns known from chronic periodontitis of natural teeth. The term peri-implantitis introduced in the 1980s, describe a destructive inflammatory process affecting the soft and hard tissues around osseointegrated implants, leading to the formation of a peri-implant pocket and loss of supporting bone (Mombelli et al., 1987).

Adhesion to a surface is the essential first step in the development of a biofilm and the sequential colonization and formation of the dental plaque is highly orchestrated (Xie et al., 2000). The association of bacteria within mixed biofilms is not random; it has been shown that there are specific associations (complexes) among bacteria in dental biofilms (Socransky and Haffajee, 2005, Socransky et al., 1998, Kolenbrander et al., 2006). In addition, these microbial complexes, can be used to describe the sequential colonization of the subgingival plaque. Some bacterial strains, mainly belonging to the genus *Actinomyces* (blue complex) and *Streptococcus* (yellow complex) have been identified as early colonizers of the dental surface, attaching and proliferating at an early stage. A second group of bacteria that functions as bridge between the early and late colonizers are formed by species belonging to the green, purple and orange complexes (i.e. *Fusobacterium nucleatum*, *Capnocytophaga sputigena*, *Eikenella corrodens*). Finally, the third group of species that appears at late stages in

biofilm development and that are considered true periodontal pathogens are species of the red complex (*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*).
This biomaterial-related infection is characterized by chronicity, persistence and lack of susceptibility to antimicrobial agents. This information suggests that the first steps on the biofilm formation on implant surfaces such as titanium (Ti) or stainless steel (SS) can be similar to the process on teeth. In fact, the experimental gingivitis model described by Löe et al. that demonstrated the cause and effect relationship between biofilm formation on teeth and gingivitis (Loe et al., 1965), has been also used to explain the implant and peri-implant mucositis (Pontoriero et al., 1994, Zitzmann et al., 2001). Studies about the peri-implant microbiota *in vivo* have examined the influence of oral health status on the presence of specific bacterial species. Some of these studies reported similar supra and subgingival microbiota on teeth and Ti implants (Shibli et al., 2008, Furst et al., 2007, Groessner-Schreiber et al., 2004). In contrast, other studies found an absence of periodontal pathogens like *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* and *Porphyromonas gingivalis* (Heuer et al., 2007) or sporadic high numbers of *Parvimonas micra* (formerly *Peptostreptococcus micros*), *Staphylococcus aureus* and *Staphylococcus epidermidis* (Furst et al., 2007, Salvi et al., 2008).
The biofilm formation process is extremely complicated and this is particularly true when multiple species are present in the biofilm as in dental plaque. This process is affected by many factors including environment, bacterial properties and material surface characteristics, such as chemical composition, surface energy, hydrophilicity and topography (Katsikogianni and Missirlis, 2004, An and Friedman, 1998, Merritt and Chang, 1991). In addition, for *in vitro* studies, the media play an important role as well, as was shown in a previous work comparing the influence of two different media on the bacterial adhesion and the initial biofilm formation (*Mycoplasma* media, a standard bacterial culture media, and sterilized human saliva); revealing different patterns of adhesion on the same surface when bacteria were cultured with different media (Almaguer-Flores et al., 2010).

2. Surface modifications to prevent bacterial adhesion and biofilm formation on dental implants

Surfaces are critical in the field of biomaterials; the nature of an implant surface determines their interaction with the biological environment. Surface modifications can be classified as physical modifications (sandblasting, patterning, etching, lithography, etc) or chemical modifications (deposition, thin films, polymer coating, etc). Often these techniques are applied in combination to alter both topography and surface energy. Surface modifications can be classified on basically three classes: (a) topographic modifications; such as size and porous distribution and roughness, (b) chemical modifications of the surface; which involve controlled cleaning and oxidation by glow discharge plasma techniques, thin film growth, and deposition of organic overlayers such as polymers, proteins and antimicrobial substances and (c) micromechanical or viscoelastic modifications of the surface, which can affect (enforce or reduce) the mechanical stress-strain fields at the interface (Bagno and Di Bello, 2004, Kasemo and Gold, 1999). These modifications offers the possibility of combining ideal bulk properties with desired properties such as increased bioactivity or prevent bacterial adhesion.
Antibacterial surface modifications have been created in order to prevent bacterial contamination and resulting infections, which can lead to the loss of the implant. These surface modifications must be designed to retard bacterial colonization or can function as

antimicrobial agents without affecting the cells and tissues adjacent to the implant; in other words, they need to be biocompatible.

Some studies have explored the *in vitro* biofilm formation on modified titanium implant surfaces; unfortunately, most of these biofilm models have only included one or two oral bacterial strains (Table 1). In the oral cavity however, the microbial ecology is complex and consists of hundreds of species (Socransky and Haffajee, 2005). For this reason, these models are good tools in order to study species-specific infections or mono-infections, but not very useful to study mixed anaerobic infections such as peri-implantitis.

Strain	Surface	Ref.
S. sanguis *S. mutans*	Ti, and TiN, ZrN, TiO_2	(Grossner-Schreiber et al., 2001)
P. gingivalis	Ti with ion implantation (Ca+, N+, F+), oxidation (anode oxidation, titania spraying), ion platting (TiN, alumina) and ion beam mixing (Ag, Sn, Zn, Pt)	(Yoshinari et al., 2001)
E. coli *P. aeruginosa* *B. cepacia* *B. subtilis*	11 different glass and metal oxide-coated glass surfaces	(Li and Logan, 2004)
S. sanguis	Ti with different roughness	(Pereira da Silva et al., 2005)
S. aureus *P. aeruginosa*	Ti coated with albumin	(Kinnari et al., 2005)
P. gingivalis	Ti and TiN thin films	(Jeyachandran et al., 2007)
S. aureus *S. epidermidis* *S. mutans* *P. aeruginosa*	Ti with bioactive polymers layers	[Maddikeri, 2008 #56]
S. sanguinis	Ti, Au, and ceramic and composite dental materials	(Hauser-Gerspach et al., 2007)
S. aureus	Ti with polyelectrolyte multilayers	(Chua et al., 2008)
S. aureus *S. epidermidis*	Ti with (P(MAA)) followed by immobilization of silk sericin	(Zhang et al., 2008)
S. aureus	Ti, Ta, Cr and DLC surfaces	(Levon et al., 2009)
S. sanguinis	Ti with two modified different roughness	(Burgers et al., 2010)
S. aureus *P. aeruginosa*	Ti with micro and nano scale surface roughness	(Truong et al., 2010)
S. epidermidis	Ti with four modified different roughness	(Wu et al., 2011)

Table 1. *In vitro* bacterial adhesion studies on Ti modified implant surfaces.

2.1 Amophous carbon films

It has been shown that all the different forms of amorphous carbon can be considered as biocompatible (Das et al., 2007, Hauert, 2003, Lettington, 1998, Du et al., 1998) and might be

adequate as a surface modification for biomedical applications, such as, dental and orthopaedic implants (Santavirta, 2003, Affatato et al., 2000). Amorphous carbon films are nanostructured materials deposited as thin films which consist of sp^2 hybridized carbon atoms, clustered within a typical size of a few nanometers, and connected among them by sp^3 hybridized carbon atoms. Depending on the fraction of sp^2 to sp^3 hybridized C atoms, the films have been name as diamond-like carbon (DLC), graphite-like carbon (GLC) or when highly hydrogenated as polymer-like carbon (PLC). The fundamental difference between graphite and diamond-like is the amount of sp^3 hybridized carbon atoms, which is very low in the first group and above 40-50% for DLC. This leads to strong differences in many of the physical properties, such as, optical gap, conductivity, surface energy, etc. (Robertson, 2002).

2.1.1 Biocompatibility

Biomaterials must satisfy certain criteria in order to be used as implants or medical devices. The most important requirement for biomaterials is that they need to be biocompatible. Several definitions of biocompatibility have been established, but in general, biocompatibility can be defined as the ability of the material, intentionally in contact or implanted into the body tissues, to perform as designed without inducing any local effect in the cells or tissue or a systemic response that elicits an immunological reaction. In addition, the biomaterial should not cause denaturalization of the proteins adsorbed on the surface or leach any substance that can induce toxicity to the cells or tissues adjacent to the implant.

In dental and orthopaedic implants, de novo bone formation in direct contact with the bone, also known as osseointegration, is desired. In order to achieve successful osseointegration, progenitor cells and osteoblasts must attach to the implant surface, differentiate into mature osteoblasts, produce an organized extracellular matrix, and finally mineralize the extracellular matrix. Several biomaterials have been applied with a broad range of success in orthopaedic and dental implants. Metallic biomaterials generally have been used for dental and orthopaedic application due to their mechanical properties. Several publications have addressed *in vitro*, *in vivo*, and clinically that metallic implant surface modifications improve osseointegration, increase bone to implant contact, decrease healing time, and are clinically successful (Schwarz et al., 2010, Stanford, Li et al., 2010, Dohan Ehrenfest et al., 2010, Karabuda et al., 2010, Schatzle et al., 2009).

During the last years, we have been investigating different aspects of the interaction between human cells (osteoblasts) and graphite-like carbon films (GLC) as possible candidates for coating dental implants. Contrary to many other research groups, we choose GLC instead of DLC films because the main interest was on the osseointegration and not on the tribological properties and graphite itself has been established as a good osteoinductor material (Rodil et al., 2003, Rodil et al., 2005). Graphite-like amorphous carbon films were produced by a hollow cathode DC magnetron sputtering system attached to a high vacuum chamber. We have obtained good results concerning the interaction to human osteoblasts and also good osteoconductive properties (Rodil et al., 2006, Olivares et al., 2004).

2.1.2 Bacterial adhesion

Nevertheless, another important factor for the success of implants (included dental implants) is to avoid formation of biofilms that might lead to implant failure or strong inflammatory process (infection). Limited studies regarding bacterial adhesion on the different carbon films have been published before (Wang et al., 2004, Ishihara et al., 2006,

Jones et al., 2006, Katsikogianni et al., 2006, Morrison et al., 2006, Kwok et al., 2007, Zhao et al., 2007, Zhou et al., 2008, Kinnari et al., 2008). These works included mainly DLC or modified-DLC films and concluded that the carbon surface has great biocompatibility properties and good resistance to microbial adhesion. However, these results could not be extrapolated to the GLC films due to the strong differences between DLC and GLC physical properties, which are known to affect the bacterial adhesion. Moreover, none of these studies include oral bacteria and in any case no more than three bacterial strains were used.

We have developed a biofilm model using nine selected species representative of all the complexes of the subgingival dental plaque, described by Socransky et al. (Socransky et al., 1998) (Table 2). We used *A. israelii* and *S. sanguinis* as early colonizers. A second group of bacteria included *F. nucleatum*, *C. rectus, E. corrodens, P. micra* and *P. intermedia,* these species are known because functions as a bridge between the early and late colonizers. *P. gingivalis* was used as a representative of the third group of species that appears at late stages of biofilm development and *A. actinomycetemcomitans* was used due to the role that seems to have in periodontal infections (Wilson and Henderson, 1995). All strains were grown under anaerobic conditions (80% N_2, 10% CO_2 and 10% H_2).

In a first study, the bacterial adhesion of these microorganisms was evaluated on amorphous carbon (a-C) films in comparison to titanium (Ti) and stainless steel (SS) control surfaces. The results showed that the oral bacterial adhesion on these GLC films was relative high in comparison to standard surfaces (Ti and SS) (Almaguer-Flores et al., 2009).

In a second experiment, (Almaguer-Flores et al., 2010), the influence of the surface roughness and culture media was investigated comparing carbon and titanium films. The surface roughness was modified by deposition of films on both rough stainless steel and silicon substrates, the roughness of the stainless steel was significantly larger than the silicon (1.89 ± 0.5 μm and 0.028 ± 0.003 μm, respectively) therefore two different roughness were compared. In addition, the study was done comparing two different media; *Mycoplasma* media (MM), which is an standard bacterial culture media, and sterilized human saliva (HS) because is the major bulk fluid in the oral cavity.

Specie	ATCC*
Aggregatibacter actinomycetemcomitans serotipe b	43718
Actinomyces israelii	12102
Campylobacter rectus	33238
Eikenella corrodens	23834
Fusobacterium nucleatum subsp. *nucleatum*	25586
Parvimonas micra	33270
Porphyromonas gingivalis	33277
Prevotella intermedia	25611
Streptococcus sanguinis	10556

* American Type Culture Collection, Rockville, MD

Table 2. Reference strains employed for the adhesion and biofilm formation assays.

AFM images of the samples presented in Figure 1 (a,b,c and d), showed that not only the roughness values were modified, but also the topographical features of the samples were different. The samples deposited on silicon showed a spiky homogeneous topography but the maximum height of the peaks is in the nanometer scale. While for the rough surfaces, the topography is like a series of non-homogeneous hills and valleys, reaching heights in the micrometer scale.

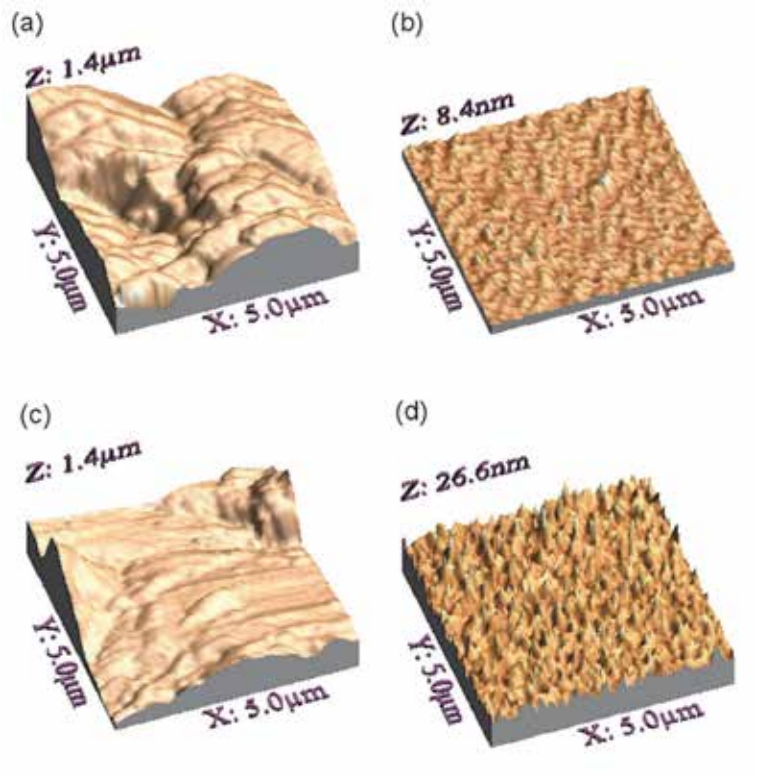
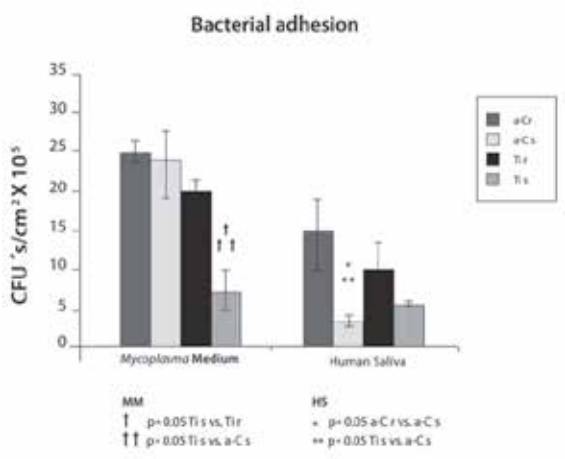


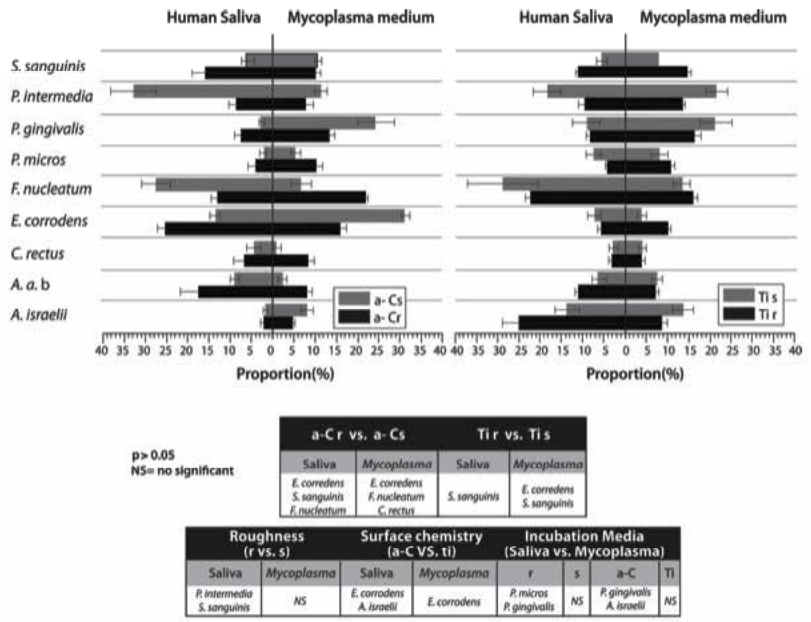
Fig. 1. AFM images of the test substrates. Vertical scale has been normalized, Z value indicate maximum height in each film. (a) a-C film deposited on the stainless steel sandblasted substrate (a-C r). (b) a-C film deposited on silicon substrate (a-C s). (c) Ti film deposited on the stainless steel sandblasted substrate (Ti r). (d) Ti film deposited on silicon substrate (Ti s).

Bacterial adhesion on the test samples varied depending on the media used, the surface roughness and the surface chemistry, data are presented in Figure 2A as the number of CFUs/cm^2 x 10^5. There were consistently more bacteria on the rough surfaces and in the surfaces cultivated with *Mycoplasma* media. The number of CFU´s was reduced on the Ti surfaces compared with the a-C surfaces. Significant differences were observed between Ti s and Ti r ($p < 0.05$) and Ti s and a-C s ($p < 0.05$). When human saliva was used, lower bacterial counts were detected on all surfaces compared to the *Mycoplasma* media. Indeed, the number of CFU's was highly reduced on the a-C s surfaces, and statistical differences were found comparing a-C s vs a-C r and a-C s vs Ti s ($p < 0.05$).



A

Fig. 2A. Bacterial adhesion (CFU's x 10^5) on rough (r) and smooth (s) a-C and Ti films, after 24h of anaerobic incubation with *Mycoplasma* medium (MM) or Human Saliva (HS).



p> 0.05
NS= no significant

a-C r vs. a- Cs		Ti r vs. Ti s	
Saliva	*Mycoplasma*	Saliva	*Mycoplasma*
E. corredens *S. sanguinis* *F. nucleatum*	*E. corredens* *F. nucleatum* *C. rectus*	*S. sanguinis*	*E. corredens* *S. sanguinis*

Roughness (r vs. s)		Surface chemistry (a-C VS. ti)		Incubation Media (Saliva vs. Mycoplasma)			
Saliva	*Mycoplasma*	Saliva	*Mycoplasma*	r	s	a-C	Ti
P. intermedia *S. sanguinis*	NS	*E. corrodens* *A. israelii*	*E. corrodens*	*P. micros* *P. gingivalis*	NS	*P. gingivalis* *A. israelii*	NS

Fig. 2B. Proportion of the nine bacterial strains in the biofilms formed on rough (r) and smooth (s) a-C and Ti films, after 24h of anaerobic incubation with *Mycoplasma* medium (MM) or Human Saliva (HS). Table at the bottom shows the statistical analysis divided according to the factors that influence the bacterial colonization. Where the bacteria name is shown, it indicates a significance of $p<0.05$ for that strain and NS means no statistical difference.

The influence of roughness was clearly observed in the number of bacteria attached to the rough surfaces (number of CFU's). Similarly, a positive correlation between surface roughness

and bacterial attachment *in vitro* has been shown (Quirynen et al., 1996). The proportions of the bacterial strains were also affected by the surface roughness and this effect seems to be more pronounced when saliva was used. The proportion of *S. sanguinis* was significantly higher ($p < 0.05$) on rough surfaces for both a-C and Ti, i.e. independently of the surface chemistry. Meanwhile, *P. intermedia* showed a higher proportion on the smooth surfaces ($p < 0.05$). Nevertheless, other studies have suggested that regarding to bacterial adhesion or initial biofilm formation; roughness appears to be a minor factor (Bos et al., 1999).

Regarding the surface chemistry, higher numbers of attached bacteria (CFU´s) were detected on amorphous carbon than on the Ti samples, confirming the results from the initial study that indicate large affinity of oral bacteria for the carbon surface (Almaguer-Flores et al., 2009). These results differ with other published papers that have reported that carbon-based films can inhibit bacterial adhesion (Wang et al., 2004, Liu et al., 2008, Zhou et al., 2008).In addition, it has been suggested that Ti has some antibacterial properties explained due to the formation of peroxides at the surface (Jeyachandran et al., 2007). Although, another study suggested that pure Ti was more colonized by two oral bacteria strains in comparison to other surfaces like TiN, ZrN or TiO_2, (Grossner-Schreiber et al., 2001).

An interesting finding was the proportion of *E. corrodens* on the biofilms formed on the a-C surfaces. This strain was found in higher proportions on the a-C samples on both, rough and smooth surfaces, for both media MM or HS, suggesting that *E. corrodens* was more sensitive to surface chemistry than to roughness or the cultured media used. This finding supported the notion that chemical surface is directly affecting the colonization of the oral bacteria (Groessner-Schreiber et al., 2004). *E. corrodens* posses an specific lectin-like substance that mediates its adherence to various host tissue cell surfaces (Yamazaki et al., 1988), so it is possible that the specificity that this microorganism show to the a-C surfaces has to be with some specific adhesion properties of this strain.

We found higher numbers of bacteria on all surfaces when *Mycoplasma* culture media was used. A possible explanation could be the differences between the components of both media; saliva contains an important presence of some antimicrobial substances, such lysozyme, lactoferrin, lactoperoxidase, and secretory IgA (Tenovuo, 1998). Meanwhile, the *Mycoplasma* media contains only nutrients and some proteins. The saliva is a more biologically significant media for the bacterial adhesion test and many studies indicate that the saliva is critical for the colonization of certain taxa (Gibbons, 1996, De Jong and Van der Hoeven, 1987), and it is determinant for the type and amount of bacteria that will attach on a surface (Gibbons, 1996, Sela et al., 2007). However, human saliva is a very complex and non-homogeneous media in comparison to the MM, and actually changes in the composition can be found from donor to donor. So, in order to study the surface-bacteria interactions, a more homogeneous media could be more convenient.

2.2 Silver – amorphous carbon films (a-C:Ag)

Searching for reducing implant infections different modifications have been proposed such as functionalization of the surface with bactericidal polycationic groups (Tiller et al., 2001, Cen et al., 2004), developing delivery systems to coating the surface with polymers loaded with antibiotic or antimicrobial substances (Shi et al., 2006, Schmidmaier et al., 2006) or covering the implant surface with quaternary ammonium compounds or silver and iodine ions (Yorganci et al., 2002, Nohr and Macdonald, 1994, Tyagi and Singh, 1997, Ewald et al., 2006).

Silver antimicrobial properties have been recognized since historic times (Klasen, 2000, Burrell, 2003), and the coating of medical devices with silver coatings (Ewald et al., 2006,

Bosetti et al., 2002, Darouiche, 1999, Schierholz et al., 1998) or the addition of silver nanoparticles (Chen et al., 2006, Kwok et al., 2007, Jung et al., 2009, Rai et al., 2009) into the material's surface might be a good method to prevent device-associated infections by physical routes instead of the chemical routes mentioned above. Silver exhibits a rather broad-spectrum antimicrobial activity *in vitro* by binding both to microbial DNA, preventing bacterial replication, and to the sulfhydryl groups of the metabolic enzymes of the bacterial electron transport chain, causing their inactivation (Darouiche, 1999).
Considering the well-known silver antibacterial activity, amorphous carbon films with silver nanoparticles inclusions were produced and the biocompatibility and antibacterial properties of such films was evaluated. Details concerning the deposition conditions and properties of the films can be found somewhere else (Garcia-Zarco et al., 2009).

2.2.1 Biocompatibility

Several assays are used to test biocompatibility; however, due to the scope of this chapter focus on common assays that we perform to test the biocompatibility of our surface modifications.
To test biocompatibility of a-C:Ag surfaces, we perform the following assays.

MTT Assay

The MTT is a colorimetric assay that measures the reduction of a tetrazolium component (MTT, 3-(4,5-Dimethylthiasol-2-yl)-2,5-diphenyltetrazolium bromide) into a insoluble purple formazan product by the cell mitochondria. This reaction only occurs in viable cells, which have metabolic activity are capable to reduce the MTT. This assay is commonly used to determine the cytotoxicity of potential medical agents and biomaterials since released molecules can cause metabolic dysfunction that decreases or abolishes MTT reduction in the mitochondria and result in cell toxicity (Mosmann, 1983, Denizot and Lang, 1986). Thus, MTT reduction is proportional to cellular metabolic activity, and decreased MTT reduction infers a possible toxicity of the biomaterial or drug tested (Chen et al., 2011, Niu et al., 2011, Sahithi et al., 2010, Bispo et al., 2010).

Cell culture

Human MG63 osteoblast-like cells are commonly used in testing metallic biomaterials. MG63 cells present an immature osteoblast phenotype, which gives them the potential to be studied as a model of osteoblastic differentiation (Bachle and Kohal, 2004) (Schwartz et al., 1999). MG63 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM, cellgro ®, Manassas, VA) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA) and 1% penicillin-streptomycin (Gibco) at 37°C in 5% CO2 and 100% humidity.

Cell viability

MG63 cells were plated at a density of 10,000 cells/cm2 on tissue culture polystyrene (TCPS) or substrates coated with amorphous carbon (a-C) or amorphous carbon/silver (a-C:Ag). Cell viability was measured using Methylthiazolyldiphenyl-tetrazolium bromide (MTT) dye after 1, 3, or 7 days in culture. MTT dye was dissolved in water to yield a 5 mg/mL solution. MTT dye was then added to culture media of each well to a final concentration of 1 mg/mL and incubated for 4 hours. The media was then removed, the monolayer rinsed twice with PBS, and formazan crystals dissolved in 500 μL DMSO. 200 μL of the resulting solution was

aliquoted into a 96 well microplate and read in an absorbance plate reader using a test wavelength of 570 nm and a reference wavelength of 630 nm.
Viability of MG63 cells cultured on a-C or a-C:Ag thin films was measured using MTT. One day after plating, cells cultured on both a-C and a-C:Ag had similar MTT activity to cells cultured on TCPS (Fig. 4). Viability at day 3 and day 7 was also similar in cells cultured on thin films than cells on TCPS. These results indicate that thin film coatings analyzed in this experiment did not affect cellular metabolism, indicating that they are not cytotoxic.

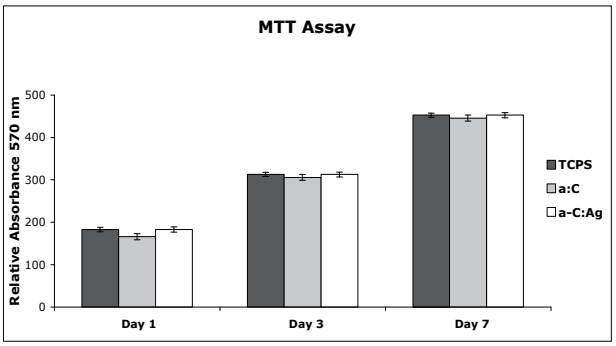


Fig. 4. Effect of thin films on MG63 cell viability. MG63 cells were cultured on tissue culture polystyrene (TCPS) or amorphous carbon (a-C) or amorphous carbon-silver (a-C:Ag) thin films. MTT activity was measured after 1 day, 3 days, or 7 days in culture. No significant differences were detected between the surfaces.

Osteoblast phenotype

MG63 cells were plated at a density of 10,000 cells/cm2 on tissue culture polystyrene (TCPS) or substrates coated with amorphous carbon (a-C) or amorphous carbon/silver (a-C:Ag). At confluence, cells were incubated with fresh media for 24h. Conditioned media was harvested and levels of secreted osteocalcin measured using a commercially available radioimmunoassay (Biomedical Technologies, Inc., Stoughton, MA). Cells were detached from the surface using two sequential incubations in 0.25% trypsin-EDTA and cell number determined using a Z2 Particle Counter (Beckman Coulter, Hialeah, FL). Cells were lysed in 0.05% Triton X-100 and homogenized by sonicating each sample for 10 s. Alkaline phosphatase specific activity was measured in cell lysates as a function of the release of p-nitrophenol from p-nitrophenylphosphate at pH 10.2 (Martin et al., 1996, Bretaudiere, 1984) and normalized to the total protein concentration (BCA Protein Assay, Pierce Chemical Co., Rockford, IL).
Whether surface modifications enhance osteoblast maturation can be assessed using three specific outcomes: cell number, alkaline phosphatase specific activity, and osteocalcin levels. Cells attach to biomaterials through the proteins adsorbed in the surface of the material. After this event, cell undergo proliferation and extracellular matrix production. It has been demonstrated that proliferation is reduced when cells undergo differentiation (Stein et al., 1990). In our experiment, cell number was lower on a-C and a-C:Ag thin films than on TCPS (Fig. 5A). Alkaline phosphatase specific activity, an early marker of osteoblast differentiation, is commonly used as a marker of bone formation. Alkaline phosphatase is an enzyme that acts on the phosphate groups of various molecules and generates a microenvironment rich in phosphate ions, which, in concert with calcium, mineralize the extracellular matrix to form bone. In osteoblasts, alkaline phosphatase increases when

proliferation is inhibited during differentiation. Our results show that alkaline phosphatase was higher in cells grown on a-C and a-C:Ag than on TCPS (Fig. 5B). However, alkaline phosphatase specific activity increases in early differentiation, reaches a maximum, and begins to decrease as mineralization is initiated (Stein et al., 1990). To establish the specific stage of osteoblast maturation, osteocalcin was measured in the conditioned media. Osteocalcin is considered a later marker of osteoblast maturation, is present in all mineralized tissues in our body, and increases in relation to total mineralization during bone formation. In our experiments, cells cultured on a-C and a-C:Ag secreted more osteocalcin than cells on TCPS (Fig. 5C). The combination of these factors allows us to gauge more precisely the stage of osteoblast maturation, and the ability of surface modifications to enhance this, than any one factor alone. Taken together, our results establish that both a-C and a-C:Ag are not toxic, and promote osteoblast maturation increasing two main factors needed for bone formation, alkaline phosphatase activity and osteocalcin.

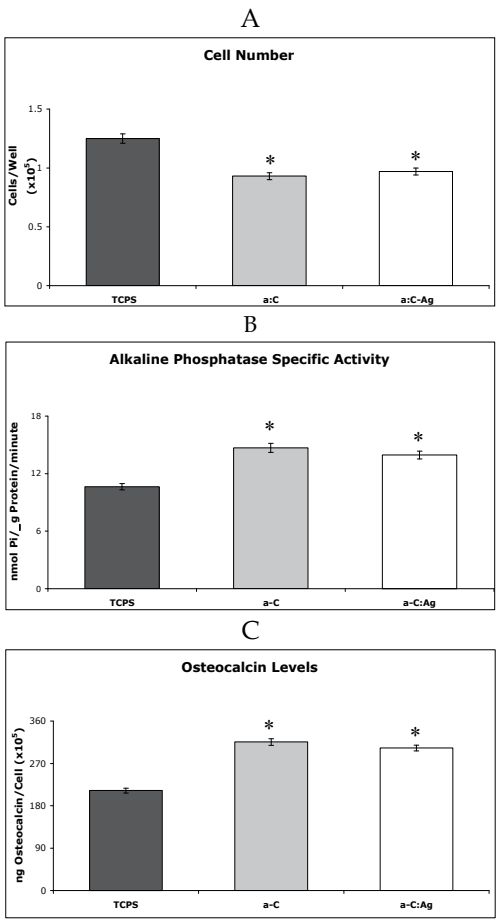


Fig. 5. Osteoblast phenotype in response to culture on thin films. MG63 cells were cultured on tissue culture polystyrene (TCPS) or amorphous carbon (a-C) or amorphous carbon-silver (a-C:Ag) thin films. At confluence, cell number (A), alkaline phosphatase specific activity in cell lysates (B), and secreted osteocalcin (C) were measured. *p<0.05, vs. TCPS.

2.2.2 Antibacterial effect

The oral bacterial adhesion and the initial biofilm formation on the amorphous carbon films modified with the Ag nanoparticles, was evaluated using our standard protocol for the oral bacteria. All surfaces (Table 3) were were incubated for 24 hours, 3 and 7 days with a mixture of the nine bacterial strains. One set of surfaces was used for determining the total counts of bacteria attached to each surface, by counting the colony forming units (CFU's) from each sample. In order to observe biofilm morphology and the surface coverage by bacteria, an additional set of samples was prepared for Scanning Electron Microscopy (SEM) following standard procedures.

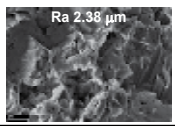
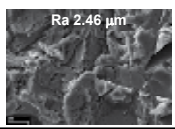
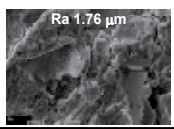
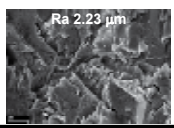
Surface	SEM image (scale 5 μm) and Ra profilometer (Scan 5 mm)	Film thickness	Contact angle
Stainless steel medical grade 316L sandblasted substrate		NA	
a-C film stainless steel medical grade 316L sandblasted substrate		53.8 nm	
a-C:Ag film stainless steel medical grade 316L sandblasted substrate		82.5 nm	
Ti film on stainless steel medical grade 316L sandblasted substrate		190 nm	

Table 3. Surface characterization.

The morphology of the four surfaces was very similar even after the film deposition. This was also confirmed by measuring the roughness before and after deposition, which remained close to 2 μm. The combined effect of the roughness value and the chemical composition lead to water contact angles, which cannot be directly related to the surface energy, but reflects the wettability of the surfaces. The more hydrophobic surface was the silver modified carbon film. The silver atomic percentage in these samples was around 6 at% and the average particle size as calculated using the Image J software (Collins, 2007) was 63.5 nm corresponding to ~10% of the surface area.

Figure 6 shows the BE images of the bacteria colonies as a function of the incubation time and for the four surfaces, using low magnification in order to observe the bacterial distribution among a large area of the surfaces (1.76 mm2). At the first day, mainly isolated attached bacteria were observed, as time went on, the formation of the biofilm was clearly observed and represents the large dark areas in the image, where the thick glycoprotein matrix was developing.

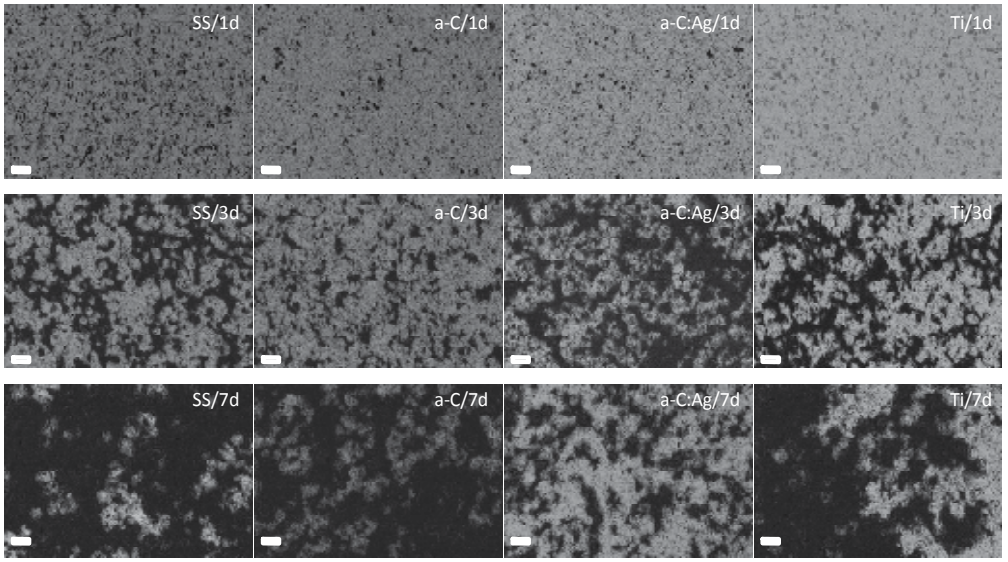


Fig. 6. Backscattering SEM images of the biofilms formed on the SS, a-C, a-C:Ag and Ti surfaces after 1, 3 and 7 days of anaerobic incubation. The scale bar corresponds to 100 µm and the magnification used was 250X.

The increment in the percentage of the surface covered by bacteria (or bacteria surface growth, BSG) as a function of time is clearly observed in figure 7, which also included the statistical analysis. Six different zones were analyzed and the area quantification was done at least three times for each zone, therefore it is possible to describe statistically the variations in the BSG. At the first day, less than 10 % of the total area was covered; actually, the bacteria were observed forming small groups (figure 6). However, at this time, a large amount of bacteria were found in the a-C:Ag films and less number was observed on the metallic surfaces. At three days, the larger number was found on the a-C films, while the other surfaces present similar coverage (~20 %). At 7 days, the amount of bacteria was highly reduced in the a-C:Ag films compared to the other surfaces.

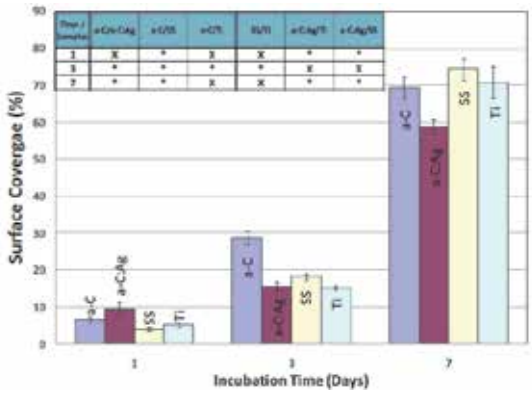


Fig. 7. Surface coverage estimated as the percentage of area covered by bacteria in the images shown in figure 6, using the ImageJ particle analyzer function. Statistically significant data are included in the table (* $p < 0.05$).

The reduction in surface coverage obtained for the a-C:Ag films after 7 days of incubation was significant lower than in the other surfaces. This is in agreement with the antimicrobial mechanism of the silver, where the active agents are really silver ions or radicals that in our samples will be produced via an erosion/corrosion process, taking place as the sample is immersed in the medium.
The addition of silver nanoparticles into the amorphous carbon matrix reduced the bacterial surface growth approximately 10% in comparison to the pure carbon matrix. The results indicated that the action of silver ions occurs in time, therefore not immediate response was observed for the bacterial adhesion.

3. Conclusions

The use of different bacterial strains from the oral cavity to study the bacteria adhesion profile on amorphous carbon have shown that it is not straightforward to reach conclusions about the anti-bacterial properties of any surface. When bacterial adhesion was tested using individual species, the adhesion profiles varied on the same surface depending of the bacterial strain.
Our results support the notion that there is a strong influence of the physical and chemical properties of the substrate in the colonization of oral bacteria, moreover when using human saliva, significantly reduced levels of bacteria were found on the a-C smooth surfaces.
In summary, it seems that Graphite-like amorphous carbon is not a suitable surface to prevent adhesion from the oral media but a-C:Ag films seems to inhibit bacteria adhesion after seven days of incubation. However, a-C films seem to be good to repel bacteria from certain oral strains, such as, *A. israelii*, *P. gingivalis* and *P. intermedia*. Although *E. corrodens* was capable to colonize in very high rates the a-C surfaces despite of their roughness or the culture media used.
Therefore, the determination of bacterial adhesion properties on biomaterials using only one or two bacterial strains is not accurate and cannot lead to general conclusions about the anti-bacterial properties of the biomaterial, at least when strains from the oral cavity are use.
Further studies are required in order to evaluate other physical, chemical and biological properties of the a-C:Ag films in order to understand the observed differences and also to analyze the sequential formation of a bacterial biofilm over these and other implant surfaces.

4. Acknowledgments

A. Almaguer-Flores thanks to Facultad de Odontología of the Universidad Nacional Autónoma de México.

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13

n-SiO_2 Embedded HA/TiO_2 Composite Coatings Deposited on Pure Titanium Substrate by Micro-Arc Oxidation

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1. Introduction

As orthopaedic and dental metallic implant materials, titanium and titanium alloys are widely used due to their relatively low modulus, good fracture toughness, excellent strenth-to-weight ration, and superior biocompatibility and corrosion resistance (Long and Rack, 1998**)**. They have become the first choice above all other candidate metallic implant materials such as Co-Cr-Mo alloys, stainless steel in recent years. But smooth titanium or titanium alloy implants are considered to have weak bioactivity and bone-bonding in vivo (Li et al., 2004; Sul, 2003; Xie et al., 2000). Therefore, a composite system including an hydroxyapatite (HA) film on the titanium or titanium alloy implant, which combines the mechanical benefits of metal alloys with the biological properties of HA, has generated widespread interest because of the HA has excellent biocompatibility and tissue bioactivity (Tkalcec et al., 2001; Weng et al., 1997). Many techniques including plasma-spraying, pulsed laser deposition and electrophoretic deposition have been studied to produce HA films over the last 20 years, and plasma-spraying was the only one that achieved commercial success (Yang et al., 2005; Cotell et al., 1992; ZHITOMIRSKY, 1997; Liu, et al., 2002; Wen et al., 2002; Gu et al., 2003; Clèries et al., 2000; Koike & Fujii, 2001). But the film formed by plasma-spraying was easily separated from the surfaces or resorbed in the body environment because of the unstable characteristics through its rapid solidification, inhomogeneous composition, melted and decomposed phases, etc (Xu et al., 2006). The other methods such as electrophoretic deposition may produce highly crystalline coatings, which are difficult to resorb in the body (Gross & Berndt, 1994).
Recently, it was reported that hydroxyapatite-containing titania coating on titanium or titanium alloy was prepared by micro-arc oxidation (MAO) technique (Barrere et al., 2002; Chen et al., 2006; Fu et al., 2002; Han et al., 2003; Wei et al., 2009; Ni et al., 2008). The obtained coating has a porous surface and exhibits perfect biocompatibility and biological activity, which is essential for orthopaedic and dental metallic implant materials. This technique is very suitable for the bioactive surface modification of titanium and its alloy implants.

2. Micro-arc oxidation technique

Micro-arc oxidation (MAO) technique, also named plasma electrolytic oxidation , micro-plasma oxidation, or anodic spark deposition developed from anodic oxidation from the

1970s. MAO is an electrochemical, plasma chemical technology in an electrolyte to obtain ceramic coating on valve-metal surfaces such as aluminium, titamium, magnesium, et al. and their alloys. During MAO treatment, the valve metal substrate is used as anode and a stainless steel plate was used as a cathode in an electrolyte cell. Sometimes, the cathode can be the stainless cell which hold the electrolyte simultaneously. When the applied voltage to the substrate immersed in electrolyte is increased to a certain point, a micro-arc occurs on the surface and a ceramic layer is formed. The method makes it possible to obtain a new coating strongly adhering to the surface and characterized by high mechanical, heat-resistant, wear resistance and other functional properties.

2.1 Micro arc oxidation of aluminium

At its earlier development stage, MAO technique was mainly studied to enhanced friction, wearing and corrosion resistance of aluminium and its alloys. It was first reported depositing oxide coating on aluminium anode by Markov and co-work, Van and co-work in 1970s (Markov et al., 1976; Van et al., 1977). Subsequently, Dittrich et al., Krysmann et al., Kurze et al.(Dittrich et al., 1984; Krysmann et al., 1984; Kurze et al., 1987) in the 1980s, and Wirtz et al (Wirtz et al., 1991) in the early 1990s contributed to the development of the MAO process. However, the MAO process gained worldwide recognition as an eco-friendly technology for deposition the tribologically superior ceramic coatings on aluminum and its alloy by the pioneering research contribution made by Yerokhin et al. of Tula State University (Russia) in the 1990s (Voevodin et al., 1996; Yerokhin et al., 1998; Yerokhin et al., 1999). After that, more researchers from United States, United kingdom, China, et al. have contributed to the further research on the Formation and Mechanism of Ceramic Coating and its properties (Krishna et al., 2007; Lukiyanchuk et al., 2002; Mertsalo et al., 2003; Rudnev et al., Shi et al., 2004; Tianet al., 2002; 2004;Xue et al., 2001).

2.2 Micro arc oxidation of titanium

MAO technique used to modify the surface of titanium and its alloys began with 2000's on the base of research development of that on aluminium. The main purposes at incipient stage were wear resistance and corrosion resistance, and quickly turned to biomaterial modification. Micro-arc oxidation can produce a porous, relatively rough and firmly adherent titanium oxide coating on titanium surface, which is beneficial for the biological performance of the titanium implants. The obtained MAO films on titanium can be used for such applications as orthopaedic or dental implants. A large number of scientists have investigated this technology and have obtained their results.

2.3 Microstructure of MAO coating on titanium

It was reported by almost all investigators that the oxide film formed using MAO on titanium surface exhibited a porous microstructure with SEM. The holes which were regarded as discharge channels of micro-arc in electrolyte were relatively well separated and homogrneously distributed over the surface (Chen et al., 2006; Han et al., 2002a, 2002b, 2003; Li et al., 2004; Ni et al., 2008). Theoretically speaking (Akin et at., 2001; Dunn et al., 1993), this micro-porous morphology of the implant surface is beneficial to bone tissue growth and enhanced anchorage of implant to bone; furthermore, a porous surface may be valuable for bioactive constituents such as growth factors or bone morphogenic proteins and has the function of an enhanced cell proliferation. The cross-sections of the oxide layers formed with different oxidation time showed that there was no obvious discontinuity

between the film and the underlying substrate, which indicated that the film could be tightly adhered to the substrate (Han et al., 2002a, 2002b, 2003; Ni et al., 2008). The morphological difference such as diameter of the pores and the thickness of the film associated with electrolyte concentration, discharge voltage and treatment time, have been found by many investigators (Li et al., 2004; Han et al., 2002a, 2003; Kuromoto et al., 2007; Yao et al. 2008).

Ti and O are two primary elements in the MAO coating analyzed by EDX analysis, which came from substrate and component or water of electrolyte respectively. In addition, the elemental component in electrolyte can incorporate into the coating too. The contents of the elements in the coating are different with the change of oxidation time, electrolyte concentration, and so on. (Chen et al., 2006; Han et al., 2002a, 2003; Li et al., 2004; Ni et al., 2008).

XRD patterns prove that the MAO coating formed by MAO is mainly composed of anatase and rutile, the peaks of which are strongly depending on the electrolyte concentration and parameter such as oxidation time, applied voltage et al (Han et al., 2003; Ni et al., 2008). The diffraction peaks of some other materials such as $CaTiO_3$, β-$Ca_2P_2O_7$ and α-$Ca_3(PO_4)_2$, which related to electrolyte composition can be detected at the same time, and this is also decided by electrolyte concentration, oxidation time, and applied voltage et al. (Chen et al., 2006; Fu et al., 2002; Ni et al., 2008, Han et al., 2003).

2.4 Properties of MAO coating on titanium

One main purpose of using MAO technique on titanium and its alloys implants is to improved its biological behavior by modifying the composition and morphology of the implant surface. So some biological and mechanical properties were measured to evaluate MAO process (Akin et al., 2001; Han et al., 2002a, 2002b, 2003; Ishizawa & Ogino, 1995; Li et al., 2004; Lim et al., 1996; Song et al., 2004 ; Sul, 2003; Wang et al., 2000; Wu et al., 2003). It also has been found that the biological behavior of the MAO samples closely related to the morphology, Elemental and phase composition, roughness, and so on.

2.4.1 Bioactivity of MAO coating on titanium-apatite-induced ability in SBF solution

The bioactivity of the MAO film can be studied by immersing the coated samples in simulated body fluid (SBF) for a period of time (Han et al., 2003; Song et al., 2004). The aim is to evaluate the apatite induction of the film in a body-analogous solution by analyzing the changes in chemistry, corrosion resistance, apatite-induced ability, and crystallinity of the coating.

In vitro bioactivity of the MAO titania-based films on titanium surface was evaluated in simulated body fluid (SBF) by Han et al. (Han et al., 2003). They found that only the film containing $CaTiO_3$, β-$Ca_2P_2O_7$ and α-$Ca_3(PO_4)_2$ could induce an apatite layer on its surface, exhibiting bioactivity. $CaTiO_3$ combined with β -$Ca_2P_2O_7$ and α-$Ca_3(PO_4)_2$ seems to be the key structural factor for MAO-formed titania-based films to be of bioactivity. Han et al. analysed the morphologies of the sample which has the apatiteinduced ability in SBF solution immersed in SBF for 40 and 50 days (Han et al., 2003). They found that after 40 days immersion, the surface of the sample exhibits the decrease of pore size and porosity, and starts to be covered with the mineralized apatite. When immersed for 50 days, original porous characterization of the sample disappears and its surface is fully covered with a dense layer of apatite. When observed at high magnification, the apatite layer is in fact composed of network structure, and the diameter of the net pores is less than 100 nm. similar results can be found in the work of others (Song et al., 2004).

Some literatures suggested that TiO_2, regardless of anatase or rutile in the MAO-formed film on titanium seems to be bioactive (Li et al., 2004; Wu et al., 2003). While others hold the view that the in vitro bioactivity is ascribed to Ti-OH group or negatively charged surface as well as to an epitaxial effect of the anatase structure (Lim et al., 1996; Wang et al., 2000; Wu et al., 2003).

2.4.2 Biocompatibility of MAO coating on titanium

Biocompatibility can be evaluated by in vitro cell tests which comprise proliferation and differentiation behavior of the cells and in vivo tests (Akin et al., 2001; Li et al., 2004). Evaluation parameters can be proliferation and differentiation behaviors (alkaline phosphatase (ALP) activity) of the cells of in vitro cell tests and the bond strength between the bone and the implant in of vivo tests.

Akin et al. found that the in vitro proliferation of human bone-derived cells (HBDC) is similar on three samples with 0.50, 16, and 50 μm diameter pores, respectively. However, higher [3H]thymidine (3H-TdR) incorporation by the HBDC is observed when they are grown on 0.50- and 16-μm pores compared to the 50-μm pores, suggesting an enhanced cell proliferation for the smaller pores(Akin et al., 2001).

In the work of Li and co-work (Li et al., 2004), the proliferation and differentiation behaviors of MAO specimens were evaluated by in vitro cell tests using MG63 and human osteosarcoma (HOS) cell lines, respectively. In their works, the proliferation behavior was determined by counting the number of cells after culturing them for 7 days. The differentiation behavior was estimated by measuring the alkaline phosphatase (ALP) activity of the HOS cells after culturing them for 10 days. The results showed Even though the proliferation rate was highest when the specimen was oxidized at the relatively low voltage of 190 V and decreased steadily with increasing voltage, the number of cells increased more than 10 times compared to the originally plated cells. However, The ALP activities of the HOS cells was not much affected by the MAO process when the applied voltage was lower than 300 V, but increased rapidly when the voltage was higher than 300 V. the result shows that the roughness and the amount of Ca and P ions incorporated into the titanium oxide layer strongly affect the cell response. Especially, the ALP activity significantly increased at higher voltages, which is deemed to be closely related to the increase in surface roughness and the increased amount of Ca and P contained in the oxide layer.

In the work of Li et al., the bond strength between the bone and the MAO-treated specimens was measured with a torque measurement device by in vivo tests on female, New Zealand white rabbits (Li et al., 2004). The removal torque of the MAO-treated Ti implants was more than three times higher than that of the as-machined Ti implant. The results showed a considerable improvement in osseointegration capability of MAO-treated specimens as compared to the pure titanium implant. This enhancement is attributable to the increase of surface roughness and to the presence of the Ca and P ions, which were incorporated into oxide layer during the MAO process.

2.4.3 Mechanical properties of MAO coating on titanium

Mechanical properties, including hardness, elastic modulus, and adhesion strength were some other important properties and were tested in some investigations (Han et al., 2002a, 2002b; Sul, 2003; Ishizawa & Ogino, 1995).

According to research results of Han et al. (Han et al., 2002a), The film prepared at 350 V exhibited a low hardness and Young's modulus which were 0.9±0.2 GPa and 32±4 GPa,

respectively. The film exhibited a significant plasticity and ductility compared to the conventional coarse-grained titania ceramics which were 9 and 230 GPa, respectively. However, the adhesion of the film to the substrates was fairly strong, as high as approximately 37±3 MPa when the film prepared at 350 V (Han et al., 2002a）. Another research received by the same authors revealed that the bond strength of the films prepared at 350 V was approximately 30±2 MPa and fracture occurred inside the films but not at the interface (Han et al., 2002b). They pointed out that this adhesive strength is much higher than that of sol–gel derived titania films, which is usually less than 10 MPa.

In study of Sul (Sul, 2003), multifactorial biocompatibility of the surface of oxidized implants by MAO method resulted in significantly improved bone reactions as evaluated by biomechanical and histomorphometrical techniques after 6 weeks of implant insertion. Mechanical interlocking and biochemical interaction, separately or together, explain the primary modes of the forces acting over the bone to implant interface. The results of the P and Ca implants point to the possibility of biochemical bonding between bone and oxidized titanium implant. Another literature, wrote by Ishizawa and co-work, proved that the sample with low contents of Ca and P had a high adhesive strength after soaking in a simulated body fluid for 300 days (Ishizawa & Ogino, 1995)

2.5 Influencing factors on MAO film of titanium

The surface morphology, elemental composition, phase components and properties of the MAO coatings on the titanium and its alloys' surface are influence largely by treatment conditions. To find the relationship between them and control them freely are always the purpose of researchers. The main influencing factors are often regarded as electrolyte factors such as its composition and concentration and operation parameters as treatment time, applied voltage, current density et al.

2.5.1 Electrolyte composition and concentration

An advantage of MAO technic is the possibility of incorporating of element in the electrolyte (e.g. Ca, P ions) into the coating by changing the composition and concentration of the electrolyte (Han et al., 2002b; Ishizawa & Ogino, 1995; Li et al., 2004; Song et al., 2004; Ni et al., 2008). The salts have been used in studies include acetate monohydrate ($(CH_3COO)_2Ca·H_2O$), sodium dihydrogenphosphate ($NaH_2PO_4·2H_2O$), sodium phosphate (Na_3PO_4), sodium carbonate (Na_2CO_3), β-glycerophosphate disodium salt pentahydrate ($C_3H_7Na_2O_6P·5H_2O$, e.g. β-GP), and calcium acetate monohydrate (CA), et.al. (Chen et al.,2006; Han et al., 2002a, 2002b, 2003 ; Ni et al., 2008; Song et al., 2004). For example, EDS spectrum of the films formed in solution containing 0.2 M calcium acetate monohydrate ($Ca(CH_3COO)_2·H_2O$) by Han et al (Han et al., 2002b) proved that Ca can incorporated into the film during the MAO process and the atomic concentration was estimated to be ~3.2%.

However, there are also some opposite results emerged by Han et al (Han et al., 2002a, 2003) that the film didn't contain element in the eletrolyte which containing sodium carbonate (Na_2CO_3). The reasons for this difference may relate to other factors such as concentration of salt, applied voltage, oxidation time, et al. which have not yet been clearly studied by investigators.

As studied by EDX analysis in our previous work (Ni et al., 2008), The films contained Ca and P as well as Ti and O. Ca and P came from electrolyte composition $(CH_3COO)_2Ca·H_2O$

and $NaH_2PO_4 \cdot 2H_2O$) and the content of which increased with increase of electrolyte concentration. The conclusion is that the increase of the electrolyte concentration was in favor of the increase of relative content of Ca and P compounded into the films. The same result was obtained by Ishizawa & Ogino (Ishizawa & Ogino, 1995) too.

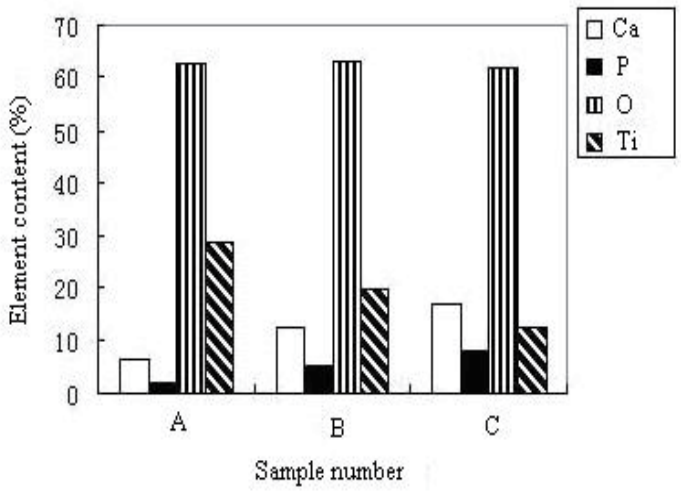

Fig. 1. The element content in coatings subjected to sample A, B, C obtained in different electrolyte concentration (A) $(CH_3COO)_2Ca.H_2O$ 0.13mol/L; $(NaH_2PO_4.2H_2O)$ 0.06mol/L (B) $(CH_3COO)_2Ca.H_2O$ 0.26mol/L ; $(NaH_2PO_4.2H_2O)$ 0.12mol/L; (C) $(CH_3COO)_2Ca.H_2O$ 0.39mol/L; $(NaH_2PO_4.2H_2O)$ 0.18mol/L (Ni et al., 2008)

Another effect of electrolyte composition and concentration to the obtained MAO film is the surface morphology of the surface (Han et al., 2003; Ni et al., 2008). According to Han et al. (Han et al., 2003), at the same voltage, the films formed in CA- and b-GP-containing electrolytic solution become more rough compared with the films formed in Na_2CO_3- and Na_3PO_4-containing solutions, and exhibit similar roughness to the films formed in CA-containing solution. It was observed in Fig.2 obtained in our previous work (Ni et al., 2008) that the discharge pores changed from clearer and bigger to unsharp and smaller when the electrolyte concentration increased gradually. The possible reason that the diameters of the pores tend to reduce with increasing of the electrolyte concentration may be incorporating of electrolyte concentration. The more the electrolyte concentration was, the more electrolyte deposited by sintering on the surface of the film and covered the edge of pores.

Apparently, because of changing of elements composition in the MAO coating on Ti substrate with altering of electrolyte concentration, the phase components of the coating would changed too. Fig.3 (Ni et al., 2008) affirmed this deduction using XRD patterns of MAO coatings formed in different concentration of electrolyte in our previous work. Except for Ti, anatase and rutile, the peak of hydroxyapatite was gradually appeared with increase of electrolyte concentration. The result imply us that hydroxyapatite can be prepared by controlling of electrolyte used in MAO process.

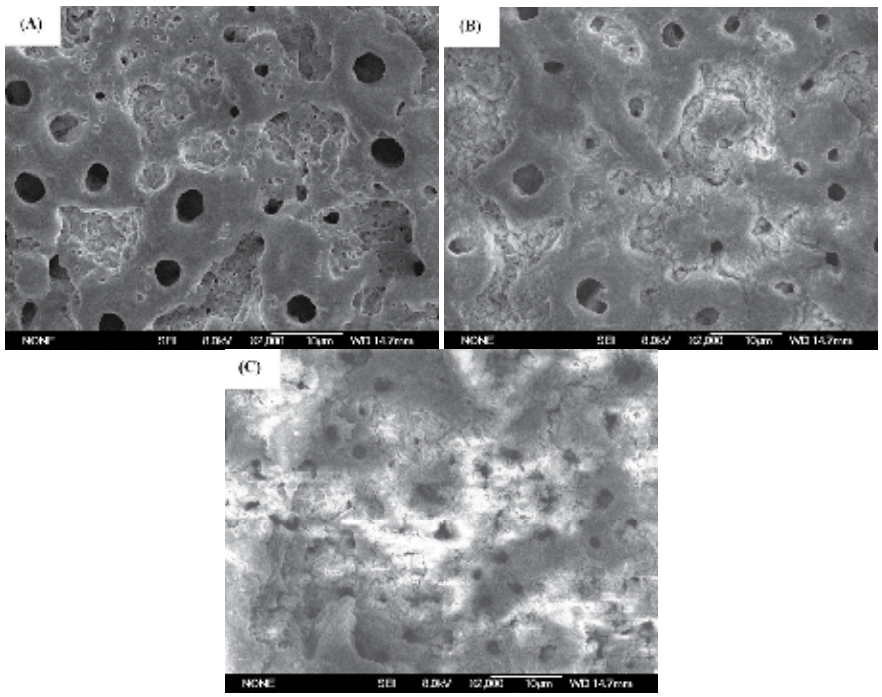

Fig. 2. SEM surface morphologies of micro-arc oxidation coatings formed in different electrolyte concentration: (A) $(CH_3COO)_2Ca.H_2O$ 0.13mol/L, $(NaH_2PO_4.2H_2O)$ 0.06mol/L; (B) $(CH_3COO)_2Ca.H_2O$ 0.26mol/L, $(NaH_2PO_4.2H_2O)$ 0.12mol/L; (C) $(CH_3COO)_2Ca.H_2O$ 0.39mol/L, $(NaH_2PO_4.2H_2O)$ 0.18mol/L (Ni et al., 2008)

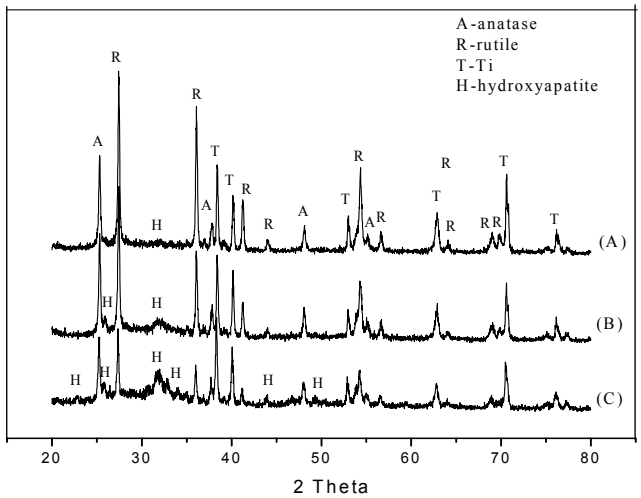

Fig. 3. XRD patterns of the MAO samples formed in different electrolyte concentration for 10min. (A) $(CH_3COO)_2Ca.H_2O$ 0.13mol/L, $(NaH_2PO_4.2H_2O)$ 0.06mol/L ;(B) $(CH_3COO)_2Ca.H_2O$ 0.26mol/L ,$(NaH_2PO_4.2H_2O)$ 0.12mol/L ; (C) $(CH_3COO)_2Ca.H_2O$ 0.39mol/L , $(NaH_2PO_4.2H_2O)$ 0.18mol/L (Ni et al., 2008)

2.5.2 Process parameters

Beside influence of electrolyte to the MAO film, process parameters such as oxidation time, applied voltage, power supply mode, and current density et al. are other factors that should not be ignored. The uppermost process parameters are oxidation time and applied voltage which can be controlled from several min to several quarters and from dozens volts to several hundred volts, respectively (Han et al. 2002a, 2002b, 2003; Ishizawa & Ogino, 1995; Kuromoto et al., 2007; Li et al., 2004; Ni et al. 2008; Song et al., 2004; Sul et al., 2002). The phase, element content, morphology, and thickness of the films were strongly dependent on the treatment time and applied voltage.

The morphological difference associated with treatment time and discharge voltage have been found by many investigators (Han et al. 2002a, 2003; Ishizawa & Ogino, 1995; Kuromoto et al., 2007; Li et al., 2004; Ni et al. 2008;; Sul et al., 2002). On the surface of MAO coating on titanium, the diameters of discharge pores tend to increase with treatment time prolonged within certain period time, and it will not increase in the time that upon the time bucket. The periods of time are not stationary because it is influenced by others process of MAO and sometimes it would not appear. The roughness of the coating also increased with increasing of voltage (Kuromoto et al., 2007; Li et al., 2004).

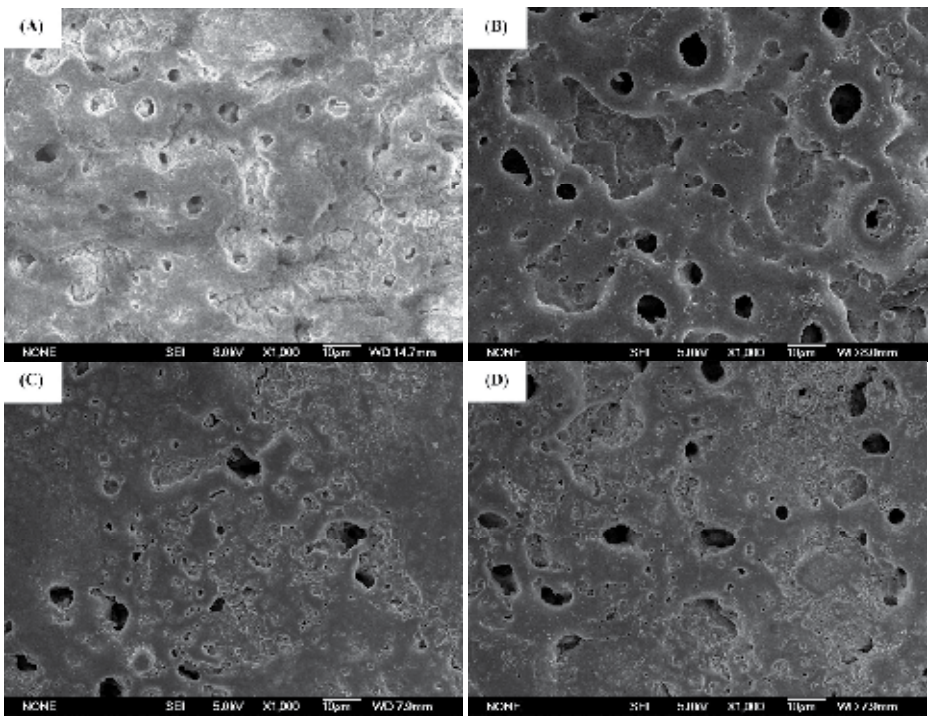

Fig. 4. SEM surface morphologies of micro-arc oxidation coatings treated for different oxidation time.(A) 10min;(B) 20min;(C) 30min;(D) 40min (Ni et al. 2008).

In the early growth stage (short treatment time) of the MAO, micro arc occurs to the fresh metallic surface or to the thinner dielectric layer (obtained at low discharge voltage) where the less accumulated electrical charge is required to activate an arc and therefore extensive small micro-arcs over the surface are facilitated. Numerous homogenously distributed tiny

pores with thin layer thickness were thus found for the specimens, as opposed to those specimens prepared at high discharge voltages or extended treatment times, where less but large arc events occur. Fig.4 demonstrated the morphological difference between coatings obtained for different treatment time in our previous work (Ni et al. 2008).

The phases of the oxide layers formed by MAO process characterized by XRD analysis are dependent on oxidation time and applied voltage, too. Generally, with increase of oxidation time, the crystal of TiO_2 become better and diffraction peaks of hydroxyapatite in the coatings were stronger. In other words, a shorter treatment time favor the growth of anatase in the MAO film. Anatase formation requires much lower activation energy than the rutile polymorphism as had been reported (Shibata et al., 1993). Under a short treatment time, the available energy is only sufficient to overcome the activation energy for the formation of anatase and the film is thus exclusively composed of this form. A longer treatment time thermodynamically favors the stable rutile phase. Fig.5 showed this tend for us by work of our previous work (Ni et al. 2008). And it agrees well with the results revealed by Lie et al (Li et al., 2004).

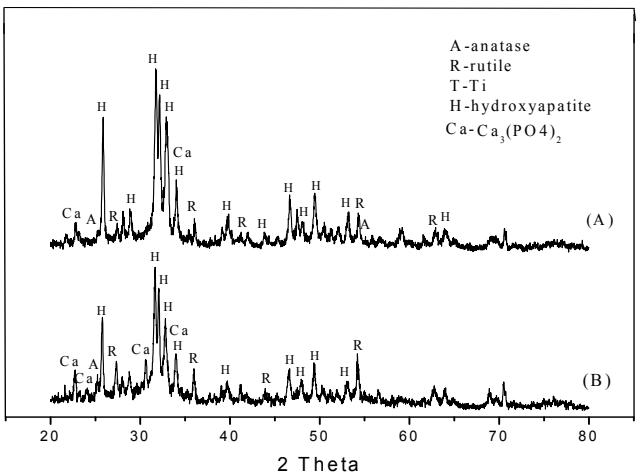

Fig. 5. XRD patterns of the MAO samples treated for (A) 30 min and (B) 40 min (Ni et al., 2008)

The same trend as treatment time was seen in discharge voltage, and the reasons can be same too (Han et al., 2003; Li et al., 2004; Shibata et al., 1993). XRD patterns of the films obtained by Han et al (Han et al., 2002a) showed that at low applied voltage, the film was composed of rutile and a small amount of anatase and with increasing the applied voltage, anatase phase gradually disappeared and rutile phase gradually increased. Sometimes, other Ca- and P-containing compounds such as $Ca_3(PO_4)_2$, $CaTiO_3$, b-$Ca_2P_2O_7$ and a-$Ca_3(PO_4)_2$ could be observed also (Ni et al., 2008; Han et al., 2003; Song et al., 2004). The possible reason for which may be higher applied voltage used (Han et al., 2003; Song et al., 2004).

As discussed above, the thickness, pore size and the content of Ca and P tended to increase with the applied voltage. And the morphology of the oxide layer was dependent on the treatment time and voltage applied during the oxidation treatment. On the other hand, the thickness and elements content changes of the MAO coating with oxidation time are not same with each other in different investigators' works. Sometimes, the thickness sostenuto thickened with the time increase (chen et al., 2006), and sometimes it thickened first and

then decreased at a certain value time (Ni et al., 2008). The tendency may be influenced by some other factors. The same condition can be seen in element content of the coating (chen et al., 2006; Ni et al., 2008).
There are also many other factors than affect the MAO film on titanium surface, such as power mode, current density, electrolyte temperature, substrate material, and so on. They all influence the microstructure, properties and of course application of the MAO coating formed on titanium and its alloy.

2.6 Summary of MAO on titanium

In a word, MAO is a simple, controllable, and cost-effective method of forming a porous TiO_2 layer on the titanium implant surface. The microstructure of the oxide layer such as amount and diameter of the pores, thickness, and roughness are easily controllable by adjusting the electrolyte concentration, voltage, processing time, current during the MAO process. Moreover, the element concentration in the coating such as Ti, O, Ca and P can also be regulated by above factors. Thus, phase composition of coating will regulate with it regulately to meet biological performance of the materials. In order to obtain HA in the coating to improve biocompatibility and biological activity of the sample, many investigators employed two-step approach such as Microarc Oxidization-Hydrothermal synthesis, hybrid treatment of micro-arc discharge oxidation (MDO) and electrophoretic deposition (Fu et al., 2002; Han et al., 2002b; Wei & Yang, 2009; Xie et al., 2000; Xu et al., 2006). Whereas, a composite film of HA and TiO_2 also can be prepared directly by MAO in Ca- and P- containing electrolyte by regulating process parameter (Chen et al., 2006; Han et al., 2003; Li et al., 2004; Ni et al., 2008).

3. n-SiO2 embedded HA/TiO2 composite coatings deposited on titanium by micro-arc oxidation

As reported, silicon plays an important role in bone mineralization and formation and is therefore incorporated into a wide variety of medical implants and bone grafts used today. The addition of silicon to HA causes a decrease in grain size that subsequently affects surface topography, dissolution–reprecipitation rates and the bone apposition process (Porter, 2006; Porter et al., 2004). In our present work, based on our preliminary works (Chen et al., 2006; Ni et al., 2008) and the function of silicon in bone mineralization and formation, a novel thought that nano-silicon dioxide particles are added to the composite coating of HA/TiO_2 formed by MAO is produced, and the primary experiment was carried out.

3.1 Experimental procedure

The material oxidized was commercially pure titanium (TA_2), the element composition of which was shown in Table 1.
The titanium plates (30×15×2 mm^3) were polished progressively using 200, 400, 800, and 1000-grit silican carbon paper and ultrasoniclly cleaned with acetone and deionized water respectively. The cleaned Ti plate was oxidized as an anode in an electrolyte containing sodium phosphate monobasic dehydrate ($NaH_2PO_4.2H_2O$, AR), calcium acetate monohydrate ($(CH_3COO)_2Ca.H_2O$, AR), a little ethylene glycol ($C_2H_6O_2$, AR) as dispersant and a small quantity of SiO_2 nano-particles. The electrolyte was treated with ultrasonic in order that the solute was dissolved sufficiently and the n-SiO_2 particles were dispersed in the electrolyte uniformly. While a stainless steel plate was used as a cathode in an

electrolytic cell. After the MAO treatment, the sample was washed with water and dried with a blower.

TA_2	Fe	O	C	N	H	Ti
Contents of impurities (<wt%)	0.30	0.20	0.10	0.05	0.015	remains

Table 1. Element composition of the pure commercial titanium

For the MAO treatment, a pulse power supply was employed. The pulse parameters (e.g. voltage, current, and duty cycle) can be adjusted independently. In this study, MAO was carried out at current density of 22~25A/dm^2 for different oxidation time as 10 min and 20 min at room temperature, and the duty cycle was 15%. The final voltage was 450V and 500V.

The surface and cross-sectional morphologies of the films were observed by scanning electron microscopy (SEM, JSM-6700F, Japan). The elemental composition was examined with energy dispersive X-ray spectrometer (EDX, INCA, Oxford) incorporated into the scanning electron microscope. The phase components of the coatings were analyzed using X-ray diffraction (XRD, D/max-γB, Japan) using CuKα radiation at 40 KV and 150mA with a scanning speed of 9^o/min and a step size of 0.02^o.

According to ISO7405:1997(E)standard (ISO 7405:1997(E)), 3-[4,5-dimethylthiazol-2-yl]-2-5-diphenyl-tetrazolium bromide (MTT) assay method was used to preliminarily evaluate the cylotoxicity of the films on L-929 mouse fibroblast. After sterilization of the samples, the extracted liquid of the films, pure titanium substrates was prepared respectively by adding the samples together with DMEM with 10% fetal bovine serum in aseptic test tube and then cultivated at 37℃ in CO_2 standing-temperature incubator for 24 h. L-929 cell suspension of 5×10^3 cells/ml was made by diluting the cells with DMEM with 10% fetal bovine serum. Aliquots of 200 uL of the cell suspension were seeded into each well of 96-well plate and cultivated at 37℃ in CO_2 standing-temperature incubator for 24 h. And then, the extracted liquid was added into the well with 200μl per well after removing the previous culture medium and washing twice with PBS. DMEM with 10% fetal bovine serum was added for negative group and 1% solution of phenol was added for positive group. After the 96-well plate was cultured at 37℃ in CO_2 standing-temperature incubator for 2d, 4d and 7d respectively, about 20μl MTT solution（5mg/ml）was added in each well for 4h at 37℃ in the CO_2 standing-temperature incubator. After washing with PBS twice, about 150μl dimethyl sulfoxide (DMSO) was added to dissolve crystals. After shaking at room temperature for 10 min and appearing of bluish violet crystals, absorbance (Optical density - OD) of each well was determined at 490 nm using a microplate reader. Cylotoxicity grade of the samples was converted by relative growth rate (RGR) according to table 2.

$$RGR = \frac{\text{average value of the samples}}{\text{average value of the negative group}} \times 100\% \quad (1)$$

RGR(%)	≥100	75~99	50~74	25~49	1~24	0
cytotoxicity grade	0	1	2	3	4	5

Table 2. Relation between cytotoxicity grade and RGR

3.2 Results and discussion

3.2.1 Morphology of the coatings

Fig.6 showed SEM micrographs of the surfaces of the MAO coatings formed at different oxidation time. In Fig.6 (A), there were micro-pores and snaky apertures on the surface of the film when the treated time was 10min. The higher magnification images indicated that there was a reticular structure at the edge of the pores while the network structure was not observed on the other area of the surface (in Fig.6 (B) and Fig.6 (C)). When the treated time extended to 20 min, the pores and the snaky apertures on the surface disappeared and the surface was covered by many micron-sized globules whose size was about 5~10μm.(in Fig.6 (D)). In the higher magnification the coating was a coralloid structure on the surface of the micro-sized globules (in Fig.6 (E) and Fig.6 (F)).

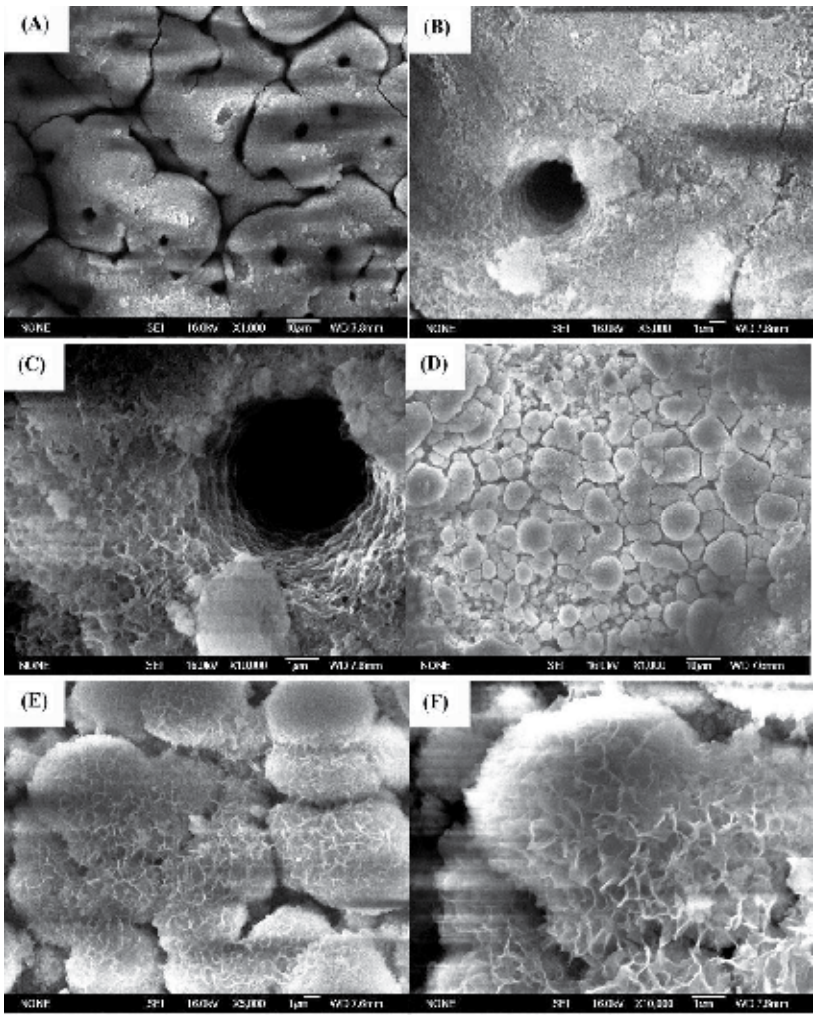

Fig. 6. SEM surface morphologies of micro-arc oxidation coatings treated for 10min (A) low magnification (×1000); (B) local magnification of (A) (×5000); (C) local magnification of (B) (×10000) and for 20min (D) low magnification (×1000); (E) local magnification (D) (×5000); (F) local magnification of (E) (×10000)

The cross-sections of the typical coating were shown in Fig.7 When the oxidation time was 10min, the thickness was about 25μm. The thickness of the film increased with the oxidation time increasing. The thickness was nearly 40μm when the oxidation time reached 20min. There was no obvious discontinuity between the deposited film and the substrate. That indicated that the film can be tightly adhered to the substrate.

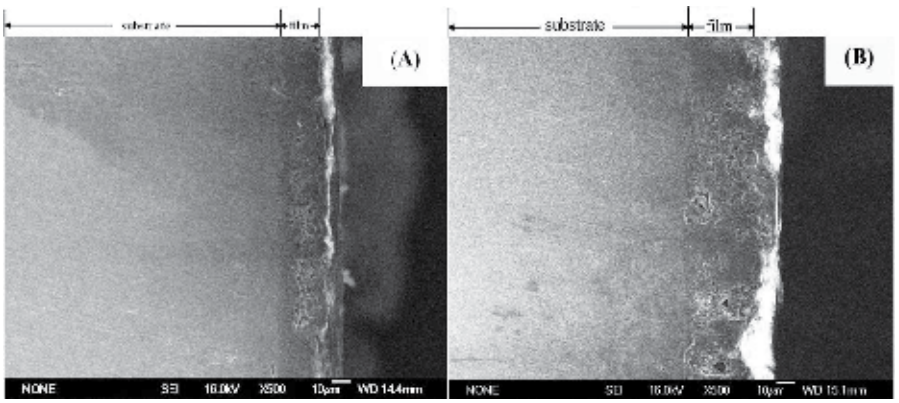

Fig. 7. SEM cross-sectional views of micro-arc oxidation coatings treated for (A) 10min and (B) 20min

3.2.2 Elemental composition of MAO coatings

The coating formed by MAO contained Ca, P, and Si as well as Ti and O, as shown in Fig.8 and in Fig.9. The atomic content of silicon was 0.74% and 1.31% respectively in films treated for 10min and 20min. It was suggested that the elemental compositions in an electrolytic solution could be compounded into the coating during MAO process. As shown in Fig.9 the content of Ca, P, Si and O were gradually reduced from the surface of the coating to the titanium substrate, while the content of Ti was gradually increased.

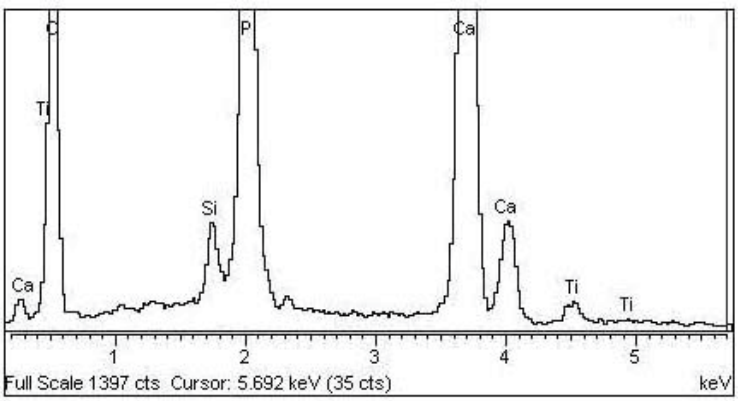

Fig. 8. EDX spectrum of Ti specimens treated with MAO

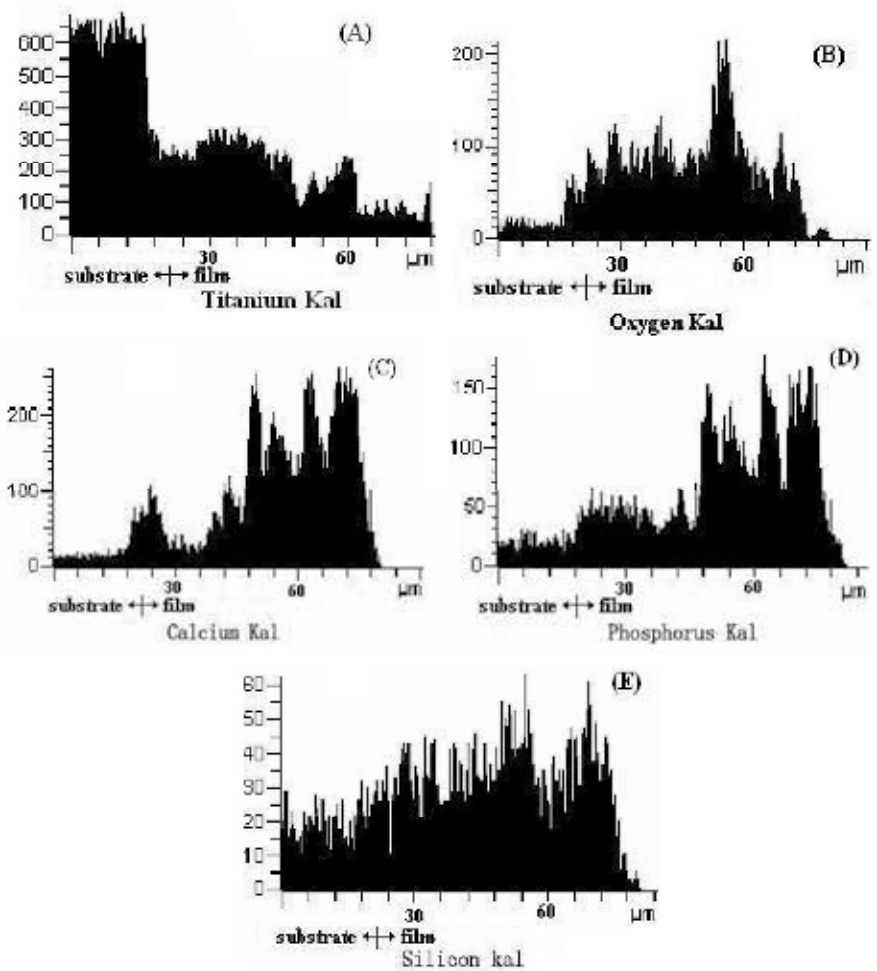

Fig. 9. Depth profiles of the elements in the oxidized coating of titanium (A) titanium; (B) oxygen; (C) calcium; (D) phosphorus and (E) silicon

3.2.3 Phase components of MAO coatings

The XRD patterns of the micro-arc oxidized samples obtained at different oxidation time was shown in Fig.10**.** When the oxidation time was 10 min, the film (in Fig. 10 (A)) was mainly composed of rutile, anatase and Ti, especially the peaks of rutile were strong. The peaks of hydroxyapatite could be observed, but the peaks were broad and weak, which implied that the crystallization of the formed hydroxyapatite was poor. When the treatment time was 20 min (in Fig. 10 (B)), the diffraction peaks of hydroxyapatite in the coatings were strong and became a predominant component in the films. It is shown that in the experiment with increasing treatment time, the applied voltage increased, the working energy increased, the HA coating formed under the effects of thermochemistry, electrochemistry and plasma-chemistry. On the contrary, the peaks of rutile weakened and the peaks of anatase and Ti were hardly observed. But the diffraction peaks of silicon or silicon oxide was not observed. It could be inferred that silicon in the coating by MAO was little.

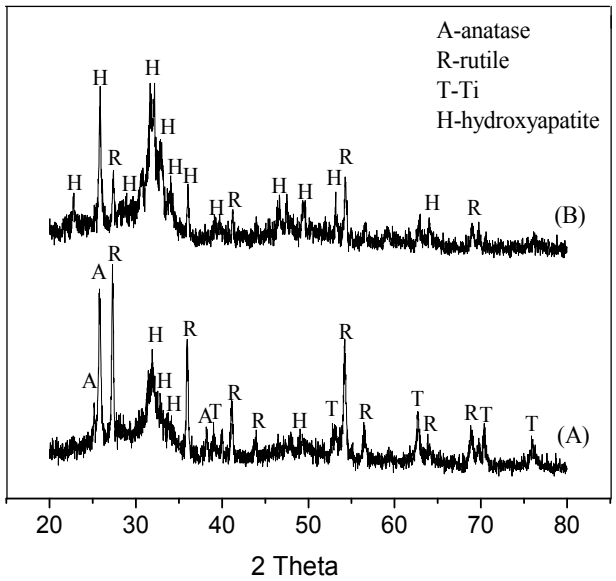

Fig. 10. XRD patterns of the MAO samples treated for (A) 10 min and (B) 20 min

3.2.4 Cytotoxicity of MAO films

The average OD-value, RGR and cytotoxicity grade of each tested group for different cultivation days were listed in table3, table 4 and table 5 respectively. The OD value increased with the increase of the cultivation time and presented its dependence on ultivation time. The cytotoxicity grade of each MAO sample was 0 and the cytotoxicity grade of titanium substrates was 0 or 1. But every cytotoxicity grade for that of the positive groups was 4. The biocompatibility of MAO films is better then that of the titanium substrate because there are HA, rutile and anatase in the film. The OD—value of each tested group were similar to the negative group and significantly different from positive group which indicated that the materials tested were safe to L-929 cells.

groups	MAO samples		titanium substrates	negative groups	positive groups
	treated for 10 min	treated for 20 min			
OD	0.275±0.026	0.281±0.028	0.256±0.031	0.235±0.025	0.055±0.007
RGR(%)	117.02	119.57	108.94	100	23.40
cytotoxicity grade	0	0	0		4

Table 3. Average values of OD, RGR and cytotoxidty grade for the tested groups at 2 days

groups	MAO samples		titanium substrates	negative groups	positive groups
	treated for 10 min	treated for 20 min			
OD	0.393±0.039	0.409±0.028	0.379±0.037	0.383±0.031	0.050±0.004
RGR(%)	102.61	106.79	98.95	100	13.05
cytotoxicity grade	0	0	1		4

Table 4. Average values of OD, RGR and cytotoxidty grade for the tested groups at 4 days

groups	MAO samples		titanium substrates	negative groups	positive groups
	treated for 10 min	treated for 20 min			
OD	0.835±0.023	0.852±0.021	0.817±0.022	0.832±0.024	0.072±0.005
RGR(%)	100.36	102.40	98.20	100	8.65
cytotoxicity grade	0	0	1		4

Table 5. Average values of OD，RGR and cytotoxidty grade for the tested groups at 7 days

4. Conclusion and remarks

According to above study, nano-silicon embedded HA/TiO_2 composite coatings can be prepared by micro-arc oxidation in Ca- and P- containing electrolyte by adding a little n-SiO_2 nano-particles in it. The surface of the coatings on titanium substrate produced a network structure without apparent interface to the titanium substrates. The thickness of the film could reach about 40μm. The film contained Ca, P, and Si along with Ti and O. As the treatment time increased, the hydroxyapatite became a predominant component in the film when the treatment time was 20min. the cytotoxicity grade of the coatings was 0 according to the MTT test and that of titanium substrate was 0 or 1, meaning that the films and substrate all had no cytotoxicity. The coatings' biocompatibility became better with increase of treatment time and were better than biocompatibility of substrate.

Based on the preparation of the composite coating of HA and TiO_2, the new work introduced a novel idea adding silicon to the composite coating in order to improve the bioactivity of the coating, and the results also proved the realizability. But, there are also many works needed to be done in depth. Although the EDS spectrum of the films revealed that the deposited coatings were composed of Ti, O, Ca, P and Si, but no siliceous crystalline was detected by XRD. The chemical status and location of the silicon are not clearly understood yet. Does silicon existe as an ion, atom, or other valence state in the HA and TiO2 coating? Or, does it constitute the structure of Si-HA? Further studies are needed to answer these questions. Primary cylotoxicity of the films on L-929 cell was evaluated and the results were perfect. However, detailed mechanical behavior and bioactivity of the coating such as apatite induction and corrosion resisting of the film in simulated body fluid (SBF), in vitro cell tests, and in vivo tests need to be researched. Furthermore, more efforts needed to pay for the optimization and mechanism study of MAO technique on titanium.

5. Acknowledgements

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14

Implant Insertion Methods and Periimplant Tissues – Experimental Study

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1. Introduction

The replacement of missing teeth with dental implants has become predictable treatment modality over the past several decades. The function of dental implants depends on the process of osseointegration, defined by Brånemark (Brånemark, 1985, as cited in Abrahamsson & Cardaropoli, 2006) as „direct structural and functional connection between living ordered bone and the surface of load carrying implant". The process through which osseointegration is achieved depends on several factors, such as biocompatibility of the metal used as well as the design and surface characteristics of the implant, the condition of the implant socket, the surgical technique used and the loading conditions applied (Abrahamsson & Cardaropoli, 2006).

Endosteal implants are available in various designs, forms and materials. The earliest implant designs were one-component devices, i.e. implant body and implant abutment were connected in a single unit. Those implants could only be inserted in one-stage surgical method and are collectively referred to as "one-stage" or non-submerged implant systems. When inserted, implants penetrate through the oral mucosa into the oral cavity thus risking the possible contamination and/or early loading that could result in implant failure.

Within the past five decades numerous types of implant designs have evolved. Almost all of them have a common characteristic: they consist of two parts – the implant body and the implant abutment or transmucosal part. The first part (implant body) is placed into the bone socket and covered with mucoperiosteal flap. The second part (implant abutment) is connected to the implant after a period of healing in the secondary surgical procedure. These designs are recommended for so called "two-stage" or submerge surgical approach, and the components of the soft tissue cover, epithelial and subepithelial tissue, act as a barrier between the internal (bone tissue) and external (oral cavity) environment.

The studies on both surgical methods have been well documented (Adell et al., 1990, as cited in Heydenrijk et al., 2002; Lindquist et al., 1996; Ericsson et al., 1996; Buser et al., 1996; Levy et al., 1996; Brägger et al., 1998; Abrahamsson et al., 1999; Hermann et al., 2001; Lindquist et al., Heydenrijk et al., 2002).

On the basis of early research in dogs, Brånemark and his coworkers introduced submerged implant placement believing it was one of the key prerequisites for osseointegration (Weber & Cochran, 1998). Namely, implants were placed under cover of the oral mucosa for a healing period of 3-6 months and after that period a second surgical procedure was

performed i.e. the implant exposure and transmucosal components (implant abutments) were placed on the implants. Implants that accidentally became exposed to the oral cavity through wound dehiscence exhibited less favorable periimplant healing than the implants that had been submerged under oral mucosa. This concept, which requires a second stage procedure, is still followed today. An opportunity exists at the second stage surgery to correct soft tissue defects, poor soft tissue relationships, poor implant placement and bone defects (Fig. 1).

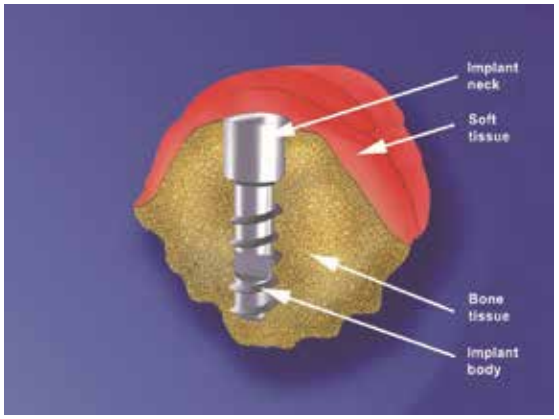

Fig. 1. Two-stage implant insertion method

However, Schroeder and al. (Schroeder, 1981, as cited in Weber & Cochran, 1998) demonstrated in the late 1970s that non-submerged or one-stage surgical method allows successful outcome of implant insertion, i.e. successful osseointegration. After the insertion the implant penetrates through the gingival tissue into the oral cavity and it is covered with the healing cap which does not interfere with the opposing or adjacent teeth. The fact that only one surgical intervention is necessary allows for soft tissue healing to the transmucosal portion of the implant by primary intention from the moment of implant insertion. This simplified method which eliminates the need for a re-entry (second surgery) is more attractive to both patients and clinicians (Fig. 2).

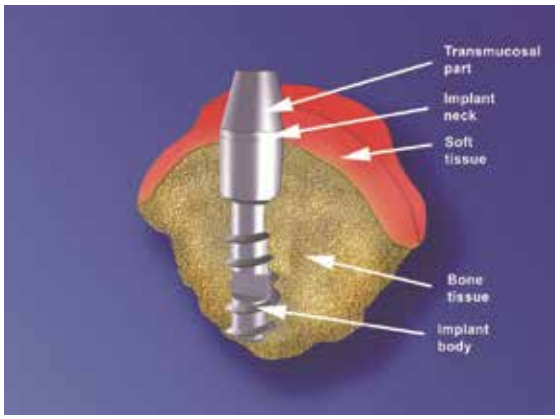

Fig. 2. One-stage implant insertion method

For a long time it was considered that implant failure was a result of soft tissue ingrowth in the coronal aspect of bony implant bed, inflammatory infiltrate, granulation tissue, bone resorption and implant mobility that was caused by the implant communication to the oral cavity.
It is now recognized that the requirement to submerge the implant during healing is not obligatory, and even has the advantages over submerged approach including: 1) the lack of secondary surgical intervention to connect the implant body and transgingival component; 2) a more mature soft tissue healing due to avoiding the second stage surgery; 3) the lack of an interface/microgap between the implant and the abutment at or below the alveolar crest level; 4) healed periimplant mucosa is not disturbed with second stage procedure for abutment placement or abutment exchanges; 5) during the osseointegration period the implants are accessible for clinical monitoring; 6) cost and time benefit advantage (Heydenrijk et al., 2002). However, one-stage implantation is not preferred treatment modality in the cases of: 1) prevention of undesirable implant loading during the osseointegration period when implants are inserted in low-density bone; 2) alveolar ridge augmentation procedures or guided bone regeneration with simultaneous implant placement when the wound has to be closed perfectly to prevent the infection of bone or membrane exposure; 3) integrated implant abutment interference with opposing jaw (in the case of one-piece implant design).
A review of literature reveals that there is no agreement as to which of these two surgical methods has achieved better outcome.

2. Experimental research

Numerous reports have compared the submerged and non-submerged implant types in animal models (Ericsson et al., 1996; Weber et al., 1996; Abrahamsson et al., 1996, 1999; Levy et al., 1996; Fiorellini et al., 1999; Moon et al., 1999; Hermann et al., 2000; Berglundh et al., 2007). The amount of scientific work was focused on the achieving and maintaining a healthy relationship of dental implants to the surrounding hard and soft tissues over extended periods. To evaluate osseointegration of dental implants by animal studies many methods have been established: radiographic evaluation of bone healing, pathohistological, immunohistochemical and histomorphometric analyses. Radiographic evaluations provide the results of periimplant bone changes, i.e. the differences of periimplant bone levels. Histomorphometric analysis is the measurement of direct bone-to-implant contact without connective tissue interposition, but all these analyses include no information about functional and structural bone architecture, bone maturity or inflammatory signs. Also, because the implants are transmucosal devices and periimplant tissues are expected to act as a barrier, pathohistological evaluations of soft tissues around dental implants were performed in order to examine the morphologic characteristics of epithelial and subepithelial tissue.

2.1 Studies on periimplant soft tissue

The role of gingival epithelium in forming a biologic seal is of great importance in implant dentistry. This seal must be effective enough to prevent the ingress of bacterial plaque, toxins, oral debris and other substances taken into the oral cavity. The initial breakdown of soft tissue was first seen around implant abutments as gingival inflammatory reaction that was followed by osteoclastic activity of underlying hard tissue and chronic resorption of the supporting bone.

James and Kelln were the first to begin a systemic study to investigate gingival attachment to the implant (James & Kelln, 1974). Although they investigated oral implants made of Vitalium (stainless steel) they concluded that an adhesion exists at the interface between the junctional epithelial cells and the penetrating implant surfaces.
Current concepts of the biologic formation of mucosal attachment are based on scientific investigations using a combination of light and electron microscopy. Periimplant epithelial tissue most closely resembles that of the natural dentition. The oral epithelium lines the lateral surface of the gingival sulcus as sulcular epithelium. Its apical part is lined with the coronal cells of the junctional epithelium which provides an epithelial union between the implant and the surrounding gingival tissue. The junctional epithelium adheres to the implant surface through a basal lamina and hemidesmosomes thus preventing external agents from moving into the internal environment of the jaw. Long-term implant maintenance depends significantly on the achievement and preservation of this attachment.
In our experimental study nine dogs (German shepherds), mean age: 4,5 years, mean weight: 32 kg were used. In the first experimental phase third and forth lower premolars were extracted bilaterally. After a healing period of eight weeks in the second phase the implants were inserted. Using split mouth design 36 c.p. (commercially pure) titanium implants were inserted using one-stage surgical method on the right mandibular side and two-stage method on the left side respectively. Three months after the implantation the animals were sacrificed and the third experimental phase – pathohistological analysis was performed.
Analyses were performed on 90 specimens from the two soft tissue regions (epithelial and subepithelial ttissue) thus comprising 180 specimens. Semi-quantitative analysis was performed for each site (evaluation of basal membrane degradation, inflammatory cell infiltration, tissue necrosis, number of blood vessels, appearance of blood vessel walls) and graded. Quantitative analyses of pathohistological findings were performed according to the established grading indices for each evaluated region. Outcomes of two surgical methods were compared using non-parametric Wilcoxon-Mann-Whitney rank-sum test for two small independent samples.
The analysis of epithelial tissue: According to the results on the basis of descriptive statistics better results were achieved by one-stage surgical method, but there were no statistically significant differences between the two applied surgical methods. Pathohistological analyses of 9 specimens inserted in one-stage approach revealed preserved basal membranes in 7 sections, massive inflammatory cell infiltrates in 2 specimens, complete tissue necrosis in 2 and partial in 3 sections (Fig. 3).

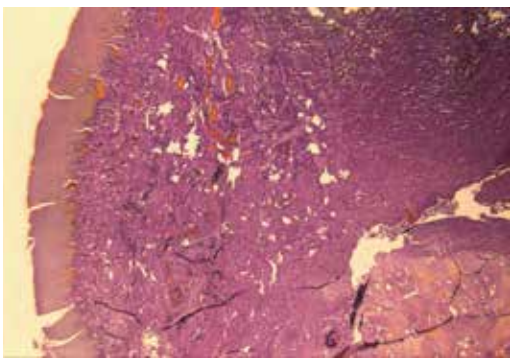

Fig. 3. Preserved epithelial tissue with basal membrane. Presence of inflammatory cells with initial necrotic lesions in subepithelial connective tissue (HE X 40).

Regarding the findings of two-stage implants, the absence of epithelial tissue, marked inflammatory infiltrate and complete necrosis were observed in 6 specimens. Basal membrane structure was preserved in only 3 sections (Fig. 4).

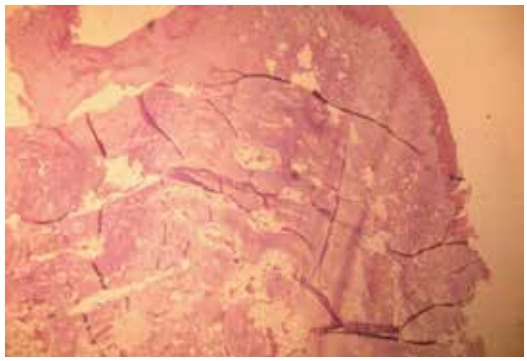

Fig. 4. Partial necrosis of gingival epithelial tissue, degradation of basal membrane, partial inflammatory cell infiltrate and initial necrosis (HE X 40)

The analysis of subepithelial tissue: According to the results on the basis of descriptive statistics, better results were achieved by one-stage surgical method regarding the number of blood vessels and vascular volume, while regarding blood vessel walls and inflammatory cell infiltrate, two methods achieved identical results. No statistically significant difference was found between the two methods. Pathohistological analyses of the implants inserted in one-stage method revealed marked neovascularization, dilated blood vessels with thick walls and inflammatory infiltrate in 3 specimens (Fig. 5).

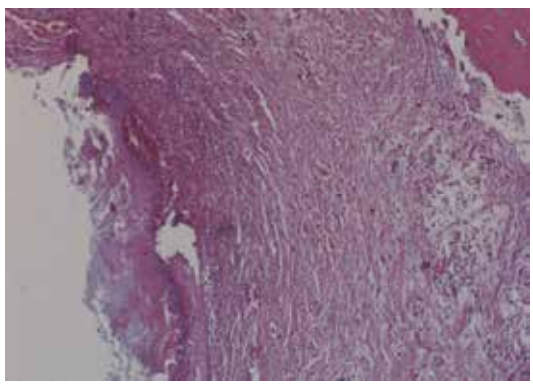

Fig. 5. Subepithelial connective tissue saturated with chronic inflammatory cells and increased vascular proliferation and dilatation (HE X 40)

Increased number of blood vessels was found in 6 specimens and marked inflammation in 4 sections of the implants inserted in two-stage method (Fig. 6).

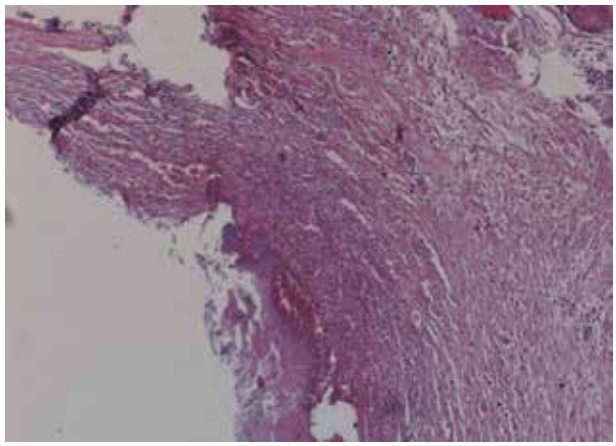

Fig. 6. Presence of partially necrotic epithelial tissue and torn subepithelial connective tissue with necrotic lesions, marked inflammatory cell infiltrate and increased vascular proliferation (HE X 40)

Dynamic changes that occur during soft tissue healing process are related to the periimplant sulcus depth, connective tissue-implant contact and the length of periimplant epithelial attachment (Buser at al., 1990; Cochran et al., 1997).

In a beagle dog study of crystal sapphire implants that were placed in one-stage procedure Fartash et al. (Fartash et al., 1990) observed discrete inflammatory infiltrate in periimplant soft tissue, while Chavrier et al. (Chavrier et al., 1994, as cited in Weber & Cochran, 1998) observed inflammatory cells in approximately half the biopsies in their study on experimental dogs. Buser et al. (Buser at al., 1990) detected the presence of periimplant epithelial attachment in one-stage implants in experimental dogs, while Abrahmsson et al. (Abrahamsson et al., 1996, 1999) in their similar experimental studies reported that surgical method had not influence on the formation and structure of periimplant soft tissue. Weber et al. comparing non-submerged and submerged implants in beagle dogs, found significant differences between implant types. In every case where the implant was initially submerged, the epithelium was found apical to the interface (microgap) between the implant and transgingival abutment. Attachment level for non-submerged implants was located more coronally compared to submerged implants. Comparing one- and two-stage implants statistically, the authors concluded that there was a significant difference in position and epithelium extension between the two methods (Weber et al., 1996).

In their review article Weber and Cochran (Weber & Cochran, 1998) described in details the significance of soft tissue response to submerged and non-submerged osseointegrated implants. Periimplant epithelium was described as junctional epithelium, in many ways analogous to the natural tooth and no difference between the methods of insertion was observed.

Moon et al. in their experimental study in dogs examined soft tissue attachment to dental implants inserted in one-stage method. The attachment was comprised of two portions: marginal junctional epithelium and connective tissue. Epithelium was observed in all examined specimens. The authors consider the connective tissue that is in direct contact with the implant surface and located apically of junctional epithelium significantly more

important. The portion of connective tissue which is next to implant surface is characterized by its absence of blood vessels and abundance of fibroblasts. The authors assume that the fibroblast rich region is „responsible" for establishment and maintenance of proper seal between oral environment and periimplant bone (Moon et al. 1999).
Herman et al. (Hermann et al., 2000) in their research on one- and two-stage implantation concluded that implant abutment/interface had an impact on gingival position and periimplant sulcus depth. Placing the microgap (the connection of the abutment and the implant body) below the bone level significantly increased the forming of infrabony periimplant pockets. If the microgap was placed 0,5 -1,0 mm below the bone level, bone resorption was increased. Microbial contamination of the microgap associated with micromovement of the implant can cause the bone resorption and chronic inflammation of the adjacent tissues. That is the reason it is not recommendable to place two-stage implants with microgap positioned apically in order to avoid negative effects on periimplant tissues. This problem has not been reported for transmucosally placed one-stage implants.
Regarding the morphogenesis of periimplant mucosal attachment, Berglundh et al. (Berglundh et al. 2007) observed epithelial proliferation and the first signs of a barrier (junctional) epithelium two weeks after implantation. Tissue maturation and collagen fiber organization was evident 6 to 12 weeks of healing.
Epithelial and subepithelial periimplant tissues were not analyzed in details in most investigations. In the experimental study in dogs Ponguarison et al. (Ponguarison et al., 2007) investigated inflammation associated with implants with different surface types inserted in one-stage method. The junctional epithelium and oral epithelium were examined for assessment of the inflammatory infiltrate. The areas of infiltrate were categorized into three grades according to the density of inflammatory cells. Pathohistological findings revealed that the periimplant tissues surrounding all types of abutment surfaces tested, exhibit some degree of inflammation. Subepithelial areas had the greatest density of infiltration (3+ grade). Implants with machined surface with circumferential groove had the largest area of inflammatory infiltrate, but there were no statistically significant differences in number of infiltrating cell types between the four investigated transmucosal surfaces, suggesting that the surface of the implant has little if any influence on the nature of the surrounding inflammation, which is in agreement with the results of numerous authors who studied the influence of implant surface on periimplant soft tissue attachment or the initiation and progression of inflammation (Abrahamsson et al., 2002; Klinge & Meyle, 2006).

2.2 Studies on periimplant bone tissue

Bone is an active biologic tissue that undergoes resorption and remodeling periods in response to various factors. Attention must be paid to maintain bone in a healthy state during the surgical procedure of implant insertion so as not to damage the cell viability and nutrient blood supply to the bone. The use of slow-speed rotary cutting instruments with internally and/or externally applied irrigation provides less amount of cell damage during surgery. Following the osteotomy, the bone must heal around the endosteal implant surface.
The concept of osseointegration arose from the studies of osseous wound healing that had started in the 1950s by Bränemark. Titanium chambers containing a transillumination system were inserted into the fibulae of rabbits to observe cellular changes during endostael

wound healing. At the completion of the study, retrieval of the titanium chamber required the fracture of bone tissue that has integrated with the chamber surface. Bränemark's team found that implants made of commercially pure (c.p.) titanium, careful bone preparation and immobilization of the implant during the initial healing phase were necessary to effect a rigid fixation of the implant to the surrounding bone tissue (LeGeros & Craig, 1993).

Surgical placement of endosteal implants elicits an osteogenic response largely driven by local factors. The initial healing response is independent of direct mechanical control because bone heals optimally in the absence of functional loading. The vascularly dependent ostegenic process can be easily disrupted by micromotion at a healing bone-implant interface. This is one of the main reasons why some surgeons advocate two-stage implant placement. Following a maturation phase of about 1 week newly formed osteoid is primarily mineralized when osteoblasts deposit about 70% of the mineral found in mature vital bone. Adequate resistance to loading in humans is achieved in about 18 weeks, but there are no quantitative data (Misch, 1999).

Two theories for the mechanism responsible for osteogenesis at implant interface exist in the literature. According to Davies et al. (Davies et al., 1991, as cited in Steflik et al., 1999) there is no fibrillar material directly at the implant-bone interface. Bone derived cells deposit calcified accretions to condition the implant surface prior bone formation, thus no collagen fibers directly interface with the implant. The second theory, based on the studies and investigations of Steflik, Albrektsson and Linde (Steflik et al., 1998; Albrektsson et al., 1981; Linder et al., 1983, as cited in Steflik et al., 1999) suggests that an unmineralized collagen fiber matrix is deposited at the implant interface and is subsequently mineralized.

The aforementioned experimental study of the authors on implant insertion in canine mandibles included pathohistological analyses of three bone regions surrounding implants: bone-to-implant contact region, bone-implant interface and bone tissue adjacent to the implant (0,03-0,04 mm) aiming specifically to provide information on bone structure, bone maturity and inflammatory reaction.

Analyses were performed on 90 specimens from each of the three bone tissue regions thus comprising 270 specimens. Semi-quantitative analysis was performed for each site (evaluation of the amount of connective – collagen tissue fibers, osteoblastic activity, number of blood vessels, inflammatory cell infiltration, bone tissue necrosis, appearance of blood vessel walls and blood vessel volume) and graded. Quantitative analyses of pathohistological findings were performed according to the established grading indices for each evaluated region. Outcomes of two surgical methods were compared using non-parametric Wilcoxon-Mann-Whitney rank-sum test for two small independent samples.

The analysis of bone-to-implant contact region: With regard to the results obtained on the basis of descriptive statistics, better results were achieved by two-stage method regarding the amount of collagen tissue fibers and this difference was statistically significant while regarding the osteoblastic activity and bone necrosis the results were identical. Pathohistological analysis of the implants inserted in one-stage method revealed increased amount of connective-collagen tissue fibers in 5 specimens. Necrosis was also observed in the same samples. In remainder moderate osteoblastic activity was found (Fig. 7).

The pathohistological findings of bone tissue in contact with the implants inserted in two-stage method revealed increased amount of connective-collagen tissue fibers in all specimens. No osteoblastic activity was observed. Partial bone tissue necrosis was found in 8 specimens and in 1 necrosis was complete (Fig. 8).

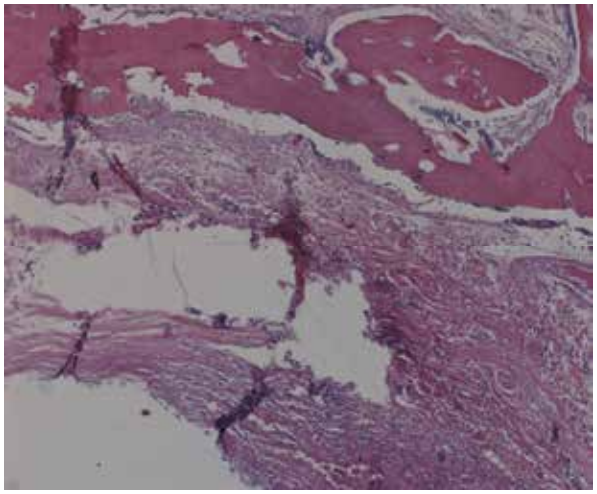

Fig. 7. Increased amount of connective tissue with marked chronic inflammatory cell infiltration, partial necrosis and marked vascular proliferation. Presence of compact bone tissue regions with osteoblastic activity can be detected (HEX40).

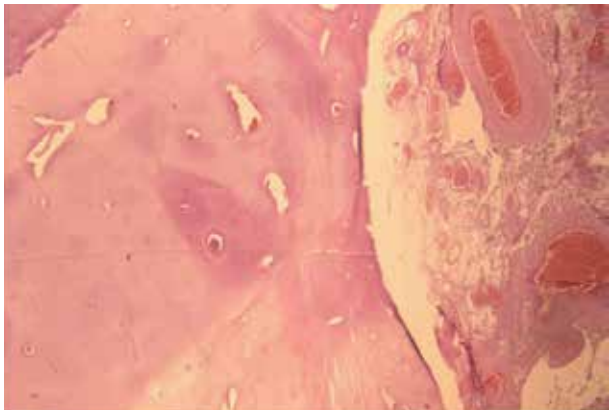

Fig. 8. On the left: compact lamellar bone. On the right: increased amount of connective – collagen tissue, with numerous dilated blood vessels, marked inflammatory cell infiltrate and partial necrotic lesions (HE X 40).

The analysis of the bone-implant interface: According to the results on the basis of descriptive statistics the results were identical regarding median values of osteoblastic activity, blood vessels, inflammatory cell infiltrate and bone tissue necrosis. Better results were achieved by two-stage method regarding the amount of collagen tissue, but there were no statistically significant differences between the methods. The pathohstological analysis of bone-implant interface of the implants inserted in one-stage method revealed increased amount of connective-collagen tissue fibers in all specimens, moderate increase of the number of osteoblasts (osteoblastic activity) was observed in 2 specimens Marked neovascularization was found in 2 and moderate in 1 specimen. Inflammation was absent in all specimens, while necrosis was found in only 1 specimen (Fig. 9).

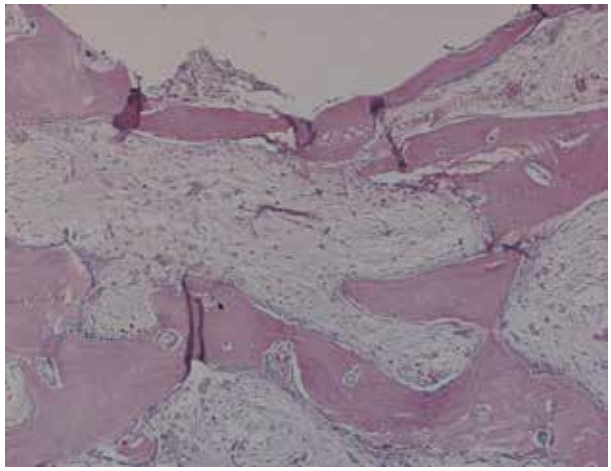

Fig. 9. Marked amount of connective - collagen tissue fibers between bone lamellae with vascular proliferation and marked osteoblastic reaction (HE X 40)

The pathohistological analysis of the implants inserted in two-stage method revealed marked increase of connective tissue fibers in 5 specimens, in 1 specimen moderate increase of osteoblasts was observed, as well as moderate vascular proliferation and marked neovascularization. Inflammation and bone tissue necrosis were also found in only 1 specimen (Fig. 10).

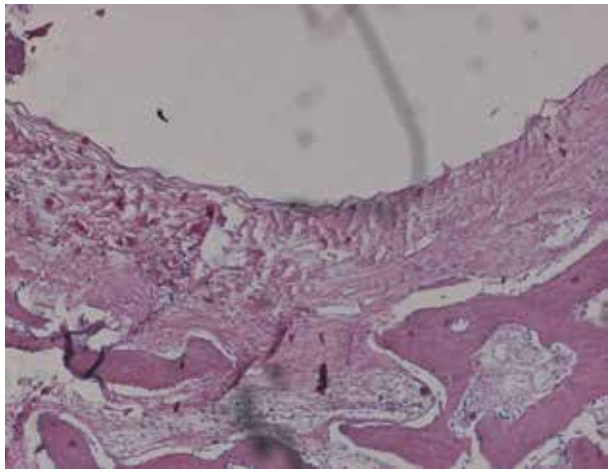

Fig. 10. Loose subepithelial connective tissue. Trabecular bone lattice with collagen tissue proliferation, marked neovascularization, poor osteoblastic activity, moderate inflammatory cell infiltrate and partial bone tissue necrosis (HEX40).

The analysis of bone tissue adjacent to the implant: According to the results on the basis of descriptive statistics the results were identical regarding the median values of amount of collagen tissue, osteoblastic reaction and bone tissue necrosis while better results were achieved regarding the number of osteocytes by two-stage surgical procedure. The latter result was also statistically significant.

Pathohistological analysis of the implants inserted in one-stage method revealed moderate increase of connective-collagen tissue fibers in only 1 specimen where also no osteoblasts and osteocytes could be observed; moderate increase of osteoblasts and osteocytes was found in 3 samples; the number of osteocytes was increased in 4 specimens. Necrotic bone tissue was not found in any of analyzed specimens. In 1 specimen bone structure was completely preserved with no connective tissue fibers, but with no osteoblasts and osteocytes as well (Fig. 11).
Pathohistological analysis of the implants inserted in two-stage method revealed moderate increase of connective tissue fibers in only 1 specimen. No osteoblastic activity could be observed in all specimens. Moderate increase of osteocytes was observed in 3 specimens and marked increase in 6 remained specimens. Necrotic bone tissue was not found in any of analyzed specimens (Fig. 12).

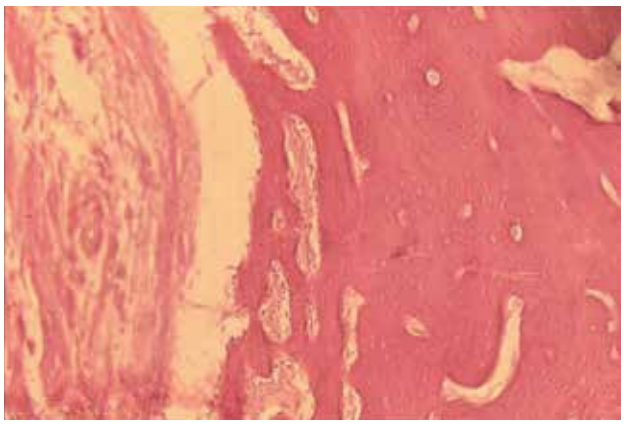

Fig. 11. On the left: loose subepithelial connective tissue, underneath scattered interconnected bone trabeculae with osteoblastic reaction, collagen tissue proliferation between trabeculae. On the right: preserved lamellar bone tissue with osteocytes in lacunae (HEX10).

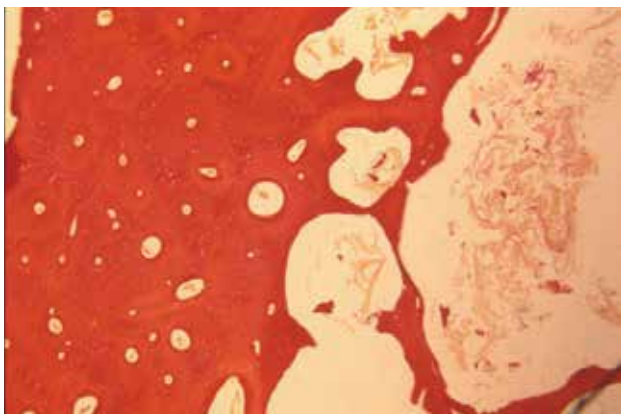

Fig. 12. Mature lamellar bone, underneath interconnected bone trabeculae and intertrabecular collagen tissue proliferation (HE X10).

Experimental study conducted by Ericsson et al. on Labrador dogs analyzed certain characteristics of periimplant tissues following one- and two-stage surgical installation of Bränemark implants. The results demonstrated that titanium implants can obtain proper bone anchorage (osseointegration) despite being exposed to oral cavity during the initial 3-month healing phase (Ericsson et al., 1996).

Implant surface characteristics have been the focus of research in implantology since 1960s. The interrelationship of surgical methods and implant surface was investigated by Levy et al. who analyzed bone-to-implant contact around porous coated root form implants placed in the canine model in one– and two-stage surgical method. After six weeks of healing, the absolute bone-to-implant contact was greater for submerged implants, although significantly on the buccal aspects only (Levy et al., 1996). Similar results were reported by Sagara et al. (Sagara et al.,1993, as cited in Levy et al., 1996) who analyzed bone-to-implant contact of submerged and non-submerged implants placed in dogs after a healing period of 12 weeks. In contrast, Gotfredsen et al. (Gotfredsen et al., 1991, as cited in Levy et al., 1996) found no significant difference between one- and two-stage implant methods in their experimental research on 6 monkeys. Namely, Gotfredsen et al. performed histological evaluations of tissue reactions to unloaded submerged implants without reopening and unloaded non-submerged ITI TPS (titanium plasma spray) implants. After 22 weeks of healing the results indicated that both groups had similar bone levels at the end of healing period and no differences were found in the histological analysis of bone-to-implant contact regions between the implant types. They concluded that osseointegration could be established regardless of the surgical approach. According to Levy et al. (Levy et al., 1996) this difference in protocol may be the important variable in that the long healing period may have provided sufficient time for bone healing to overcome any early inhibitory factors affecting non-submerged implants. Fiorellini et al. (Fiorellini et al., 1999) evaluated the radiographic changes following the insertion of submerged and non-submerged implants in beagle dogs and concluded that there were no differences between the implants on the overall amount and rate of periimplant bone loss, although distinctive patterns of bone resorption existed between the implant types.

The region of bone adjacent to the implant has been investigated in not so many studies. This region is considered a key zone for continued osseointegration of the implant. One of the studies investigating the bone tissue supporting the implant is the one conducted by Meenaghan (Meenaghan, 1974, as cited in Steflik et al., 1999) who first described a triple layer of osseous cells in the remodeling process close to blade implant. He suggested that an outer dense cellular layer existed comprised of mesenchymal–like cells interfaced with the implant. A middle layer of highly vascularized osteogenic tissue existed to a third layer of osteoblasts associated with osteoid matrix and bone. Similar findings were reported by Steflik et al. who observed osteoblastic activity in the zone adjacent to implant. (Steflik et al., 1994, as cited in Steflik et al., 1999).

Investigating crestal bone changes around titanium submerged and non-submerged implants in canine mandibles histomorphometrically, Hermann et al. (Hermann et al., 2000) concluded that bone changes were not dependent on the surgical technique (one- and two-stage insertion).

Koch et al. (Koch et al., 2010) investigated the osseointegration of the implants of different materials inserted in one- and two-stage surgical procedure in dogs. Healing modalities did

not influence the rate of bone-to-implant contact between the implants, among which were the implants of c.p. titanium.

2.3 Future research

Oral implants have developed rapidly. New technical and technological improvement upon diagnostic tools and medical equipment have considerably reduced the risk of operative complications. Basic studies within physical and biologic sciences have resulted in development of various surgical implant systems where special attention has been paid to surface topography and technology as well as to the processes of wound healing along biomaterial interfaces.

Currently direct bone-to-implant contact is considered the optimal healing outcome around endosteal implants. The character of tissues around the implants and the quality of tissue attachment to the implant surface influence the biomechanical response of this integration.

The interfacial interactions between recipient tissues and implanted material are limited to the surface layer of the implant and a few nanometers into the living tissue. The surface property has been recognized as important factor for successful osseointegration because it can promote early and secure bone formation around dental implants. Titanium is by far the material of choice for dental implants and various surface treatments have been proposed to enhance the osseointegration: blasting of the surface, acid or fluoride etching or anodizing. In recent years chemically modified surfaces have been proven to enhance bone-to-implant contact. These modifications increase the hydrophilic property of titanium surfaces and accelerate and enhance the initial adsorption of extracellular matrix proteins (Rupp et al., 2004; Buser et al., 2004, as cited in Jimbo et al., 2011). In their experimental study on Japanese White rabbits Jimbo et al. (Jimbo et al., 2011) investigated bone apposition during the early phases of osseointegration of anodized porous titanium implants modified with hydrogen fluoride solution and ultraviolet irradiated. Histomorphometric analyses revealed that photo-induced hydrophilic surface enhanced bone apposition thus shortening the healing period. In addition, photo-reactive implant surface provide photo-induced sterilization as well as a decontamination of infected implant parts (eg. transmucosal abutments) by simple irradiation.

Recently, much attention has been given to implant surface treatment with biologically active substances. These include purified proteins and synthetic peptides that mediate the binding of osteoblast cell adhesion receptors. When dental implants inserted into the bone a sequence of cellular and molecular events are involved. Undifferentiated mesenchymal cells proliferate and migrate into the implant socket from adjacent marrow, endosteum and periosteum. These cells differentiate into osteoblasts or osteoclasts under the influence of locally acting growth factors, leading to bone maturation and mineralization. Bone morphogenetic proteins (BMPs) are members of the TGF-β (transforming growth factor) - β super family, a group of cytokines with the expression in various tissues. They have numerous functions, among which is to induce the osteoblasts to differentiate and form bone tissue (Asahina et al., 1996; Yamaguchi et al., 1996).

In their study on the influence of recombinant human BMP-2 (rhBMP-2) on bone-implant osseointegration, Lan et al. (Lan et al., 2007) investigated whether osseointegration can be enhanced by the use of rhBMP-2. Titanium implants were coated by rhBMP-2 with

polylactic acid as a carrier. The results showed that osseointegration was improved in quality as well as in quantity, however further studies are necessary to evaluate the optimal dose range of rhBMP-2 and research new carriers.

Most studies evaluating growth, differentiation and matrix factor as potential agents in support alveolar bone augmentation have focused on rhBMP-2, but it is not the only member of BMP family of proteins approved for clinical use. Susin et al. (Susin et al., 2010) have used rhBMP-7 (also known as recombinant human osteogenic protein - rhOP-1) coated onto the titanium porous-oxide surface implants to attain vertical alveolar ridge augmentation and implant osseointegration. Two different doses of BMP-7 were compared. No statistically significant differences were observed. Both doses produced clinically relevant bone formation. The authors concluded that rhBMP-7 coated onto titanium implants exhibits a convincing potential to stimulate local bone formation. Lee et al. (Lee et al., 2010) also investigated osseointegration of implants coated with rhBMP-2 and concluded that significant bone formation was achieved. Huh et al. (Huh et al., 2010) investigated the effects of anodized implants coated with Escherichia coli-derived rhBMP-2 (ErhBMP-2) on alveolar ridge augmentation in beagle dogs. The results suggest that ErhBMP-2 can induce vertical bone augmentation when placed in the vertical defects of alveolar bone that has been healed after tooth extraction thus improving the stability of implants and excluding the need for additional bone transplantation.

Functioning arrangement of connective tissue around endosteal implants that would act as a periodontal ligament does not exist, but the efforts are made in order to make an implant resemble a natural tooth. One direction of investigations is to regenerate periodontal ligament from periodontal cells (for example in immediate implantation) and the other direction is from mesenchymal stem cells or periodontal progenitor cells.

Early experimental studies on new formation of periodontal ligament have started in 1980s.Those studies indicated that only cells residing in PDL (periodontal ligament) were capable of forming new cementum with inserting collagen fibers on exposed root surfaces. Periodontal ligament is lost with the natural tooth loss, it cannot be restored after implant placement. Buser et al. (Buser et al., 1990) analyzed the wound healing around titanium implants in the presence of retained root tips. The experiment was performed on 5 monkeys. After a period of 12 months pathohistologic examination revealed that in regions the implants were placed close to retained roots, a periodontal ligament had formed around large portions of the implant. In polarized light it was apparent that the collagen fibers were oriented perpendicularly to the implant surface and were inserted in the cementum on the implant surface as well as in the opposing bone.

The discovery of a periodontal ligament around titanium implants opens new perspectives in implantology. Parlar et al. (Parlar et al., 2005) performed unique experiment on mongrel dogs to explore the formation of periodontal tissues around titanium implants using maxillary canine roots. They created chambers within the roots and prepared slits in the cavity walls to connect the chamber to the periodontal space. Implants were placed into the center of each chamber. Following 4 months of healing pathohistological analysis was performed. Fibrous encapsulation was observed around most implants, but cellular cementum was deposited on 2 implants, most likely formed through cementoconductivity rather than by differentiation of periodontal ligament cells upon contact with the implant surface. Partial generation of periodontal ligament on endosteal dental implants in dogs was

also observed by approximating a tooth–to-implant contact using orthodontics (Jahangari et al., 2005).

In a pilot experimental study Craig et al. (Craig et al., 2006) reported on the effect of autogenous periodontal cell grafts on the implant-connective tissue interface with and without enamel matrix derivative (EMD). After 8 weeks histomorphometric and microscopic analyses revealed that EMD and the type of cell population present in the implant wound-healing environment may alter the implant-connective tissue interface.

Technically implant carrying periodontal ligament (PDL) may be inserted in the extraction socket of the missing tooth. One of the recent investigations regarding a tissue-engineered periodontal ligament around dental implants was performed by Gault et al. (Gault et al., 2010). They developed so called "ligaplant" which is the combination of PDL cells with the implant biomaterial. Following implant placement a new PDL-like tissue is produced. The authors claim that ligaplants have the capacity to induce the formation of new bone tissue. Investigations were performed in animal and human models. Hydroxyapatite (HAP)-coated titanium implants were combined with canine-derived PDL cells and inserted into the jaws of respective cell donors. The dogs were sacrificed after 20 weeks and pathohistological analyses were performed on 11 implants. The results indicated that the formation of PDL-like tissue can be attained *in vivo*, if a suitable implant surface and sufficient amounts of PDL cells are provided. A PDL-like tissue was formed with perpendicular fibers between the implant and alveolar bone. However, formation of this tissue is a slow process.

Dental and non-dental derived stem cells have the periodontal regeneration capability. As Liu et al. suggested in their study on miniature pigs, autologous periodontal ligament stem cells (PDLSC) are capable of forming bone, cementum and PDL if they are transplanted onto to HA/TCP (hydroxyapatite/tricalciumphosphate) carrier into surgically created periodontal defects (Liu, 2008, as cited in Batistella et al., 2010). PDLSC used with HA/TCP as a carrier showed the capacity to differentiate into cementoblasts and to form cementum/PDL-like structures (Batistella et al., 2010). These cells (PDLSC) also have the capacity to form collagen fibers similar to Sharpey's fibers and suggest the potential to regenerate PDL attachment.

Non-dental derived stem cells have also been the source of periodontal regeneration cells. Marei et al. (Marei et al., 2005) investigated the formation of periodontal structure around titanium implants utilizing bone marrow mesenchymal stem cells (MSCs). In their pilot experimental study on goats they placed immediate implants after canine teeth extractions bilaterally. Experimental side received a porous hollow root-form poly DL-Lactide co-Glycolide scaffold with autogenous bone-marrow-derived mesenchymal stem cells. The results showed that periodontal–like tissue with newly formed bone was observed. The authors concluded that undifferentiated MSCs were capable of differentiating to provide three critical tissues required for periodontal tissue regeneration: cementum, bone and periodontal ligament. Adipose-derived stem cells were used in a work of Tobita combined with platelet-rich plasma (PRP) and implanted into the periodontal tissue defects in rats. Partial alveolar bone regeneration and a periodontal ligament-like structure were observed 8 weeks after implantation (Tobita et al., 2008, as cited in Batistella et al., 2010).

However the precautions must be taken, since using stem cells may have some risks. Carcinoma-associated fibroblasts (CAF) play important role in the growth of epithelial solid

tumors. Although the cell type of origin of CAFs has not been conclusively established it has been shown that they may be marrow derived. When cultured in conditioned medium MSCs) derived from cancer cell lines for prolonged periods, assume CAF properties. These activated MSCs have increased expression of CAF markers and in addition, support growth of breast cancer cells *in vitro* as well as *in vivo* in xenograft model (Mishra et al., 2009).

3. Conclusion

Over the past few decades, an improved understanding of the various parameters that influence osseointegration has resulted in high predictability and clinical success of dental implants. One- and two-stage implant insertion give acceptable results regarding certain important characteristics of investigated soft and bone periimplant tissues (epithelial basal membrane structure, inflammatory reaction, tissue degradation and/or necrosis, osteoblastic activity, collagen tissue proliferation, neovascularization). Most of the experimental study designs area based on a limited number of investigated samples, so there are no significant differences between the two methods.

Numerous other phenomena that occur after the functional loading of implants are common for all implant systems, irrespective of the method of their surgical placement. Moreover, it seems that surgical methods are of lesser importance for the long term implant maintenance compared with implant design characteristics, especially those related to their surface. Ultimately, with the increasing knowledge about tissue engineering, when tooth loss does occur, regeneration of the entire tooth may be advantageous in comparison with replacement by implants.

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15

Bacterial Leakage Along the Implant-Abutment Interface

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1. Introduction

Titanium implants have been successfully and increasingly used for the substitution of dental elements in the treatment of total or partial edentulism, exhibiting success rates frequently above 90%, since the earliest reports on this technique in the 1960 decade (Lang et al., 2004; Pjetursson et al., 2007; Jung et al., 2008). When treatment failures are calculated based on patients who lost implants and not on implant lost by the population in general, success rates may be considerably lower (Lambrecht et al., 2003; Stavropoulos et al., 2007; Esposito et al., 2010).

Excessive premature loading, occlusal trauma and poor bone support are considered the main factors associated with early implant loss (Esposito et al., 2000; Piattelli et al., 2003). Recent reports demonstrated that microorganisms in the oral cavity, especially the ones involved in periodontal diseases, together with unfavorable occlusal factors are considered as the main causes of unsuccessful treatment with implants (Mombelli & Lang, 1998; Covani et al., 2006).

A direct correlation between presence of microorganisms and disease of the peri-implant tissues has been demonstrated. Gram-negative, anaerobic species like *Fusobacterium spp, Prevotella spp* and spirochetes are frequently found in large quantities in affected sites. In contrast, healthy sites are predominantly colonized by Gram-positives (Mombelli & Lang, 1998; Quirynen et al., 2006). Periodontitis in proximity to implants and presence of periodontal pathogenic bacteria in the peri-implant sulci are considered risk factors to the success of dental implants (Mombelli et al., 1995; Mombelli & Décaillet, 2011). Surface characteristics, physical properties, as well as biological factors involved in this type of treatment may facilitate bacterial colonization and growth of potentially pathogenic microorganisms at the implant sites (Mombelli et al., 1995; Jansen et al., 1997; Covani et al., 2005).

Another risk factor to the peri-implant tissues is the presence of marginal discrepancies between prosthetic crowns and implants abutments, although it is a controversial issue. The assessment of these discrepancies varies largely depending on the material employed for crown fabrication, type of cement, measuring methods, etc. Studies using human extracted teeth have reported marginal discrepancies ranging from 5 to 430 μm (Abbate et al., 1989; Felton et al., 1991; Valderrama et al., 1995; Kosyfaki et al., 2010). The highest values, varying from 110 to 160 μm, are frequently associated to feather–edge cast gold crowns (Marxkors,

1980; Diedrich & Erpenstein 1985). In contrast, clinical evaluation of ceramic crowns have shown smaller discrepancies, ranging from 32-145 μm (May et al., 1998; Boening et al., 2000; Kokubo et al., 2005). Acceptable discrepancies between abutments and fixed prosthesis should not be higher than 120 μm (Jemt & Book, 1996; Kosyfaki et al., 2007), although some authors report success in patients having misfits of around 30 to 200 μm (Boeckler et al., 2005).

The great majority of current implant systems contain two parts connected by screws, the intraosseous cylinder and the abutment, showing, as a result, an interface and empty spaces between the components. Localization levels of the implant-abutment interface in relation to the alveolar bone crest during implant placement, as well as the type of connection may vary between the different implant systems. Depending on the system, the implant-abutment interface is positioned either at the bone or gingival level. In bone level systems, a second screw, or cementing agent, connects the prosthetic structure to the abutment, thus introducing a second union interface. Gingival level implant systems have the implant-abutment interface many times covered by the prosthetic structure, which results externally in a single implant-prosthesis interface that are positioned about the gingival level. Frequently, the implant and abutment connection surfaces are machine made while prosthetic structures may be machined or cast. In general, more regular surfaces and precise marginal adaptations can be achieved with machined components in comparison with in comparison with cast ones. On the other hand, independent parts connected by screws are prone to micro-movements, which may alter the adaptation between components and inevitably originate hollow spaces (Steinebrunner et al., 2005). In some systems the abutment is connected to the implant using a cementing agent, which fills the existing interfacial gaps (Piattelli et al., 2001; Scarano et al., 2005). Jansen et al. (1997) have demonstrated that the presence and sizes of gaps between implants and abutments may vary according to the type of connections and the structural characteristics of the abutments. Vertical marginal discrepancies between implant and abutment of approximately 0 to 10 μm (Jansen et al., 1997; Bondan et al., 2009) and horizontal average discrepancies of 60 μm (Byrne et al., 1998; Kano et al., 2007) have been observed. Even wider variations were found when methodological differences are considered, particularly in comparisons involving unitary and multiple prosthetic elements. Although considerably smaller than the spaces between implants and cast or synterized ceramic crowns, discrepancies between machined components are still larger than the corpuscular dimensions of various bacterial species.

The hollow spaces between implant and abutments may act as reservoir for commensal and/or pathogenic bacteria, especially anaerobic or microaerophilic species, representing a potential source of tissue inflammation. Hence, microbial colonization of the interfacial gaps may ultimately result in bone resorption (Quirynen et al., 1990; Quirynen et al., 1994; Mombelli et al., 1995).

Several *in vivo* and *in vitro* studies have shown bacterial leakage through the implant-abutment interface, either from the external sites to the inner parts of the implants or vise-versa (Quirynen et al.,1994; Jansen et al., 1997; Rimondini et al., 2001; Steinebrunner et al.,2005; Callan et al., 2005; do Nascimento et al., 2009a; Cosyn et al. 2009; Aloise et al., 2010). Other studies, have demonstrated the leakage of dyes and/or bacterial endotoxins through the implant-abutment interface (Gross et al., 1999; Piattelli et al 2001).

Bacterial contamination of two-part dental implants has been well described in non-loading conditions but several questions remain on the biomechanical principles that control the whole system during masticatory function (Brunsky et al. 2000). Lack of component

adaptation and passive connection are potential causes of mechanical damages, like screw loosening and fracture, as well as the development of mucositis and peri-implantitis due to biofilm retention (Quirynen et al., 1994; Mombelli et al., 1995).
Mechanical loading of the prosthetic abutments is another factor that may affect adaptation of implant and components and in consequence, leakage of microorganisms through the implant-abutment junction. Reports by Steinebrunner et al. (2005) indicate that bacterial penetration between implants and abutments connected by screws varies according to the number of loading cycles applied to the abutment. The leakage of microorganisms and fluids to the inner parts of the implants is considerably increased by load application, which generates micro-movements and interfacial gaps (Khraisat et al., 2006).
Bacterial species harboring the internal surfaces of the implants and components is not surprising, since the size of oral bacterial species ranges in average from 1.1 to 1.5 μm in length and 2 to 6 μm in diameter. Smaller species like spirochetes (diameter of 0.1 to 0.5 μm) may also be found in the oral microbiota (Jansen et al., 1997).
The presence of bacterial contamination of implants and components has traditionally been detected by conventional microbiological cultures, which have inherent deficiencies, particularly in the identification of fastidious species and strict anaerobes (Rolph et al., 2001; Moraes et al., 2002).
The last two decades witnessed the development and extensive use of new molecular techniques to detect, identify and quantify microbial species dwelling in the oral cavity. These rapid, sensitive and specific techniques revealed an enormous, hitherto unknown, microbiota. (Sakamoto et al., 2005; Haffajee et al. 2009). Improvements in new implants systems are being rapidly developed, for instance in their connection mechanism, surface treatment and physicochemical properties.
Considering the increased use of dental implants in restorative dentistry and recognizing the impact of bacterial leakage along the implant-abutment interface on the health of peri-implant tissues, this chapter intends to review and discuss the literature on the subject.

2. Biofilms and bacterial colonization of dental implants

2.1 Biofilm formation

Bacterial colonization of the the oral cavity in humans starts at birth and remains constant through life (Carlsson et al., 1970; Rosan & Lamont, 2000). A saliva film is initially formed composed basically of a cell-free matrix and proteins. Subsequently, bacterial colonization of this film is followed by selective adhesion on the different substrates (Gibbons, 1984). Large quantities of lactobacillus spp, responsible for biofilm adhesion, and streptococcus spp (mainly *S. sanguinis, S.oralis, S.mitis* and *S. sobrinus*),which promote biofilm growth, are initially found. Actinomyces spp and Gram-negative species are found in low proportion at this phase (Rosan & Lamont, 2000), and cell viability and adhesion capacity are fundamental requirements for the success and keeping of bacterial colonization (Gibbons & Van Houte, 1975).
A variety of bacterial species are transitory in the oral cavity. However, the characteristics of existing surfaces may facilitate adherence and prolong their permanence. Indeed, there are reports suggesting that some of the colonizing strains may remain stable for years while others may fluctuate (Alaluusua et al., 1994; Emanuelsson & Thorqvist, 2000). The profile of the oral microbiota is shaped, in addition to environmental factors, by significant interactions between bacterial species, inhibiting or stimulating each other (Grenier & Mainard, 1986). Bacterial antagonism with predominance of pathogenic species may be the

determinant of health or disease status of the supporting tissues (Hillman et al., 1985; Socransky et al., 1988).

According to Quirynen et al. (2002), gaps in the implant-abutment interface may act as a trap for bacteria, favoring the development of biofilm with varying composition and impact on periodontal tissues. *Agregatibacter actinomycetemcomitans*, *Tannerella forsythia and Porphyromonas gingivalis*, many times present in these biofilms, are pathogens intimately related to the development and maintenance of periodontitis and peri-implantitis. Other pathogens with relevant participation in these diseases are *Prevotella intermedia, Campylobacter rectus, Peptostreptococcus micros, Fusobacterium nucleatum, Eubacterium nodatum, Streptococcus intermedius* and spirochetes (Quirynen et al., 2002; Socransky & Haffajee, 2002).

Periodontal disease affects tissues that support teeth and is characterized by loss of periodontal ligament insertion, and resorption of adjacent alveolar bone. This disease has multifactorial etiology, which includes biofilms as having an essential role in its pathogenesis (Lamont & Jenkinson, 1998). The term peri-implantitis, established in the Periodontia European Workshop, 1993, characterizes diseased implant supporting tissues (Albrektsson & Isidor, 1994). The initial bacterial colonization of peri-implant sulci is characterized by an increasing number of facultative anaerobic *streptococcus*, although Gram-negatives anaerobes may be occasionally found but in smaller numbers (Monbelli et al., 1988). With time, strict gram–negative anaerobes as *Fusobacterium spp and Prevotella spp* become increasingly predominant (Mombelli & Mericske-Stern, 1990).

Several reports on the microbiota detected around dental implants were published in the last decade (Haffjee et al .,1998; Botero et al., 2005; Cosyn et al., 2009; Van Brakel, et al. 2010). Presence of potential periodontal pathogens colonizing the peri-implant grooves in unsuccessful implants has also been described (Laine et al., 2005; Shibli et al., 2007; Persson et al., 2010). Other studies suggested that periodontal pathogens and/or their metabolic products might be involved in peri-implant bone losses (Lindhe et al., 1992, Persson et al., 1996). However, information on the diversity of bacteria colonizing the internal surfaces of two-part dental implants, abutments and implant prostheses and on their correlation with periodontal and peri-implantar sulci species is still lacking. . Cosyn et al. (2009), in a short clinical evaluation, have found similarities in the microbiota of samples collected from the internal parts of implants and from their related peri-implant sulci.

Another investigations indicated that the existing oral microbiota prior to implant placement is determinant to the establishment and maintenance of the implant related microbiota.. Partially edentulous patients with a history of periodontal disease show high incidence of putative periodontal pathogens in implant sites (Mombelli et al., 1995; Quirynen et al., 2002, Quirynen et a., 1996a). Laine et al. (2005), reported that the bacterial biofilm composition in the peri-implant sulcus changes during the healing period following implantation and shows constant alterations subsequently. Pathogenic and non-pathogenic species have been found surrounding peri-implant sites (Callan et al., 2005; Quirynen et al., 2006). De Boever & De Boever (2006) suggested that periodonto-pathogenic species isolated from the implant sites during the first 6 months after placement there might be no clinical consequence. However, the presence of these pathogens in the immediate healing phase may have future consequences. Bacterial species found in the wound healing of implants that failed immediately after surgery are similar to the ones seen in acute infections (Laine et al., 2005). Lately, when the implants have been exposed to oral microorganisms for a long

period, the microbial biofilm changes into a pattern similar to the one found in chronic periodontal disease (Mombelli & Lang, 1998).

2.2 Design features and marginal fit of implant components

One of the most common causes of dental implants failure is poor adaptation of implant and prosthetic components, irrespectively of the implant system and connection design. Notwithstanding the constant efforts to overcome this drawback, the hollow spaces produced by poor adaptation act as traps for bacteria of the oral cavity resulting in inflammatory reactions of the peri-implant tissues (Mombelli et al., 1995; Quirynen et al., 2002). Both the microorganism and their metabolic products may be responsible for inflammation and bone loss (Lindhe et al., 1992; Quirynen & Van Steenberghe, 1993). Ultimately, uncontrolled local inflammation may lead to generalized peri-implantitis and compromise the long-term success with dental implants (Ericsson et al. 1995).

Two-part dental implant systems with screw-retained abutments are still largely used, principally due to their well reported protocol, high success rates, and broad spectrum of indications. However, gaps and cavities in the assemblies after abutment attachment are frequently related to peri-implant tissue problems. Figure 1 illustrates the gaps resulted from attached components of an implant system. These gaps favor bacterial biofilm accumulation between components, generally in the interfaces implant-abutment, abutment-prosthesis, and on the exposed surfaces of abutments, prosthesis and implants to the oral environment. Under loading, where the abutment components are subjected to eccentric forces, the number and size of gaps can be augmented. Moreover, micro-movements of implant components during function may allow the initiation of a pumping effect that facilitates bacterial leakage through the implant-abutment interface (Steinebrunner et al., 2005). Also, the abutment screw loosening may contribute to joint instability, screw fracture, and clinical failure. Increased percentages of abutment screw loosening and the consequent increase in micro-movements between components are more common in implants with external hexagonal connections (Binon et al., 2000).

Sahin & Cehreli (2001), in a revision study, evaluated the clinical significance of the passive fit on the final marginal fit of implant-supported restorations. The authors recommend that the implant-abutment assembly should result in a passive connection, not inductive of tension in implant components and adjacent bone. However, according to the authors, this is not possible since clinical and laboratory procedures utilized in the fabrication of the super structures are not adequate. The lack of dimensional precision and may introduce strains to the fixation screws, potentially causing screw or abutment fracture.

Studies on the adaptation between implant and components have been related their failed adaptive conditions to the presence of bacterial infiltration (Jansen et al., 1997; Steinebrunner et al., 2005). Quirynen & Van Steenberghe (1993) investigated the presence of microorganisms in the internal screw threads of Branemark implants. In nine patients, the apical part of two intermediate screws installed three months before was examined through contrast phase microscopy, showing a significant quantity of microorganisms, mainly coccus (86.2%) and nonmotile rods (12.3%). Motile species (1.3%) and spirochetes (0.1%) were scarce. Another similar study observed a microbiota mainly composed of anaerobes and facultative streptococcus, Gram-positive anaerobic rods such as *Propionibacterium, Eubacterium and Actinomyces* besides Gram-negative anaerobes, including *Fusobacterium, Prevotella and Porphyromonas* (Persson et al., 1996).

Several implant systems with different designs are currently available in the market. The Figure 2 and 3 illustrate three different implants and platform connections of an implant system. They have different shapes, surfaces, sizes and distances between screw threads, as well as different types of connections between implants and abutments. The external hexagonal platform proposed by Branemark et al. (1969), was until recently the most widely used and well documented. The system has a connecting hexagonal platform acting as an anti-rotational mechanism that is, together with the connecting screw, responsible for the mechanical stability of the implant-abutment set. Despite the wide acceptance and use, the system shows an external connection concentrating considerable more strength in the threads of the abutment screw, which may lead to fractures and implant failures (Norton et al., 1997; Finger et al., 2003; Bernardes et al., 2009). This type of connection shows the highest vertical and horizontal implant-abutment discrepancies described in the literature (Binon et al., 1996).

Studies on the precision of the connection between components of different systems showed that some can be interchanged with an accuracy similar to the one observed in connections with components of the same system (Hagiwara et al., 1997; Dellow et al., 1997).

Scanning electron microscopy examinations of the implant-abutment interface, showed vertical and horizontal maladaptations between implants with external hexagonal connections and their respective abutments varying, on average, from 1 to 100 μm, (Binon et al., 1996; Jansen et al., 1997). In general, machined abutments show superior adaptation in relation to laboratory cast ones, suggesting that stricter standard controls should be held on components obtained by casting procedures (Byrne et al., 1998). The type of metal alloy in cast components has also a relevant role on the final adaptation of the assemblies. Components cast in Ni/Cr or Cr/Co are more variable in respect to the marginal dimensional stability when compared with Au or Ag/Pd alloys cast components (Binon et al., 1995; Byrne et al., 1998).

Bone crest changes around implants were studied in dogs by Hermann et al. (2001), by observing the influence of marginal gaps in the implant-abutment interface and occurrence of micro-movements. Gap sizes were in the range of 10-100 μm and micro-movements were abolished in some groups by laser welding of interfaces The results showed that resorption in crest bone was significantly influenced by movements between abutments and implants but not by gap sizes along the interface.

New connection designs were proposed to overcome the drawbacks of the hexagonal precursor system. Hexagonal platforms with larger interfacial area as well as internal connection systems with varied shapes were developed to improve adaptation and stability of the implant components. However, in contrast to the well-standardized features of the external hexagonal precursor, the internal connections are usually unique for each developer and thus difficult to standardize.

Clinical and in vitro evaluations indicate that connections between components are more stable in the internal hexagonal system, in which the larger contact area between internal implant and abutments walls favors force distribution and preserves the abutment screw (Norton et al., 1997; Mollersten et al., 1997; Binon et al., 2000; Finger et al., 2003). Microbiological studies, however, do not show significant differences between hexagonal internal or external connections, when bacterial leakage is concerned (Jansen et al., 1997; Steinebrunner et al., 2005; Duarte et al., 2006).

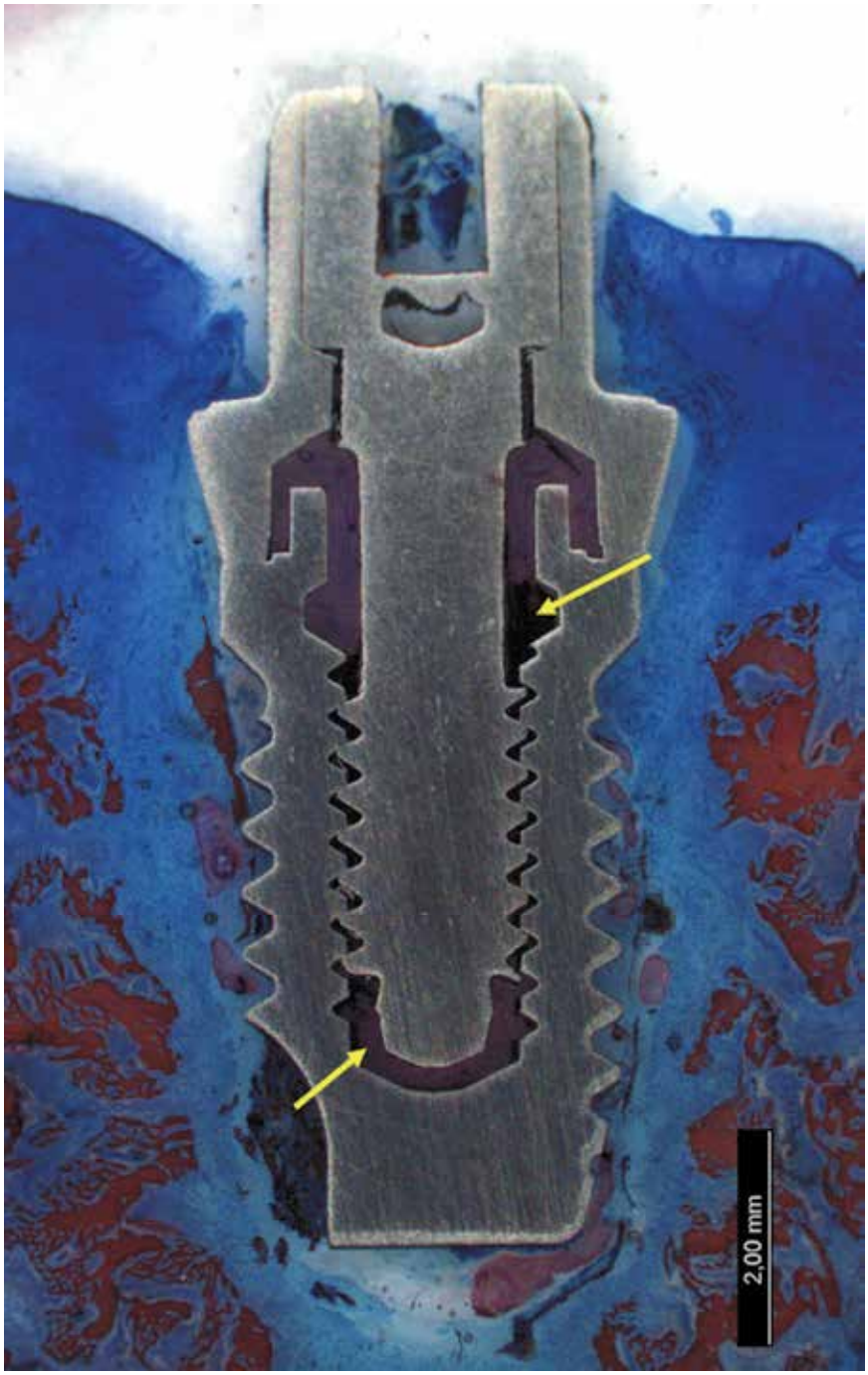

Fig. 1. Histological Photomicrograph showing the gaps and hollow spaces (arrows) resulted from the poor adaptation of the implant and prosthetic components in the two-part implant systems (MKIII TiUnit and it respective abutment, Nobel Biocare- regular external hexagonal platform with 3.75 mm in diameter and 7 mm in length).

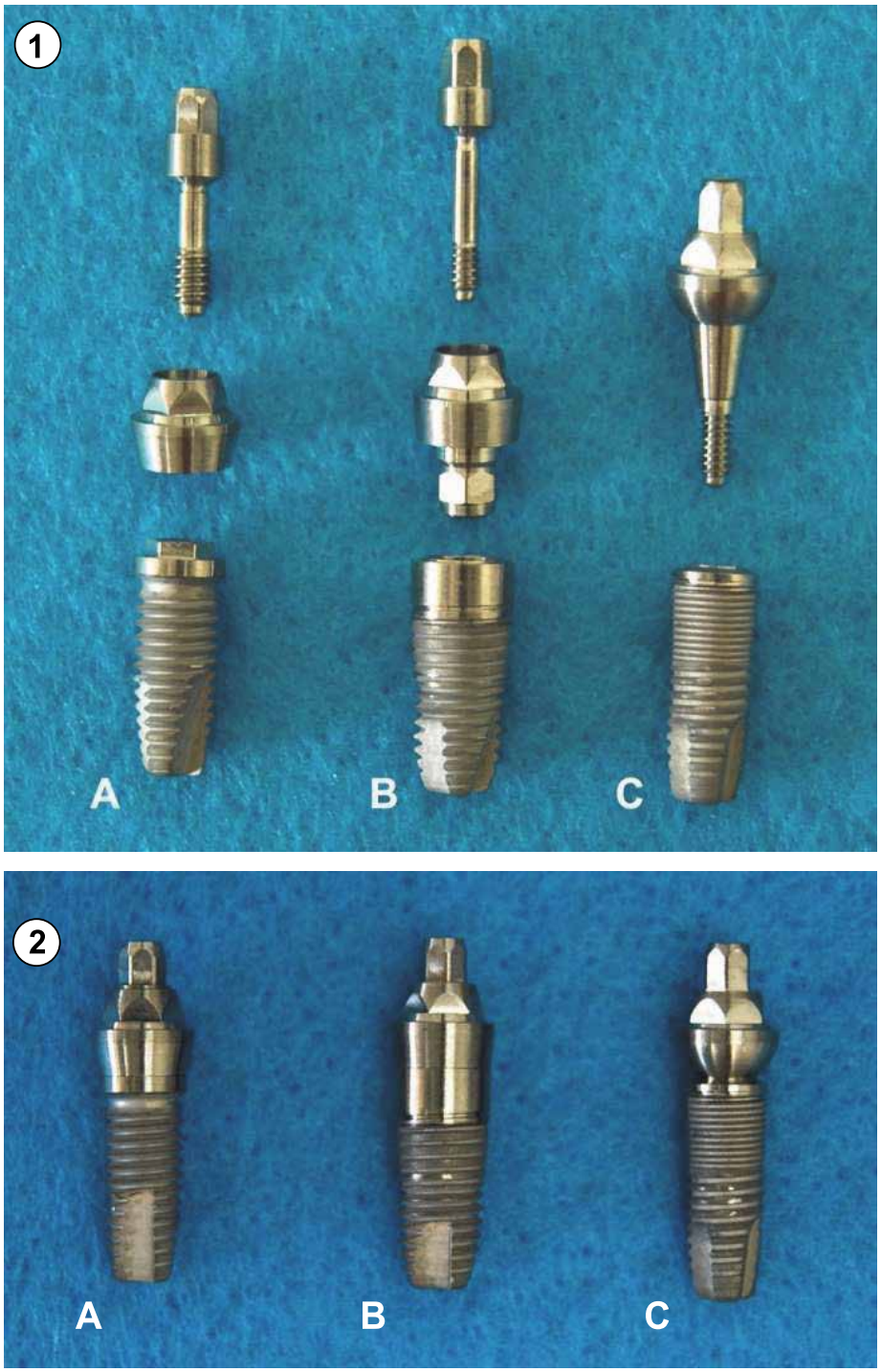

Fig. 2. Illustration showing 3 different implants and abutments before (1) and after (2) connection: (A) External Hexagonal, (B) Internal Hegonal and (C) Morse Cone.

The Morse cone connection system has recently been introduced and is becoming increasingly popular in implant dentistry. Due to its tapered design, the system is considered mechanically more stable and efficient in preventing bacterial leakage. The Morse cone connection, compared with other types, is more efficient in the dissipation of forces exerted on the prosthesis and consequently on the supporting bone tissue (Merz et al., 2000). It does not completely inhibit the passage of bacteria and fluids through the interface, but microbiological evaluations show that there is a more efficient bacterial sealing between the Morse cone implant and its abutment, revealed by lower bacterial counts in comparison to other connection systems (Pautke et al., 2009; Aloise et al., 2010). The intimate adaptation between contacting surfaces obtained with this type of connection seems to produce a frictional locking, which restrains micro-movements. Concurrently, minimized spaces at the interface seem to be related to decreased levels of bacterial contamination, probably by preventing leakage and reducing the available volume for bacterial growth. Further investigations are necessary, however, in order to determine the biomechanical behavior of these components in long-term studies, since most of these findings were obtained in short term studies.

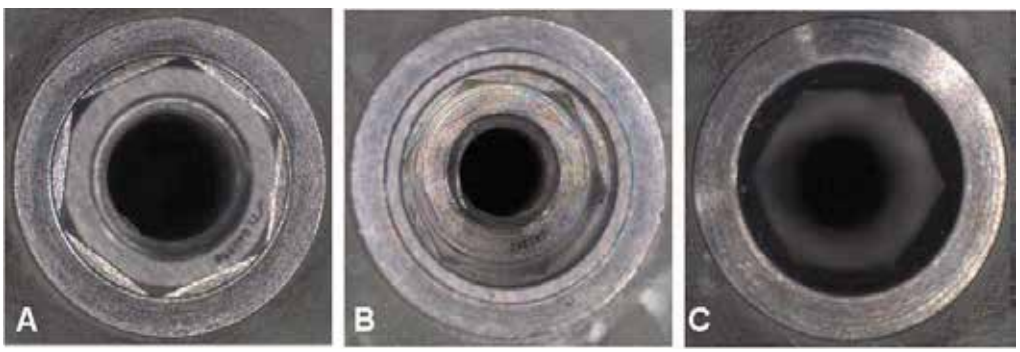

Fig. 3. Microscopy picture showing the External Hexagonal (A), Internal Hexagonal (B) and Morse Cone (C) platform.

2.3 Abutment materials

Peri-implant soft tissues act as a protection barrier between the oral cavity environment and the peri-implant bone (Welander et al., 2008). The type of material used in the abutment fabrication seems to be crucial in determining the final quality of the mucosa in contact with theabutment surface (Abrahamsson et al., 1998). It has been observed that when titanium abutments are used, the surrounding mucosais mainly composed of epithelial and connective healthy tissues. Healthy soft tissues should promote an effective barrier against the passage of microorganisms and/or their products, preserving the adjacent bone from being damaged (Welander et al., 2008).

Precious and basic metals, as well as ceramic materials, are also used in the manufacture of abutments. Ceramic materials, such as zirconium dioxide, or simply zirconia, are popular materials, increasingly used in prosthetic abutments. They are similar in color to dental structures and have potential advantages over metallic materials (Brodbeck, 2003; Watkin & Kerstein, 2008) besides other properties such as higher translucency, (Denry & Kelly, 2008), good tissue adhesion (Pesskovᡠet al., 2007), less tissue discoloring effect (Bressan et al., 2010), lower bacterial adhesion and growth (Scarano et al., 2004) and lower toxicity (Uo et al., 2003).

Zirconia has excellent mechanical properties besides being material of choice for esthetic and biological reasons. It has high resistance against fractures (Anusavice et al., 2007; Manicone et al., 2007; Denry & Kelly, 2008) which was demonstrated in short term (3-4years) *in vivo* studies with satisfactory clinical results (Glauser et al., 2004; Canullo et al., 2007; Ekfeldt et al., 2011).

Although abutments with esthetic properties are continually sought, conclusive clinical evidence of their ability to maintain healthier periimplant tissues is still lacking in the current literature (Linkevivius & Apce, 2008). Few studies have compared bacterial adhesion in metallic and non-metallic components. While some authors claim that bacterial adhesion is lower in zirconia components (Scarano et al., 2004), others show that there is no difference between zirconia and titanium components (Salihoglu et al., 2010). Results from a study in animals (Abrahamssom et al., 1998) and a recent one in humans (Van Brakel et al., 2010), indicate that zirconia and titanium abutments may exert a similar healthier effect on peri-implant tissues.

On the other hand, the use of zirconia abutments in esthetic components can be limited in cases of cemented prosthesis, due to the low adhesion of the cement to the ceramic material, although the use of recently developed primers may minimize the problem. Another recent proposal to improve the treatment outcome with implants by taking advantage of the best properties of different materials is the use of hybrid connection systems, composed of titanium abutments surrounded by a zirconia-overlaid collar. Future studies are expected to evaluate the biomechanical behavior of these systems.

2.4 Effects of surface characteristics (chemistry, free energy and roughness)

The surface physico-chemical properties of implants and abutments, like roughness, chemical treatments and free energy have an important role in the formation and maintenance of bacterial biofilms (Grossner -Screiber et al., 2001).

Bacterial adhesion to hard surfaces in the oral cavity is highly influenced by rugosity, which in turn is related to the number of colonies formed (Quirynen et al., 1996b). Scanning electron microscopy analysis of bacterial behavior on different materials used in implant and prosthetic components, indicated that rough surfaces have higher indexes of bacterial adhesion (Grossner-Schreiber et al., 2001; Amoroso et al., 2006). Implants and components showing an average roughness lower than 0.1 μm partially inhibited bacterial biofilm formation and growth after 24 hours (Rimondini et al., 1997). High surface rugosity and consequent high hydrophobic properties (low wettability) tend to favor biofilm formation and bacterial adhesion (Drake et al., 1999). Steam-autoclave sterilization of titanium increases the oxide layer thickness, accumulation of impurities on the metal surface, and hydrophobicity, as shown by Drake et al. (1999). The authors demonstrated that surfaces that were steam-autoclaved 10 times showed significantly greater CFU/mm2, specially of *S. sanguis*, than other methods of sterilization, regardless of surface roughness. .

Surface treatment of components has a relevant impact on bacterial adhesion and colonization. Titanium implants treated with titanium nitrate (TiN) or zirconia nitrate (ZrN) followed by thermal oxidation or laser irradiation produced significant reduction of biofilm formation and bacterial adhesion when compared to surfaces of polished titanium (Grosner-Schreiber et al., 2001). Other surface treatments proposed to inhibit microorganism adhesion such as, antibacterial coverings (Ge et al., 2010), electro-chemical treatments (Visai et al., 2008), and new metallic alloys (Pautke et al., 2009) still need further evaluations to warrant applicability.

Biofilm formation is directly, but less intensely, related to component surface free energy (SFE) (Burgers et al., 2010). SFE results from the interaction of cohesion and adhesion forces, responsible for the wettability property of surfaces. SFE facilitates accumulation of bacterial biofilm, by promoting firm adhesion to contact surfaces besides selecting specific bacterial species, a property intimately related to the acid-basic characteristics of bacterial cell walls. In a literature review, Quirynen & Bollen (1995) conclude that smooth surfaces with low SFE can potentially reduce the occurrence of caries and periodontitis. In contrast, rough surfaces promote plaque formation and maturation, and high-energy surfaces are known to collect more plaque and to select specific bacteria.

3. Culture and culture-independent methods focusing on oral microbiota of dental implants

Identification of microorganisms inhabiting peri-implant crevices and the internal parts of implants has been of relevant importance in respect to the outcome of the treatment with dental implants, since several studies showed a correlation between bacterial species of the oral cavity, especially those involved in periodontal diseases, and the occurrence of failure in the treatment with implants (Ong et al. 1992; Shibli et al., 2007). Periodontal pathogenic bacteria in peri-implant crevices and teeth with periodontitis close to dental implants are considered risk factors for the success of dental implants (Gouvoussis et al., 1997; Saito et al., 1997). To date, a large number of microbial species related to periodontal and peri-implant diseases have been identified and can be quantified by different methods.

3.1 Conventional cultures

Bacterial culture is a well-known method historically used to characterize the oral cavity microbiota, and considered a classical reference method in microbiology. Traditionally, culture-dependent methodologies are used to isolate, enumerate and detect probiotic organisms, especially from mixed cultures (Charteris et al., 1997). Several variables in culture technology, especially an appropriate sample collection technique and media selection, have been recognised as having a significant impact on the sensitivity and specificity of the test, mainly on the organism recovery rates and time for reporting results (Riedel & Carroll, 2010). This method constitutes an important epidemiological tool, with results that serve as a base for building an empirical therapeutic strategy. Also, this methodology is essential in the initial phase of several culture-independent techniques, where bacterial growth and isolation is necessary to DNA probes confection.

These methods are essentially designed around the recovery and (or) enumeration of viable bacteria in the contaminant media. Detection of viable bacteria is traditionally performed by implementing a means of culturing growth of individual species. The use of non-selective media such as trypticase soy agar or standard methods agar, known as the aerobic or standard plate count, is routinely applied in this methodology. In addition, in specific conditions, the increased sensitivity of these standard media has been achieved using a selective agar overlay approach designed to recover a larger proportion of bacteria from contaminant media (Specket et al. 1975; Harrigan, 1998).

Most studies describing the microbial leakage through the implant-abutment interface are based on results with conventional culture method (Jansen et al., 1997; Piatelli et al., 1999; Steinebrunner et al., 2005; Pautke et al., 2009; Aloise et al., 2010). However, an inherent limitation of microbiological cultures is that the difficulties to identify strict anaerobes,

frequently associated with periodontal and peri-implant diseases, as well as fastidious species (Barbosa et al., 2009; Roças et al., 2010). It is estimated that 50% of the oral microbiota is not cultured by conventional methods and several of these species are directly related to infectious processes in the oral cavity (Arank et al., 1969; Paster et al., 2001; Parahitiyawa et al., 2010). Furthermore, non-viable cells, still able to produce aggressive compounds against peri-implant tissues are not detected by culture methods.

Despite of the efforts to optimise broth composition, enhance the growth of microorganisms and prevent contamination during procedures, the methodology is time-consuming and the microbial viability is essential to confirm the presence pathogens. Cell killing and degradation by bacteriocin as well as degradation of DNA by proteolytic enzymes and endonucleases has been demonstrated in several studies (Loyola-Rodriguez et al. 1992, Cowman & Baron 1993, Cascales et al. 2007). These substances may cause deleterious effects on the peri-implant support tissues. Therefore, despite the advantages of these familiar culture methods in detecting viable bacteria, such as ease of use and low cost, assay sensitivity is still relatively low compared with alternative methods (such as molecular-based approaches).

Quantitative bacterial measurements are widely used in microbiology. Many years of research studies using quantitative microbiology on solid media have demonstrated that such measurements provide clinically valuable information. For example, bacterial load is predictive for the occurrence of complications (Yagupsky & Nolte, 1990). However, the bacteria quantitation in conventional culture method is difficult to achieve and is rarely practised in clinical laboratories because it requires subsequent plating on solid media rather than incubation in liquid media. The time required for liquid culture bottles to become positive provides some suggestion of bacterial load, but is a weak quantitative measure and varies with the microorganisms present. Also, each bacterial must be individually evaluated with a specific media.

3.2 Culture- independent methods

In the last two decades great advances in molecular diagnostic methods were achieved, which have been extensively used in the detection and identification of microbial species inhabiting the oral cavity (Sakamoto et al., 2005; Haffajee et al., 2009; Costa et al., 2010). These techniques are more rapid, sensitive and specific when compared to the conventional culture methods. Species showing diverse phenotypic behavior may be identified by their genomic characteristics, which are not dependent on cell viability, a great advantage in studies evaluating anaerobic infections, when cell death may occur during sample collection or transportation (Whelen & Persing, 1996; Pitt & Saundres, 2000). These techniques have also promoted advances in the knowledge of the microbiota in other parts of the human body (Eckburg et al., 2005; Dethlefsen et al., 2007; Grice et al.,2008; Oakley et al., 2008) revealing a great quantity of bacterial species not cultured, whether associated or not to infectious processes (Turnbaugh et al., 2007; Mallard, 2008)

3.2.1 Checkerboard DNA- DNA hybridization

Methods based on cellular DNA characterization, used for microbial detection and quantitation have been documented in the current literature. The Checkerboard DNA hybridization technique utilizes genomic DNA probes to identify and quantify several bacterial species simultaneously in a great number of samples from the oral cavity, and has been largely employed in studies on the oral microbiota (Socransky et al., 1994). Several

areas in dentistry have recently been using the technique to evaluate the composition of bacterial biofilms in health or disease conditions (Aberg et al., 2009; Teles et al., 2010; Kim et al., 2010, Vettore et al., 2010), and in studies on the association of local and systemic factors that can affect biofilm formation (Borges et al., 2009; Demmer st al., 2010). The technique is also employed to evaluate bacteria associated with endodontic lesions (Roças & Siqueira, 2010) and to verify changes in biofilm composition as a result of periodontal treatments (Haffajee et al., 2009).

More recently the DNA hybridization technique has been used to identify and quantify multiple bacterial species associated to dental implants. *In vitro* studies show that several bacterial species colonizing the internal surfaces of implants may be consistently identified and quantified (do Nascimento et al., 2009; Barbosa et al., 2009). The DNA Checkerboard method is significantly more sensitive in the bacterial detection than conventional cultures according to Loesche et al. (1992). Barbosa et al., (2009) compared the two methods in an *in vitro* study involving the investigation of *F. nucleatum*in the interior of implants with an external hexagonal connection. The microorganism counts were significantly higher with the DNA –Checkerboard method leading to the conclusion that it is more sensitive than conventional cultures and allows identification of bacteria that are viable or not. Considering that bacterial cell structure and its degradation products may act as nutrients to other opportunistic species, the identification of non viable species may be relevant to risk determination associated to peri-implant tissues.

3.2.2 PCR-based techniques

Other molecular diagnostic methods utilize amplified genetic material by the Polymerase Chain reaction (PCR). These methods are more specific and sensitive and may be useful to complete results obtained by DNA hybridization. PCR-based techniques are very sensitive and specific allowing the identification of species in the interior of implants even when they are present in very small quantities (Haffajee et al., 2009). Susceptibility of contamination due the amplification procedure is the main limitation of this method.

PCR methods involve the enzymatic synthesis of a specific DNA segment of the target species by DNA polymerase. The reaction develops the annealing and enzymatic extension of an oligonucleotide pair utilized as initiators, the so-called primers, which delimit the DNA sequence of the double strand targeted by the amplification. Three variants of the technique can be employed in dentistry to identify bacterial species in the oral cavity: PCR (conventional or qualitative), RT_PCR (Real time PCR or Quantitative PCR) and 16SrDNA-based PCR.

The high specificity of amplification by the qualitative PCR technique discriminates numerous species of microorganisms in the oral cavity, including pathogens not easily detected by other methods. However, detection is limited to the plateau reaction and it is unable to determine the precise number of bacteria present in a determined site (Higushi et al., 1992; Jervoe-Storm et al., 2005). Real-Time PCR precisely quantifies the species in the study. The number of product molecules synthesized in this technique depends directly on the number of molecules used as standards. Quantification data are collected in the exponential phase of PCR, producing a precise quantification of the number of target DNA copies when internal and external standard are used. Real Time PCR has been used as a complement to conventional cultures or DNA Checkerboard to quantify main periodontal pathogens, as for instance, *P. gingivalis, A. actinomycetemcomitans, T. denticola* and *T. forsythia.*

The sequencing of the DNA ribosome gene (rDNA) 16S, found in the bacteria genome, has recently been used to evaluate the microbial diversity of the oral cavity (Gu et al., 2009), esophagus (Macfarlane et al., 2007), stomach (Li et al., 2009), intestine (Hill et al., 2010, colon (Mäkivuokko et al., 2010) and vagina (Oakley et al., 2008). In contrast with molecular methods, which employ DNA probes, like the DNA Checkerboard, this methodology identifies large populations of non-cultured species, many times associated to the disease conditions. Through these methods it was possible to characterize the subgengival microbiota of the periodontal pouch as being composed of more than 700 diverse species isolated from different individuals. The great majority does not have a DNA totally identified and sequenced and, thus, cannot be investigated by methods using DNA probes (Roças et al., 2010).

4. Conclusions

Implant materials, connection systems, and surface properties have all relevant impact on the initial formation of biofilm and on the characteristics of the peri- and intra- implant microbiota. These factors can influence bacterial adhesion to implants and prosthetic abutments, with varied impact on the peri-implant tissues, and may interfere on the success with this type of treatment.

More stable connections, showing smaller and less frequent free spaces after component adjustments, have shown to be more efficient in containing bacterial leakage through the interfaces. Modifications of the titanium surfaces, or the surfaces of other materials used in the manufacture of implants systems, may reduce biofilm formation and consequent outcomes. Diagnostic techniques currently used to evaluate bacterial contamination of implant related structures have specific characteristics and applications, and thus, should be considered as complementary.

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16

The Current Knowledge of Genetic Susceptibility Influencing Dental Implant Outcomes

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1. Introduction

Oral diseases are still a major global health burden, in spite of big efforts in research and dental services, where disbursement on treatment may exceed that for other diseases, including major illnesses such as cancer, heart disease, stroke, and dementia (Williams, 2011). In this context, tooth loss is a topic of public health concern, since it is the final result of the first and second most prevalent diseases in dentistry: caries and periodontitis (Pihlstrom et al., 2005; Pitts et al., 2011). Although the prevalence of edentulism has decreased over the last decades, there will be a relevant proportion of edentulous individuals worldwide (Polzer et al., 2010a).

Tooth loss is a problem complex to be solved all over the world which affects children, adults and elderly. Complete edentulism prior 65 years old was associated with all-cause mortality, an evidence supporting the notion that poor oral health is an important public health issue across the lifespan (Brown, 2009). Although edentulism is not a life threatening condition, tooth loss impairs several orofacial structures, such as bony tissues, nerves, receptors and muscles. Consequently, most orofacial functions are diminished in edentate subjects (Polzer et al., 2010a). Regarding partially edentulous people, tooth loss is found in 5-20% of most adult populations all over the world (Petersen et al., 2005). Quality life levels were reported to be direct related to the number of remaining teeth (Polzer et al., 2010a). Thus, edentulism was found to be a global problem, with estimates for an increasing demand for oral rehabilitation in the future (Felton, 2009). In this context, oral health restoration should aim to restore function and esthetics. Dentistry has the challenge of improving the access and quality of oral rehabilitation (Tilman, 1985), although oral health care is still being conducted without a solid research evidence base (Pang et al., 2011).

2. Oral rehabilitation

A wide variety of treatments is available to replace tooth loss. Osseointegrated dental implant is the gold standard treatment modality to replace missing teeth in terms of

function and aesthetics (Davarpanah et al., 2002; Fugazzotto, 2005). It is estimated that over 10 million implants are installed all over the world annually (Hospitalar, 2007).
The term osseointegration was coined by Branemark to define a structural and functional contact between titanium surface and bone (Albrektsson et al., 1981; Branemark et al., 1969). Since 1982 in the Toronto Conference in Clinical Dentistry, when the guidelines for Implantology were proposed, they remain mainly unaltered. The main protocol change was regarding different times for loading (immediate and early load), which seem to be positive if patients present high degree of primary implant stability (high value of insertion torque) (Esposito et al., 2009). Other treatment differences regarding modification of implant macro and microstructure have limited evidence of clinical improvement supported by longitudinal studies (Esposito et al., 2007).
Dental implants are considered a very predictable rehabilitation procedure in dentistry, with a success rate above 90% showed in longitudinal studies (Adell et al., 1990). In spite of the high success rate, the absolute number of dental implant failure becomes significant and causes economic and social impact for patients and dental professionals. Dental implant failure has been extensively studied during the last years (Esposito et al., 1998a; b). The comprehension of the implant failure process may provide novel insights into the mechanisms underlying osseointegration (Mengatto et al., 2011).

3. The challenges of implantology

Dental implant failure could include surgical complications, patient aesthetic concerning or implant functional disability. A significant reduction in the bone-implant contact may jeopardize the osseointegration process and lead to implant loss. Implant loss is considered a complex, multifactorial trait, investigated by several clinical follow-up and retrospective studies (Esposito et al., 2004; Fugazzotto, 2005; Graziani et al., 2004).
The process is divided into early and late events: early failure occurs before implant load, and late failure takes place after the implant has received occlusal load (Esposito et al., 1999). Early failures have been related to smoking (Ganeles and Wismeijer, 2004), aging (Moy et al., 2005), systemic diseases (Quirynen and Teughels, 2003; Weyant and Burt, 1993), bone quantity and quality (Degidi and Piattelli, 2005; Rosenberg et al., 2004; Stanford, 2003), surgical trauma (Gruica et al., 2004), and contamination during surgical procedure (Kuttenberger et al., 2005; van Steenberghe et al., 1990). Late failures have been related to peri-implantitis (Rosenberg et al., 2004), and occlusal overload (Misch et al., 2004).
Although many studies have provided an important contribution to the understanding of the implant failure process, in some situations, clinical factors alone do not explain why some present implant loss (Deas et al., 2002; Montes et al., 2007). The goal that should be achieved by modern implantology research is developing tools able to predict the patient biological response to treatment before implant surgery intervention.

4. Physiopathology of dental implant loss

Inflammation surrounding implant placement area is a crucial physiopathological process that permits the elimination of local tissue damage and substitution for a viable tissue; process termed regeneration. An augmentation of this inflammatory process is directly related to the quantity of tissue that may be substituted (Thomas and Puleo, 2011).

A complete stabilization between implant pins and surrounding bone is required to achieve a successful implant osseointegration. Primary stability is a mechanical feature achieved during surgical implant placement, which helps stabilization at early phases, leading to a desirable outcome (Szmukler-Moncler et al., 1998). When the process is developing in the post-surgery timeline, implant stability in relation to surrounding bone tends to decline, with the lowest implant stability quotient values being reached at 3 weeks (Han et al., 2010). After the bone regeneration process, stability reaches the maximum value when the osseointegration is achieved.

The two of the main factors for achieving predictable success of osseointegrated oral implants are lack of stability and micromovements (Albrektsson et al., 1981; Turkyilmaz et al., 2009). An abnormal exacerbated inflammatory process may lead to an abnormal decline in implant stability. Micromovements of implants in this crucial phase may result in the formation of a conjunctive tissue between implant and surrounding bone, process known as implant encapsulation (Lioubavina-Hack et al., 2006). Encapsulated dental implants do not become integrated to the bone, not reaching sufficient stability, and sometimes causing patient local pain. Those implants cannot be used as support for a tooth prosthesis and need to be removed, representing the major cause of implant failure.

4.1 Interleukin (IL)-1 as a key inflammatory cytokine

Implant surface biological aggregates interact with the cell membrane-bound proteins or receptors, eventually initiating cell attachment to the implant surface (Kasemo and Gold, 1999). Studies have shown that the coating material of the implants, considered innocuous, can stimulate cells to produce immunogenic inflammatory mediators in vitro (Harada et al., 1996; Perala et al., 1992).

Interleukin-1 (IL-1) is considered a pro-inflammatory mediator with central importance in the initiation and maintenance of acute inflammatory responses (Hoffman and Brydges, 2011). Its name as an interleukin, which means "between leukocytes", is misleading because IL-1 is synthesized not only by leukocytes but by other cell lineages as well. Besides acting as a mediator of local inflammation, IL-1 can produce systemic effects (Dinarello, 2007). IL-1 is the term for two polypeptides: IL-1α and IL-1β that possess a wide spectrum of inflammatory, metabolic, physiologic, hematopoietic, and immunologic properties (Pelegrin, 2008). IL-1β has been particularly studied as a critical determinant of tissue destruction due to its proinflammatory and bone resorptive properties and increased levels of IL-1β in gingival crevicular fluid were correlated with the severity of periodontal disease (Bloemen et al., 2011; Goutoudi et al., 2004; Hellmig et al., 2005; Stashenko et al., 1991). Although both forms of IL-1 are distinct gene products, they recognize the same cell surface receptors and share the various biologic activities. The IL-1 natural occurring inhibitor, the interleukin 1 receptor antagonist (IL-1ra), acts by binding the IL-1 receptors (IL-1R) inhibiting biological responses (Lennard, 1995). Produced and secreted by almost all cells expressing IL-1, IL-1ra functions as a competitive receptor antagonist, binding to IL-1 receptors, but not activating target cells (Molto and Olive, 2010). Today, IL-1 family is recognized to include 11 total members (Smith, 2011), which play particular roles in immune-inflammatory aspects of the host response. IL-1 is thus a "cytokine" and this term is used to connote that the sources and actions of IL-1 and related polypeptides include several different cell types. Moreover, IL-1 belongs to a group of cytokines with overlapping biologic properties such as tumor necrosis factor (TNF). IL-1 and TNF share the ability to

stimulate T and B lymphocytes, augment cell proliferation, and initiate or suppress gene expression for several proteins (Laurincova, 2000). IL-1α, IL-1β, and TNF-α were observed to signal fibroblasts to produce matrix metalloproteinases (MMPs) that induce connective tissue degradation (Kornman, 2006; Takashiba et al., 2003). Circulating levels of IL-1 are elevated in a variety of clinical situations and, together with elevated levels of TNF, correlate with the severity of some diseases, suggesting that these cytokines participate in the host response to or development of illnesses.

There is a dramatic increase in IL-1 production by a variety of cells in response to infection, microbial toxins, inflammatory agents, products of activated lymphocytes, complement, and clotting components. At the site of inflammation, IL-1 acts on macrophages, further increasing the production of IL-1 and inducing the synthesis of IL-6. In endothelial cells, increases the expression of surface molecules that mediate leukocyte adhesion (Abbas et al., 1998). This cytokine also acts on fibroblasts, stimulating their proliferation and transcription of collagen type I, III and IV. Thus, the development of fibrosis appears to be partly mediated by IL-1 (Dinarello, 1988). Indeed, production of IL-1 in tissues is thought to contribute to local effects such as fibrosis and tissue matrix breakdown, besides the influx of inflammatory cells (Stevens et al., 2009). IL-1 has also significant effects on bone, increasing constitutive receptor activator of NF-kappa-B ligand (RANKL)/osteoprotegerin (OPG) ratios (Stein et al., 2011). In vivo experiments indicated that IL-1 cytokine plays potent activity in bone resorption (Polzer et al., 2010b). Osteoclasts possess surface receptors for IL-1, which, when activated, stimulate the production of prostaglandins and IL-1 itself, modulating gene expression of several other cytokines. Thus, it is suggested that IL-1 participates in the pathogenesis of diseases involving bone tissue (Masada et al., 1990; Tatakis, 1993).

Many studies have investigated tissues surrounding unsuccessful dental implants in order to clarify the implant failure mechanisms (Aboyoussef et al., 1998; Ruhling et al., 1999). The bone-implant interface area is first occupied by red blood and inflammatory cells, degenerating cellular elements, then is gradually replaced with spindle-shaped or flattened cells, with initiation of host bone surface osteolysis (Futami et al., 2000). An abnormal immune-inflammatory response involving different cell types such as macrophages, polymorphonuclear neutrophils, T and B lymphocytes, endothelial cells, fibroblasts, keratinocytes, osteoclasts, and osteoblasts can impair periodontal and peri-implant tissues (Seymour et al., 1989). If activated, these cells can synthesize and release cytokines such as IL-1, which mediates both inflammatory and bone resorption processes (Gainet et al., 1998).

Searching for diagnostic markers to monitoring implant health status, levels of interleukins have been measured in diseased implant sites (Boynuegri et al., 2011). Higher levels of IL-1β were found in diseased implant sites when compared with healthy ones (Aboyoussef et al., 1998), suggesting that such inflammatory mediator is associated with implant failure (Salcetti et al., 1997). Higher levels of TNF-α also showed association with unfavorable clinical outcomes at 2 and 14 days after implant placement, being proposed that TNF-α gene expression may predict clinical complications (Slotte et al., 2010). The association between implant surface modification and inflammation molecular markers has been investigated and suggested as an additional indicator of implant clinical outcome (Monjo et al., 2008).

4.2 Extracellular matrix (ECM) on the osseointegration process

Osseointegration has been considered not the result of an advantageous tissue response but rather the lack of a negative tissue response (Mavrogenis et al., 2009). Some research has

been progressed in order to better understand the implant-bone tissue interface and the kinds of matrix produced on an implant surface (Wierzchos et al., 2008), although those events have only been characterized at a morphological level, using several histological approaches (Linder et al., 1983; McKibbin, 1978; Schenk and Perren, 1977; Winet and Albrektsson, 1988). Extracellular matrix (ECM) appears to vary morphologically between different material surfaces, suggesting that the extracellular response can be affected by the implant surface. However, ECM investigations, valuable in determining the vascular and morphological changes occurring in the healing site, suffer from the inability to biochemically evaluate the cellular response around a fixture (Winet and Albrektsson, 1988). Only more recently, studies aiming to characterize ECM at a molecular level start dissecting the structural components during implant osseointegration process and a cartilage ECM gene was found to be expressed (Mengatto et al., 2011). Patient individual response to implant treatment, in terms of the interfacial response of cells in contact with the implant surface, seems to impact clinical outcomes (Huang et al., 2004).

4.2.1 Matrix metaloproteinases (MMPs) and other mediators involved in ECM remodeling

Matrix metalloproteinases (MMPs) represent the major class of enzymes responsible for ECM metabolism (Kerrigan et al., 2000). They are metal-dependent proteolytic enzymes that contribute to the degradation and removal of collagen from damaged tissue. MMPs are secreted by inflammatory cells in response to stimuli such as lipopolysaccharide and cytokines (Birkedal-Hansen, 1993). Specific enzymes of this family can function beneficially during tissue remodeling and during formation of the ECM (Fanchon et al., 2004). However, MMPs may increase the adverse effects of cardiovascular disease (Kukacka et al., 2005), cancer metastasis (Nemeth et al., 2002), caries process (Sulkala et al., 2001) and periodontal disease (Liu et al., 2006) by destruction of collagen and other proteins of the ECM.

Previous studies have also shown that MMPs (e.g. collagenases, gelatinases) are present in peri-implant sulcular fluid (Apse et al., 1989; Ingman et al., 1994; Ma et al., 2000; Teronen et al., 1997) and may play a pathologic role in peri-implant bone loss (Golub et al., 1997).

Matrix metalloproteinase-1 (MMP-1), also known as collagenase-1, is a key mediator of the degradation of collagen, which is abundant in connective tissue and bone matrix (Yamada et al., 2002). MMP-1 degrades types I, II, III, and IX collagen, which are the most abundant protein components of extracellular matrices (de Souza et al., 2003; Dunleavey et al., 2000). An enhanced secretion of MMP-1 was verified in peri-implantitis fibroblasts compared to healthy and periodontitis sites (Bordin et al., 2009). Gelatinase B (MMP-9) is particularly active against gelatins, denaturing type I collagen, and type IV collagen, a major component of the basement membrane. It also acts proteolytically against proteoglycan core protein and elastin, which are resistant to degradation by some other MMPs (Birkedal-Hansen, 1993). MMP-9 is produced by inflammatory cells as well as by stimulated connective tissue cells (Foda and Zucker, 2001) and has been identified in many human cancers both in neoplastic tissues and in the surrounding stromal and inflammatory cells (Crawford and Matrisian, 1994). Related to dental implants, zymography studies showed increased activities of MMP-9 in cells exposed to titanium particles between 48 to 72 hours (Choi et al., 2005). Transforming growth factor-beta 1 (TGF-β1) is a member of a large family of growth factors and cytokines, which are synthesized by a wide range of cells and therefore are distributed

in many different tissues (Massague, 1990). There are at least three homologous TGF-β isoforms in humans: TGF-β1, TGF-β2, and TGF-β3. TGF-β1 is the best characterized TGF-β isoform, and its primary sequence is highly conserved throughout evolution (Syrris et al., 1998). It is synthesized as precursor latent forms, and the active form consists of two identical disulfide linked polypeptide chains (Derynck et al., 1985; Syrris et al., 1998). TGF-β possesses some major activities: it inhibits proliferation of most cells, but can stimulate the growth of some mesenchymal cells; exert immunosuppressive effects and reduction of inflammation; is involved in extracellular matrix deposition, and promotion of wound healing (Lawrence, 1996; Santos et al., 2004a). In the health organism TGF-β is involved in wound repair processes and in starting inflammatory reactions and then in their resolution. The latter effects of the TGF-β derive in part from their chemotactic attraction of inflammatory cells and of fibroblasts (Lawrence, 1996). In periodontal diseases, TGF-β concentration was directly associated with plaque index and probing pocket depth. Moreover, decreases in gingival crevicular fluid concentration levels after surgical treatment of periodontitis sites was also found (Sattari et al., 2011). Cytokines such as TGF-β and MMPs can affect the attachment and synthetic activities of osteoblasts and cause the reduction of bone matrix and mineral deposition (Kwon et al., 2000; Kwon et al., 2001).

4.3 Bone metabolism on implant healing

The properties of bone are directly related to the features of the mineralized ECM adjacent to implants in two ways. First, the implant geometry and the insertion approach (surgery technique) determine the principal bone–implant relation. Second, the properties of bone homeostasis and turnover have a major impact on the load-related characteristics of the microenvironment adjacent to implants (Joos et al., 2006). The cortical part of bone provides the mechanical and protective functions, whereas cancellous bone is also involved in metabolic functions (e.g. calcium homeostasis). Both aspects (structural and metabolic) are closely related to the features of the mineralized extracellular matrix at implant surfaces. Trabecular bone fills the initial gap and arranges in a three-dimensional network at day 14 (Franchi et al., 2005). The de novo formation of primary bone spongiosa offers not only a biological fixation to ensure secondary implant stability (Ferguson et al., 2006) but also a biological scaffold for cell attachment and bone deposition (Franchi et al., 2005). After 28 days, delineated bone marrow space and thickened bone trabeculae with parallel-fibered and lamellar bone can be found within the interfacial area. After 8 to 12 weeks, the interfacial area appears histologically to be completely replaced by mature lamellar bone in direct contact with titanium (Berglundh et al., 2003).

Osseointegration has been defined as the direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant (Branemark et al., 1969). Since Branemark's initial observations, the concept of osseointegration has been defined at multiple levels such as clinically (Adell et al., 1981), anatomically (Branemark et al., 1977), histologically, and ultrastructurally (Linder et al., 1983). When an implant is placed, the space between the fixture and bony crypt will heal with new bone by reparative osteogenesis resulting in clinical fixation of the implant. The bone turnover is also evidenced by bone changes during the first 6-year period *in vivo*, resulting in an increased thickness (up to 200 nm), which contain increased levels of organic and inorganic (Ca, P, S) material. This may suggest the potential for a surface reactivity not

usually associated with titanium and supports the concept of a dynamic definition of osseointegration (Stanford and Keller, 1991).
Patients have a mean time to repair an implant surgery in terms of bone, and create new mineralized tissue around implants. The meantime repair does not have to apply to all patients because they present different bone turnover rate (Chang et al., 2010; Courbebaisse and Souberbielle, 2011). This issue is not clinically measured and may have an impact on implant osseointegration. Quality and quantity differences in proteins that are classically involved in bone metabolism may modulate bone remodeling (Alvim-Pereira et al., 2008b). Guidelines taking into account bone tissue repair and turnover rate are desirable to ensure a successful osseointegration. These characteristics are nowadays based only clinically, considering medical and dental history. In this way, it cannot be established a clear cutline across patients that are suitable or have an increased risk for dental implant therapy in terms of host response. Therefore, it has been proposed that progression of osseointegration may be accelerated by growth factors and modification of implant surface, and functional integration of peri-implant structure may be feasible to predict the implant function during osseointegration (Chang et al., 2010). Although it is important to study extrinsic factors which could impair or accelerate osseointegration, there is still a lack of understanding over inter-individual differences on host physiologic response.

4.3.1 Bone metabolism proteins

Bone is one of the classical target tissues for vitamin D action. Vitamin D regulates calcium homeostasis by influencing intestinal calcium absorption, renal calcium reabsorption, and bone calcium metabolism (Binkley, 2006). Vitamin D is ingested or cutaneously produced upon exposure to ultraviolet B radiation in an inactive form. To be activated, vitamin D is transported in the blood bound to a vitamin D-binding protein, hydroxylated in the liver and the resulting metabolite is further hydroxylated mainly at the kidney, resulting in the active form called 1,25-dihydroxyvitamin D3 (Panda et al., 2004). In target tissues, 1,25-$(OH)_2$D3 is believed to exert most of its actions by binding to the vitamin D receptor (VDR), a member of the nuclear steroid hormone receptor superfamily, and by regulating the transcription of vitamin D target genes (Haussler et al., 1998). VDR also plays a complex role in the control of bone homeostasis and recruits co-regulators, which may have activating or repressing effects. In VDR knockout growing mice, the primary defect of calcium metabolism is at the intestine; loss of VDR causes calcium malabsorption and rickets that can be prevented by a high-calcium diet. Additionally, VDR knockout mice reveal that VDR plays a role in suppression of bone formation (Fleet, 2006). Functionally, in experimental model, vitamin D analogs dramatically increase bone mass, size and strength in rodents (Slatopolsky et al., 2003). Observations suggest that bone integration around implants may be critically impaired by vitamin D deficiency (Mengatto et al., 2011).
Bone morphogenetic protein (BMP) family, as a member of the TGF-β superfamily, has a variety of functions in the development and reparation of bone tissue (Rider and Mulloy, 2010). The hallmark of the BMPs is their ability to induce bone formation *in vivo* by promoting osteoblast differentiation (Rider and Mulloy, 2010). BMP-2 has been shown to stimulate bone ingrowth, gap healing, and implant fixation in several animal studies (Cochran et al., 1999; Sumner et al., 2004). BMP-2 recombinant protein application showed a good potential in terms of regeneration and decreased morbidity as compared with bone

autografts (Tonetti and Hammerle, 2008), also suggesting an important role on bone regulation in oral reparation sites.

Calcitonin is a peptide hormone that rapidly, transiently, and reversibly inhibits osteoclast-mediated bone resorption and also modulates calcium ion excretion by the kidney (Pondel, 2000). The physiological effects of calcitonin are specifically mediated by high affinity calcitonin receptors (CTRs), which belong to the class B subfamily of seven-transmembrane domain G protein-coupled receptors. The effect of calcitonin drugs during the period of bone maturation around titanium implants was investigated in animal model, and a positive time effect was verified improving bone mass (Januario et al., 2001). Moreover, it was shown that implant surface modifications might alter the expression of calcitonin receptor gene in osteoclasts (Monjo et al., 2008).

The system RANK/RANKL/OPG has been described as a central regulator of bone metabolism. RANKL was shown to bind its receptor, RANK, on osteoclast lineage cells to induce osteoclastogenesis. The molecule blocked by the soluble receptor OPG was identified as the key mediator of osteoclastogenesis in both a membrane-bound form expressed on preosteoblastic/stromal cells as well as a soluble form. The RANK/RANKL/OPG regulatory axis is also involved in inflammatory bone destruction induced by pro-inflammatory cytokines such prostaglandin E_2 (PGE_2), IL-1β, IL-6, and TNF-α (Boyle et al., 2003). In addition, a number of other mediators of bone metabolism, such as TGF-β (Takai et al., 1998), parathormone (PTH) (Lee and Lorenzo, 1999), 1,25-dihydroxyvitamin D3 (Kitazawa et al., 1999), glucocorticoids (Hofbauer et al., 1999), and estrogen (Hofbauer et al., 1999; Saika et al., 2001) exert their effects on osteoclastogenesis by regulating osteoblastic/stromal cell production of OPG and RANKL. RANKL and OPG concentrations were significantly higher at the crevicular fluid sampling sites of patients presenting peri-implantitis, suggesting an increased risk of alveolar bone loss around dental implants (Arikan et al., 2011).

5. A research focus on the host genetic susceptibility

The knowledge that implant loss i) is not totally explained by clinical conditions and ii) tends to cluster in subsets of individuals (Montes et al., 2007; Weyant and Burt, 1993) may indicate that specific host response characteristics, that disturb the osseointegration process, are influenced by genetic factors (Alvim-Pereira et al., 2008a).

Gene polymorphisms are a mechanism by which individuals may exhibit variations in DNA sequence. These variations may impact specific protein production and/or function (Hu et al., 2005) that in turn could alter host response modulating disease susceptibility or implant treatment outcome. Most polymorphisms are single nucleotide exchanges (SNPs) that occur in a high frequency in the human genome (Venter et al., 2001). Functional polymorphisms may account for variation in the production or function of proteins (Hu et al., 2005; Pociot et al., 1992). Those resultant slight changes in the immunoinflammatory response modulation might influence implant loss (Lachmann et al., 2007; Laine et al., 2006).

The focus of studies investigating genetic susceptibility to dental implant failure has been limited to candidate gene, population-based association analysis (Alvim-Pereira et al., 2008a). In this approach the physiology and involved metabolic pathways of healing and osseointegration process are the basis to search for candidate genes underlying host susceptibility to implant failure. But, a number of biologic mechanisms is involved in the osseointegration complex process, some of which have not yet been identified (Mengatto et

al. 2011; Montes et al., 2007). Although some similarities between osseointegration and tooth extraction socket were seen, different pathways of transcription and growth factors, extracellular matrix molecules, and chemokines were proposed (Lin et al., 2010). A recent study with rats showed a possible network of genes that associated with success and failure of implant osseointegration (Mengatto et al., 2011).
In the PUBMED library available literature, a total of twenty-three original papers analyzing genetic polymorphisms in candidate genes in humans related to implant outcomes were found. The main data and results of each study are summarized in Table 1.

5.1 Genetic polymorphisms and dental implant failure

The most commonly studied polymorphisms in genetics of implant failure are funtional variations in the interleukin-1 (IL-1) gene cluster in several populations. Because of IL-1 proinflammatory and bone resorbing properties, a role has been suggested for this cytokine in controlling genetic risk of implant failure. The association of *IL1A* gene (which codes for IL-1α) polymorphisms with dental implant outcome was investigated in several studies (Campos et al., 2005c; Feloutzis et al., 2003; Gruica et al., 2004; Jansson et al., 2005; Lachmann et al., 2007; Laine et al., 2006; Lin et al., 2007; Rogers et al., 2002; Shimpuku et al., 2003b; Wilson and Nunn, 1999). Since *IL1B* gene (which codes for IL-1β) was seen to be up-regulated at early stages of healing and then down-regulated at later stages (Lin et al., 2010), polymorphisms in *IL1B* gene were also investigated for association with implant failure susceptibility (Campos et al., 2005b; Dirschnabel et al., 2011; Feloutzis et al., 2003; Gruica et al., 2004; Jansson et al., 2005; Lachmann et al., 2007; Laine et al., 2006; Lin et al., 2007; Melo et al., 2011; Montes et al., 2009; Rogers et al., 2002; Shimpuku et al., 2003b; Wilson and Nunn, 1999). Moreover, *IL1RN* gene (which codes for IL-1ra) was also searched for association to implant failure (Campos et al., 2005c; Laine et al., 2006; Montes et al., 2009). Even though *IL1* gene cluster is the most frequent analyzed inflammatory candidate genes, the results are divergent, yet not conclusive and generally not replicated (see table 1 for review). However, genotype 2/2 of *IL1RN* polymorphism was significantly more frequent in patients who presented multiple losses. Some other functional polymorphisms in inflammatory candidate genes were also analyzed: *IL2* (Campos et al., 2005a), *IL6* (Campos et al., 2005a; Melo et al., 2011), and *TNFA* (Campos et al., 2004; Cury et al., 2007; Cury et al., 2009) (Table 1).
Also, genes involved in the regulation of ECM such as *TGFB1* (Santos et al., 2004a), *MMP1* (Leite et al., 2008; Santos et al., 2004b) and *MMP9* (Santos et al., 2004b) have been investigated (Table 1).
It has been suggested that polymorphisms in the *VDR* gene significantly alter expression and/or function of VDR, which may interfere in mineral bone density (Shishkin et al., 2010). So far, only one paper has investigated the association of *VDR* gene polymorphisms with dental implant loss with no association evidenced between a functional polymorphism and implant loss (Alvim-Pereira et al., 2008b) (Table 1).
Bone morphogenetic protein 4 (*BMP4*) gene expression was reported to be slowly increased during osseointegration and the bone healing process (Lin et al., 2010), and a gene polymorphism in this gene was associated with marginal bone loss around dental implants (Shimpuku et al., 2003a) (Table 1). Another polymorphism in the *CTR* gene was also associated with marginal bone loss in the jaw, but not in the maxilla (Nosaka et al., 2002) (Table 1). Polymorphisms in *CTR* gene were also associated with bone metabolism regulation in postmenopausal women (Masi et al., 1998; Nakamura et al., 2001) and severe periodontitis (Suzuki et al., 2004).

Authors	Year	Polymorphisms	Case (n) / Control (n)	Mean age (years)	Smoking Yes / No	Population	Results
Wilson and Nunn	1999	*IL1A* (-889) and *IL1B* (+3953)	17 / 38	57	27 / 35	?	Not associated with implant failure
Nosaka et al.	2002	*CTR* (+1377)	15 / 20	54.8	15 / 20	Asian Japanese	Associated with marginal bone loss in mandible, but not in maxilla
Rogers et al.	2002	*IL1A* (-889) and *IL1B* (+3953)	19 / 31	66	?	Australian Caucasian	Not associated with implant failure
Shimpuku et al.	2003	*IL1A* (-889) and *IL1B* (-511, +3954)	17 / 22	55.1	14 / 25	Asian Japanese	Associated with marginal bone loss
Shimpuku et al.	2003	*BMP4* (+538)	21 / 36	52.6	24 / 38	Asian Japanese	Associated with marginal bone loss
Feloutzis et al.	2003	*IL1A* (+4845) and *IL1B* (+3954)	?	59.5	41 / 39	European Caucasian	Smoking + *IL1* positive genotype associated with marginal bone loss
Campos et al.	2004	*TNFA* (-308)	38 / 38	47.2	0 / 66	Brazilian	Not associated with early implant failure
Santos et al.	2004	*TGFB1* (-509, -800)	28 / 40	46	0 / 68	Brazilian	Not associated with early implant failure
Santos et al.	2004	*MMP1* (-1607) and *MMP9* (-1562)	20 / 26	45.9	0 / 46	Brazilian	*MMP1*: associated, and *MMP9*: not associated with implant failure
Gruica et al.	2004	*IL1A* (+4845) and *IL1B* (+3954)	34 / 176	25 to 90	53 / 127	European Caucasian	Smoking + *IL1* positive genotype associated with late infection
Campos et al.	2005	*IL1A* (-889), *IL1B* (-511, +3953), and *IL1RN* (intron 2 - 86 bp repeats)	28 / 34	47.5	0 / 62	Brazilian	Not associated with early implant failure
Campos et al.	2005	*IL2* (-330) and *IL6* (-174)	34 / 40	46.3	0 / 74	Brazilian	Not associated with early implant failure
Jansson et al.	2005	*IL1A* (-889) and *IL1B* (+3954)	6 /16	54	10 /12	European Caucasian	Smoking + *IL1* positive genotype associated with implant loss
Laine et al.	2006	*IL1A* (-889), *IL1B* (-511, +3953), and *IL1RN* (intron 2 - 86 bp repeats)	71 / 49	67.2	84 / 36	European Caucasian	*IL1RN* associated with peri-implantitis
Lin et al.	2007	*IL1A* (-889), IL1B (-511, +3953)	29 / 30	42.8	32 / 27	Asian Chinese	*IL1B* associated with marginal bone loss
Cury et al.	2007	*TNFA* (-308)	36	46	0 / 36	Brazilian	Not associated with marginal bone loss
Lachmann et al.	2007	*IL1A* (-889), *IL1B* (+3953)	11 / 18	66	?	European Caucasian	Not associated with levels of peri-implant crevicular fluid nor with cytokine concentration
Alvim-Pereira et al.	2008	*VDR* (exon 9, *Taq* I)	80 / 137	51.7	43 / 174	Brazilian	Not associated with implant failure
Leite et al.	2008	*MMP1* (-1607 and -519)	44 / 60	?	0 / 104	Brazilian	*MMP1* associated with early implant failure
Montes et al.	2009	*IL1B* (+3954) and *IL1RN* (intron 2)	90 / 176	51.5	58 / 208	Brazilian	*IL1RN* associated with multiple implant loss
Cury et al.	2009	*TNFA* (-308)	49 / 41	?	?	Brazilian	Not associated with peri-implantitis
Dirschnabel et al.	2011	*IL1B* (-511)	92 / 185	53.6	61 / 216	Brazilian	Not associated with implant loss
Melo et al.	2011	*IL1B* (+3954), *IL1B* (-511), and *IL6* (-174)	31 / 16	?	?	Brazilian	Not associated with peri-implantitis

Table 1. Studies investigating the association between genetic polymorphisms and dental implant failure.

5.2 Future insights in dental implants genetic research

Although candidate gene, association analysis has proved to be a promising tool for the dissection of the nature of the genetic component controlling dental implant failure, the design is limited by the fact that just a small segment of the genome is analyzed. Moreover, the sample sizes are often small; therefore, findings must be replicated in larger populations (Alvim-Pereira et al., 2008a). As a consequence, genetic susceptibility to osseointegrated implant failure remains widely unknown.

Despite these promising advances, the exact number, identity and role of regulatory factors that lead to a successful implant osseointegration and its maintenance are still largely unknown, which limits genetic analysis approaches based on functional candidate genes. The challenge then is to map all the involved genes (Bosse et al., 2004), a considerably difficult task given that the human genome is composed of at least 30,000 genes (Baltimore, 2001), reaching 4.1 million of SNPs catalogued in public databases markers.

Genomewide association scans (GWAS) are a fully automated technology that allows genotyping hundreds of thousands of SNPs in a single experiment (Thomas et al., 2005). This extremely high throughput SNP genotyping technology is making possible the development of association-based case-control design covering the entire genome (Hirschhorn and Daly, 2005). However, some limitations do exist. Those analyzes are still very expensive and need cutting-edge genotyping technology (Detera-Wadleigh and McMahon, 2004) and tremendous amount of raw data demands adequate statistical methods of analysis (Devlin et al., 2001). False-positive results are likely to increase (Marchini et al., 2004), in this context, replication in independent populations becomes mandatory (Neale and Sham, 2004). On the other way around, the great amount of failure in identifying significant associations of complex traits and diseases with common variants across the genome may indicate that those complex phenotypes may possibly be determined by rare gene variants. In this context, the whole genome sequencing of a few individuals, whose phenotype is very well characterized and extreme, may be cheaper and offer more valuable results.

Another development in the genetic field is called next-generation sequencing (NGS) technology, which includes genome sequencing and resequencing, transcriptional profiling (RNA-Seq) and high-throughput survey of DNA–protein interactions (ChIP-Seq) (de Magalhaes et al., 2010). The advantages may change the landscape of genetics by a reasonable cost and high throughput (Mardis, 2008). In spite of giving rise to new challenges, in particular in processing, analyzing and interpreting data, this type of application may clarify and increase knowledge over physiologic pathways of bone remodeling and osseointegration.

6. Conclusion

Despite the difficulties, the motivation to continue applying traditional and new approaches for genetic analysis to the effort towards a better understanding of dental implant physiology and failure mechanisms is clear. For example, genetic studies may shed new light not only upon the physiopathology of dental implant failure, but also upon broader, related processes, such as bone healing. In addition, a direct result of such studies may be the definition of potential targets for effective screening, prevention and maintenance of dental implants.

7. References

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17

Implant Complications

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1. Introduction

Implant treatment is regarded as a safe technique with high rates of success (Adell et al., 1981; Adell et al., 1990; Buser et al., 1997; Buser et al., 2000; Wennström et al., 2005). Nevertheless, it has, as every surgical procedure, several complications that can occur and that must be known in order to prevent or solve them. Implantology is an ever growing field that is reaching the practice of general dentists due to the simplification of technical procedures. Specialists in oral surgery now perform more demanding procedures, along with general dentists, they must pay special attention during the learning curve in order to avoid risks.

The osseointegration process is considered to be safe and durable by both patients and dentists, for this reason, implants are considered as the first option to treat almost all cases of complete or partial edentulism. Regarded as a problem free procedure, it´s demand is increasing. Despite the severe complications index is low, risk evaluation must be conducted systematically during the preoperatory stage based on clinical history, thorough exploration and if necessary consulting other specialists, dentists or physicians. Only by using a good work protocol, we can detect the local and systemic risk factors that could determine the success of the treatment and allow us to implement preventive measures if needed.

Parameters concerning systemic risks and their categories were well established in the 2nd ITI Consensus's Conference (Buser et al., 2000) there are also excellent systematic reviews (Bornstein et al., 2009). If our interest is to know about local factors that can negatively interfere in the success of the treatment or even cause serious local or general complications, we can consult several reviews or case reports (Cochran, et al., 2009).

It is mandatory to classify all those clinical complications that can arise in this chapter, so we should differentiate between two concepts: accidents and complications (Annibali et al, 2009).

- Accidents always happen during surgical procedures.
- Complications appear lately, once surgery is already performed. There are two kinds of complications, depending on the time they emerge: early and late.
- Failures occur when the professional and/or the patient do not obtain the desirable results.
- Iatrogenic acts are regarded as accidents, complications or failures caused by a deficient praxis of the professional.

The majority of problems that can arise in an implantology treatment are accidents, complications or iatrogenic errors, and are a consequence of an inadequate indication, poor quality or quantity of bone, an erroneous surgical technique, infections, lack of oral hygiene, smoking habit, systemic diseases that were poorly controlled, et cetera. Failures of implants normally occur once they are correctly osseointegrated, have developed an acceptable masticatory function and are the consequence of lost of bone support derived from a periimplantitis produced by the presence of bacterial plaque and/or overload.
Early complications can be included in the following group: infection, edema, ecchymosis and hematoma, emphysema, bleeding, dehiscence of the area and sensitive alterations; and in the group of late complications we can distinguish: mucoperiosteal flap perforations, maxillary sinusitis, mandibular fractures, loss of osseointegration, bone defects, periimplant lesions and infections (Annibali et al., 2009). Nevertheless, some of them, like bleeding, could appear at any moment during the treatment. We will not comment prosthetic complications or periimplantitis because they will be explained in other chapters.
Other authors classify complications as general or local, depending on their etiology. We prefer to describe these topics as they arise in the course of the treatment.

2. Intraoperative accidents

As we said previously, we define intraoperative accidents as those events that can occur during surgery. Their severity ranges from minimum to maximum values.
Among accidents we can include: Badly placed implants, bleeding episodes, lesions of soft tissues, and on an adjacent tooth, lack of primary stability, dehiscences and osseous fenestrations, implant displacement to maxillary sinus, mandibular fracture, broken instruments, aspiration or swallowing of instruments:

2.1 Malposition or angulation of an implant

It is advisable to assess the characteristics of the edentulous zone subject to rehabilitation using clinical and radiological CT, or cone beam CT imaging (Dreiseidler et al., 2009) at the time of devising an implantology treatment. Sometimes, this is not possible and the option then is to use short or tilted implants that can increase the availiable bone length by 50% when tilted aproximately 30º or" by avoiding anatomical structures (menton nerve, maxillary sinus).
A wrong planning that involves a malposition or an overangulation, would represent an obstacle for carrying out the prosthetic restoration, while it also would deteriorate long-term implant viability throughout the implant treatment.
Angulation, in the case of a single implant, increases tension forces between the implant and the bone. However, angulation of implants located at very distal positions reduce forces supported by the periimplant bone (Bellini et al., 2009).
In the event that, as a result of a negligence or bone deficit, an implant may have been placed with an angulation that makes it dysfunctional, it is suggested the use of a repositioning system that has yielded excellent results and which is based on the osteogenic distraction of a bone fragment containing the integrated implant (Gotta et al., 2008; Mendonca et al.,. 2008; Oduncuoglu et al., 2011). All of which improves esthetic effects, as well as the biomechanical behavior of the implant by correcting crown-root proportion, contour of soft tissues and the relation with neighboring teeth. The authors reported that

the distraction rate is the usual value (1 mm per day) and the suggested consolidation period is 8 weeks, and confirm implant stability in time (Oduncuoglu et al., 2011).

2.2 Bleeding

Bleeding excessively is a common accident that can happen in some surgeries as a consequence of local-anatomical or systemic causes. Sometimes, patients are more prone to bleeding, since they are under a platelet antiaggregant treatment on a daily basis or have coagulation disorders (Garfunkel et al., 1999). This clinical situation is defined in the Group 2 of medical-systemic risk, where other disorders are also classified: irradiated patients (radiotherapy), those with Diabetes Mellitus (specially type I), patients with coagulation disorders (anticoagulated patients or those with haemostatic disorders) and severe smokers (Buser et al., 2000). Group I includes high risk patients: patients with serious systemic diseases (rheumatoid arthritis, osteomalacia, imperfect osteogenesis), immunodepressed (HIV, immunosupresory treatments), drug addicts (alcohol, etc.), unreliable patients (mental or psychological disorders).

Patients treated with anticoagulant medication usually have history of vascular or cardiac pathology (fibrillation, myocardial ischemia, valvular diseases or prosthesis, or thromboembolisms) (Lang et al., 2000). Probably, these patients would be included in the P1 or P2 risk classification groups of the ASA (American Society of Anesthesiology); P3 and P4 groups cannot undergo any surgical intervention until they are under treatment and included in a lower group. In general, the elderly are the majority of patients who need implantology solutions, which means that the probability of comorbidity is higher and therefore it is mandatory to know their medical history.

Generally, therapeutic options in these patients comprise two approaches: decrease or eliminate the anticoagulant therapy once patient and physician have assessed risks and benefits.

However, invasive treatments can be performed, as in the case of implantology procedures, without the interruption of medication and as long as values concerning International Normalized Ratio (INR) are > 4, and adequate hemostatic measures are followed and, at the same time, efforts are made to use atraumatic surgery techniques; in this situation, the risk of bleeding should be similar to that seen in healthy patients who are not under surgical outpatient treatment (Bacci et al., 2010). Anyway, it is advisable that any discontinuation of the anticoagulant therapy before any aggressive surgery (bone graft, extensive flaps, and maxillary sinus elevation) is discussed with the patient's physician, whereas patients should always be informed on the risk of procedures. It is recommendable to temporarily discontinue the anticoagulant treatment for 1-2 weeks, with previous physician's knowledge, when it is based on platelet antiaggregants (acetylsalicylic, acid, clopidogrel, ticlopidin, etc.) (Brennan et al., 2007; Sanz et al., 2009).

Data from recent studies also suggest that the risk of postoperative bleeding in this type of patients is low or almost negligible, while any bleeding complication can be handled with local intraoperative or postoperative measures suggested for dental extractions or dental implantations, where this is the standard procedure to assure local hemostasis (suture, compression, the use of hemostatic microfibrilar collagen gauzes, oxidized cellulose, re-absorbable fibrin, or mouth rinsing with 4,8% of tranexamic acid) (Bornstein et al., 2009; Napenas et al., 2009). In more severe cases of bleeding, it is proposed to use a nasal spray of desmopressin acetate (Nahlieli et al., 2011).

The same procedure could be useful in those cases where bleeding is caused by a mild variety of Von Willebrand disease (Garfunkel et al., 1999). The rest of coagulation pathologies resulting from a lack of coagulation factors should be managed in conjunction with the Hematology Department to settle the adequate preoperative therapeutic measures.
Patients should be advised, once intraoperative bleeding has been put under control and surgery procedures have concluded, to follow some postoperative instructions at home, which include the application of ice, compression, and the intake of a soft and cold diet; they also should be provided with a contact telephone in case of any emergency.
Other infrequent causes of bleeding are as follows: lesions in any sublingual, lingual, peri-mandibular, or submaxillary artery when performing surgeries in the lower and anterior area of totally edentulous patients who have a deficit in the quality and quantity of bone. Sometimes bleeding is less acute and can be perceived as a sublingual hematoma detected after few hours of performing the surgery (Mardinger et al., 2007). In other cases it involves an emergency that threatens patient's life and which requires a bimanual compression and subsequent referral of the patient to an hospital. A total of 18 clinical cases have been reported in the literature with this problem. Dubois et al. (Dubois et al, 2010) reported two cases that required a tracheotomy to solve an obstruction of the upper respiratory system due to a perforation of the lingual cortical that broke several sublingual vessels. Therefore, it is essential to do an exhaustive preoperative radiological exam that should include regular computerized tomography to appreciate the particular anatomy of each mandible, especially when placing implants in the premolar and canine area to avoid concavities and follow the major axis to the maximum (Dubois et al., 2010; Frenken et al., 2010). The onset of this complication is easily determined by a remarkable lingual protrusion caused by the swelling of the area below the tongue. In the event of a wound, it should be compressed by a ligature of the vessel involved as soon as it has been identified.
There is also a case report that describes an uncontrolled bleeding following a sinus lift procedure that caused a lesion on the artery that anastomoses the posterior superior alveolar artery with the infra-orbital one. Another case on a sinus lift procedure described an uncontrolled bleeding resulting from a lesion involving an artery that joined by anastomosis the posterior superior alveolar artery and the infra-orbital artery. Both arteries are branches of the maxillary artery that finds its way through an intraosseous passage located at a distance of 16.4 mm from the bone crest (Elian et al., 2005). The process of opening the lateral wall of the maxillary sinus, to connect the upper and lower osteotomies with vertical right and left osteotomies, can produce a lesion on this vessel that could lead to an intense and pulsating bleeding in a remote and difficult place to access (Testori et al., 2010). Lee (Lee, 2010) reported a case of a bleeding of an 8,1 mm artery above the alveolar crest that was finally controlled (after multiple attempts of compression and using bone wax) with the administration of local anesthesia and vasoconstrictor agents, as well as the application of gauzes soaked in bovine thrombin to help in the last coagulation process of transforming fibrinogen into fibrin. Once again, it is strongly recommended to carry out an exhaustive tomography study of this area before performing this surgical technique.

2.3 Soft tissue lesions

A series of lesions can occur in soft tissues, such as burn lesions on the labial mucosa resulting from overheating of the hand piece head, flap tear due to an excessive traction or as a consequence of an incorrect use of instruments or any abrupt movement by the patient or the surgeon, among other causes (Ozcelik et al., 2005). These lesion can be mostly avoided

through a careful management of tissues or by sedating the patient and thus prevent the occurrence of stress situations for both surgeon and patient.

2.4 Lesions of adjacent teeth

The malposition of an implant may lead to the lesion of an adjacent tooth, where this involves a lesion on the radicular surface or the root apex and a subsequent post-operative pulpitis, or periodontitis, that must be treated, in the majority of cases, by endodontic means, while sometimes it involves the non-integration of the implant because of the inflammation. It is of the utmost interest to study the axis of those teeth delimiting the edentulous space to be rehabilitated with implants to decide, before surgery, implant axis and thus choose the most convenient one, or reduce its length to curb its convergence and thus prevent this type of dental iatrogenic lesions. On some other occasions, the inflammatory-infectious origin in the apical zone is a tooth adjacent to the implant and this is especially due to the proximity of the tooth to the implant and to the time elapsed since the endodontic procedure on the tooth was performed, so the risk of a retrograde periimplantitis increases when the distance between tooth and implant apexes is shorter and when the lapse of time between the endodontic procedure and the implantation is also shorter (Quirynen et al., 2005; Tozum et al., 2006; Zhou et al., 2009).

2.5 Lack of primary stability

Primary stability is determined by bone density and cortical bone thickness, a fact that explains why it is easier to obtain a better stability in mandibular implants than in maxillary ones (Seong et al., 2009) as it entails preparing the implant bed (incorrect tapping, too-wide ostectomy) or in those cases in which the implant is immediately placed after an exodontic procedure (Becker et al., 2011; Cooper, 2010) and becomes manifest by insertion torque values, so a low insertion torque value (<10Ncm) will determine a higher risk of osseointegration failure (type bone IV), whereas a too-high torque value (>45Ncm) could lead to a bone compression which would result in a bone necrosis (type bone I), and in an osseointegration failure (Neugebauer et al., 2009).

Cooper (Cooper, 2010) described, in a study on 1084 implants, that there was a 6.43-fold lower risk of primary implant stability failure in the anterior mandible than in other locations. The maxilla had a 2.7-fold higher risk of primary stability failure versus the mandible. Females had a 1.54 higher risk of primary implant stability failure versus men; implants less than 15 mm in length had a 1.49-fold higher risk of primary implant stability failure versus longer implants.

Rough surfaces, a cone design of implants, and the use of osteotomes in the management of the implant bed can increase primary stability on low-quality bones (Javed et al., 2011; Padmanabhan & Gupta, 2010). When it is not possible to implement the aforementioned solutions, it would then be advisable to replace the unstable implant for a rescue implant, with a wider diameter and/or a longer length, or wait, as a final resort, 6-8 weeks before a surgical re-intervention.

2.6 Dehiscences and fenestrations

Osseous dehiscences and fenestrations in the vestibular cortical bone during the placement of implants constitute a risk factor for the healing process of periimplant tissues (fig. 1). In

these cases, bone regeneration in parallel with implant placement is therefore mandatory and can be achieved using different materials, such as autologous, allogenic or xenolog bone, or phosphocalcium materials, such as hydroxypatite or tricalcium phosphate, in conjunction with non-reabsorbable or reabsorbable membranes (Becker et al., 2009; Jung et al., 2009; Oh et al., 2003; Rosen & Reynols, 2001; Mayfield et al., 1997), in addition to associated growing factors, or in combination with different materials (Hassan, 2009.)

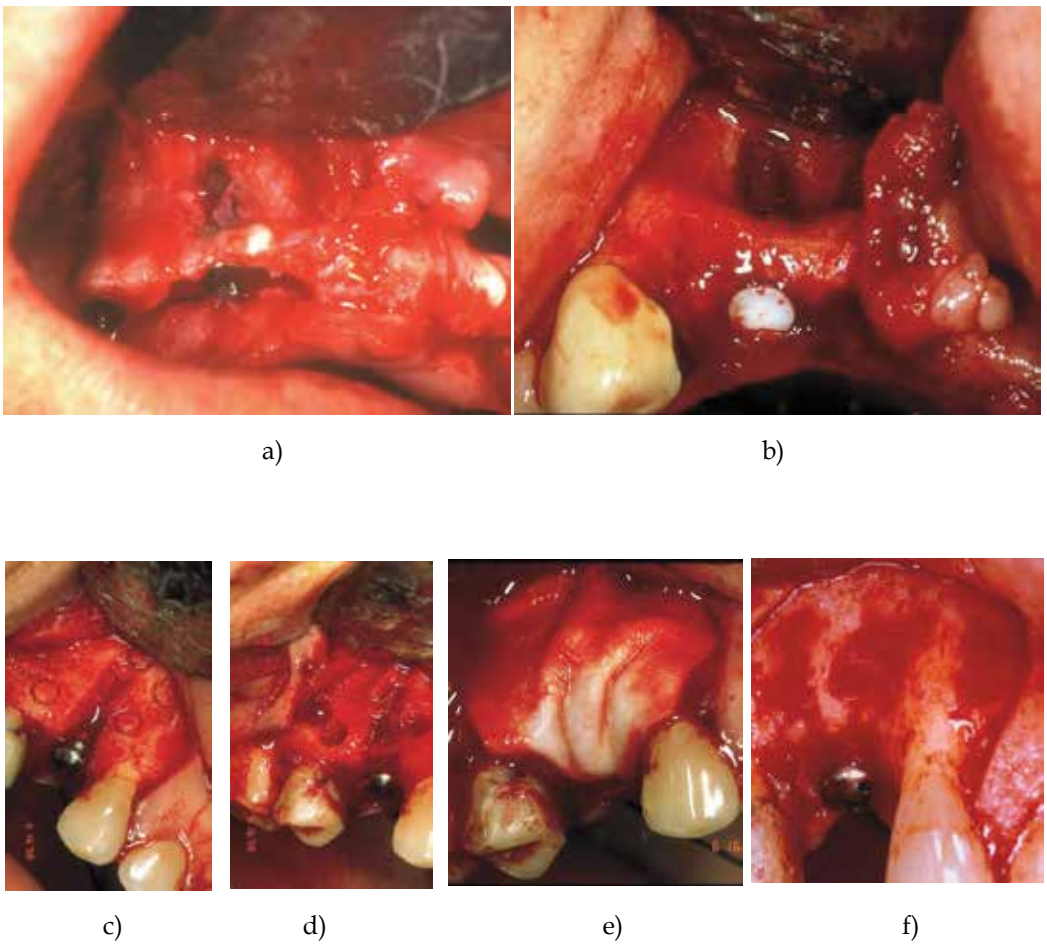
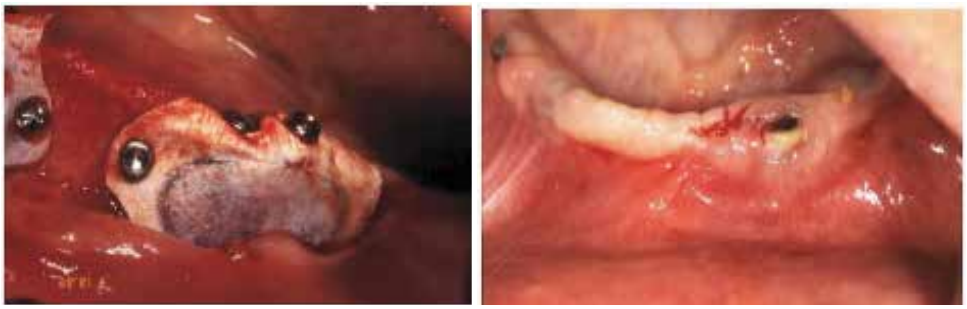


Fig. 1. (a, b) Osseous fenestration. (c) Osseous dehiscence. (d) Autologous bone chips. (e) Collagen membrane. (f) Clinical result at 5 months.

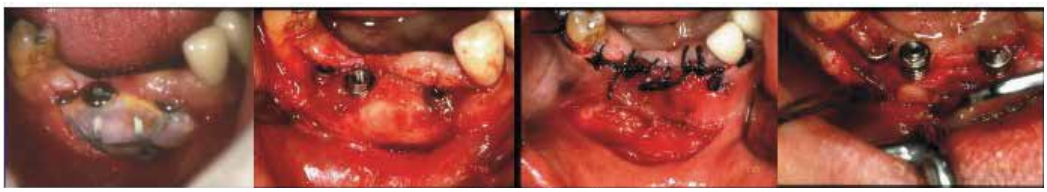
Results are subject to the magnitude and morphology of the defect to be regenerated, the stability of both the material and the membrane, the maintenance of the space, the reabsorption time of the membrane, on the assumption that it is reabsorbable since its efficiency, or that concerning the fenestration of non-reabsorbable membranes (it could trigger the onset of an infection). All these factors determine the final results (fig. 2).



(a) (b)

Fig. 2. (a) PTFE membrane covering a bone defect. (b) Membrane fenestration at 15 days control.

These accidents happen when implants are placed in a prosthesis-guided axis-position, but even though guided bone regeneration principles may have been followed, the main problem lays in a good wound-closure that may allow a primary scarring. It is necessary for the design, and the management and release of the flap to allow a wider extension, and thus achieve a better cover and tension-free surgical site (Hur et al., 2010). Smoker and diabetic patients have an added risk factor since scarring is more difficult and this can significantly jeopardize results, since the success of bone regeneration in non-smokers may reach 95% compared to 65% in those who smoke; furthermore, inflammation and exposure of membranes is greater (Garg, 2010; Abt, 2009; Cochran et al., 2009; Lindfors et al., 2010) (Fig. 3).



a) (b) (c) (d)

Fig. 3. (a) Wound dehiscence in a GBR with PTFEe Ti reinforced at 5 weeks of maintenance. (b) Image after the membrane was retrieved. (c) Bipedicular flap covering the new tissue. (d) Clinical result after 5 months.

Osseous dehiscences and bone fenestrations can pass unnoticed in those cases when an implant is immediately placed following an exodontic procedure or a transmucosal flapless surgery (Steigmann, 2008); so, as to values concerning probing depth and insertion level, results are less favorable when compared with implants placed in integral alveolar crests (Siciliano et al., 2009). This risk should be prevented by a correct exploration of the alveolus before inserting the implant (Nahlieli et al., 2011).

It is not recommendable to carry out guided bone regeneration (GBR) during the intra-operative period when the exposed surface of the implant is, in the event of dehiscence, equal or greater than 2/3 of its length.

Consequences could be more severe in the event of perforating the lingual cortical layer of the inferior jawbone in the inter-mental region, as abovementioned, so it is necessary to adequately arrange the implant axis in this situation and in all possible situations. This situation, though, could be prevented by requesting CT imaging studies (Parnia et al., 2010).

2.7 Implant displacement

The invasion of the maxillary sinus by an implant can occur during or after the surgery as a result of an insufficient primary stability. It has been reported a case of an implant which had been implanted 9 months earlier during a maxillary sinus lift surgery, and which was found inside the sinus only a few days after having placed the fixture (Chappuis et al., 2009). Therefore, any implant could undergo a displacement at any time after having been fixed with a cover screw (osseointegration period), even avoiding regenerative techniques in a spontaneous and asymptomatic way, or even afterwards, at the time of connecting the healing abutment (Ridaura-Ruiz et al., 2009) (Fig. 4).

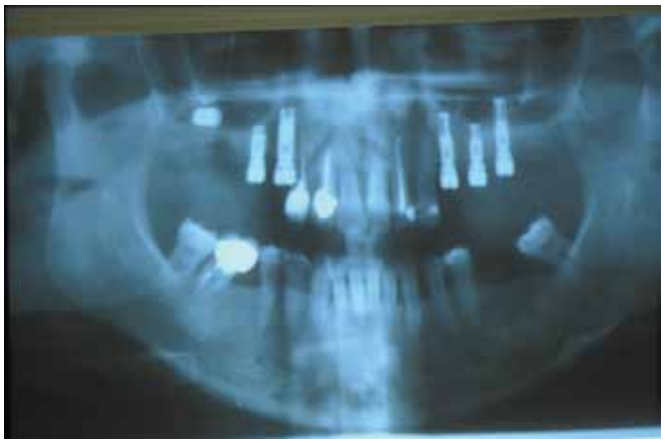
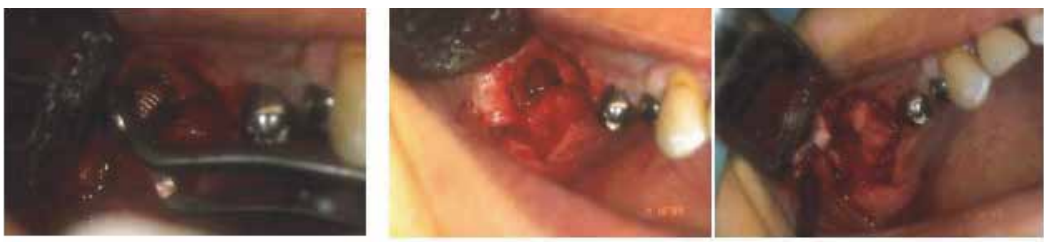


Fig. 4. Implant displaced within the course of a second stage surgery.

In general, implant displacements occur sometimes after an implant placement because there is an absence or loss of osseointegration and, therefore, of stability (low osseous density around it). Nevertheless, some authors report the displacement of implants into the maxillary sinus during their installation (Chappuis et al., 2009; Felisati et al., 2007; Haben et al., 2003). When this happens, the implant remains, in almost all the cases, lodged inside the sinus (Chappuis et al, 2009; Borgonovo et al, 2010), and can be removed a few days later by opening the lateral wall of the maxillary sinus (Haben et al., 2003) (Fig. 5), or by endoscopic via through a nasal window; a process that allows a considerable good access to the zone and a lower postoperative morbidity than when it is performed intraorally (Haben et al., 2003; Felisati et al., 2007; Galindo et al., 2005; Ramotar et al., 2010).



a) (b) (c)

Fig. 5. (a) Implant retrieval through a lateral window. (b) Oro-antral communication. (c) Membrane covering the window.

In these intra- or postoperative cases, authors recommend the immediate removal of the implant lodged in the maxillary sinus (Ramotar et al., 2010; Galindo et al., 2005; Felisati et al., 2007; Haben et al., 2003) through an intraoral or trans-nasal via that will prevent further associated complications; although it is in general asymptomatic condition, most patients suffer from a marked sinusitis. Another complication, in addition to the acute or chronic sinusitis, is the implant intrusion that occurs when it is not removed during its migration Felisati et al. and Haben et al. (Felisati et al., 2007; Haben et al., 2003) reported two cases of intrusions in which they found that two fixations had displaced from the maxillary sinus into the sphenoidal and ethmoidal sinuses, respectively, when they tried to remove them; the solution, though, should be to use an endoscopic nasal via. There is also a particular case describing a migration that ended in the orbital floor (Griffa et al., 2010) in its way from the maxillary sinus, and after an attempt to install a fixture in the alveolar bone. The article describes how the authors tried to aspirate the implant, which had penetrated deep into the zone and perforated the thin cortical bone of the orbital floor to end up lodged between the bone and the inferior rectus orbital muscle causing pain and diplopia. It was finally removed with general anesthesia and nasal endoscopy (Fig. 6).

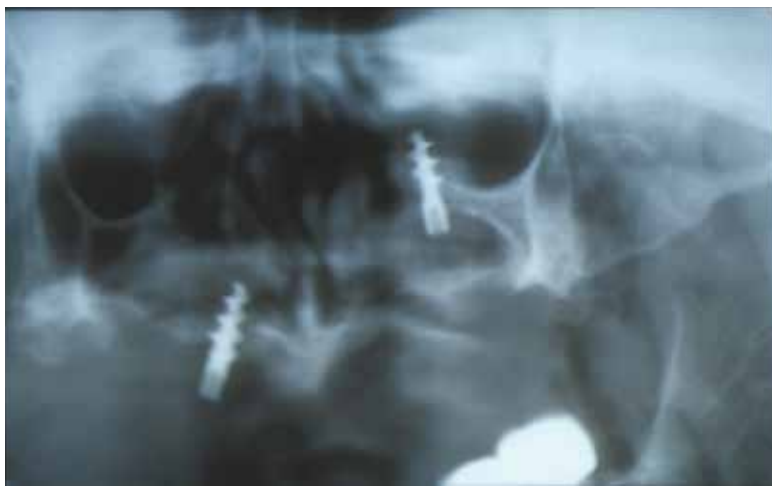
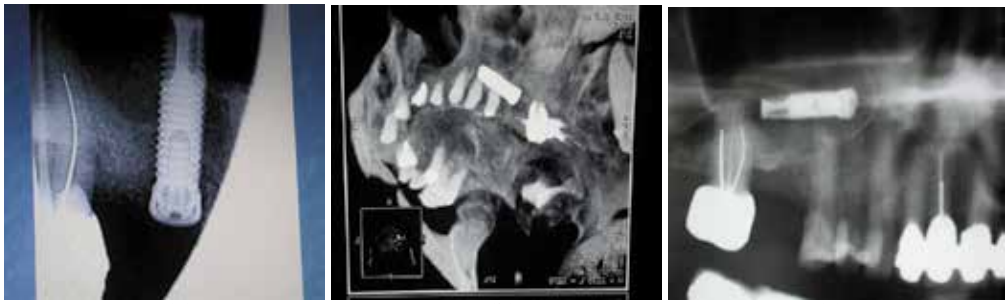


Fig. 6. Image of an implant migrated to the orbital floor.

To conclude, the positioning of an implant into the sinus is an infrequent event that does rarely occur during the placement of dental fixtures, or in the second phase. It is usually detected in the postoperative period and is in general ascribed to a lack of primary stability of the implant (a poor bone quality) (fig. 7). This complication could be prevented with an accurate surgical technique that would include using osteotomes to prepare the implant beds or a drill with a smaller diameter to that of the fixture, or using implants with a conical compressive form. If this complication finally occurs, it must be solved immediately and carefully in order to avoid any migration of this foreign body to a more harmful anatomical space.



(a) (b) (c)

Fig. 7. (a) Implant installed (Summer's technique). (b) Control CT Scan after displacement and before second stage surgery. (c) Change of position.

Finally, it is worth mentioning a case report describing the migration of a zygomatic implant to the cranial fossa. This is a major complication that can end up with a cerebral lesion or an infection that must be prevented with a preoperative and postoperative three-dimensional radiographic study (Reychler & Olszewski, 2010).
There are no publications about cases where the cortical nasal floor ended up perforated and the implant has subsequent migrated to this cavity. The fact of anchoring the fixture bicortically does not constitute any risk as long as the mucosa is preserved, whereas if it became perforated it would then lead to an infection complication because it would enable a direct contact with a septic cavity.

2.8 Mandibular fracture

Mandibular fracture, during implant placement, is associated with atrophic mandibles. The central area of the mandible has a greater risk for this complication because it has a poor vascular irrigation, which sometimes makes very difficult for the bone and periosteum to provide enough blood for the healing process derived from an implant placement (Chrcanovic & Custodio, 2009). The bone in this area is usually sclerotic and undergoes severe resorption as a consequence of a large period of edentulism and also as a result of the pressure exerted by the prosthesis, which does accelerate the process. Technically, it is difficult to perform implant perforations in this zone without running the risk of overheating the surrounding bone; moreover, sometimes surgeons resort to bi-corticalized implants in this area, which increases the risk of a mandibular fracture, so patients should be informed before starting this surgical procedure (fig. 8).

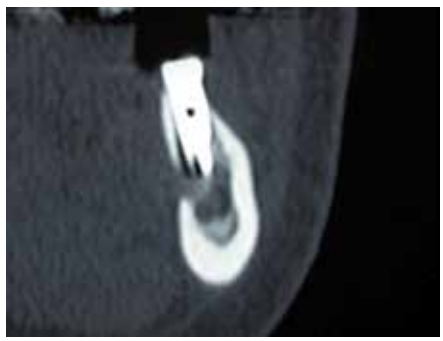
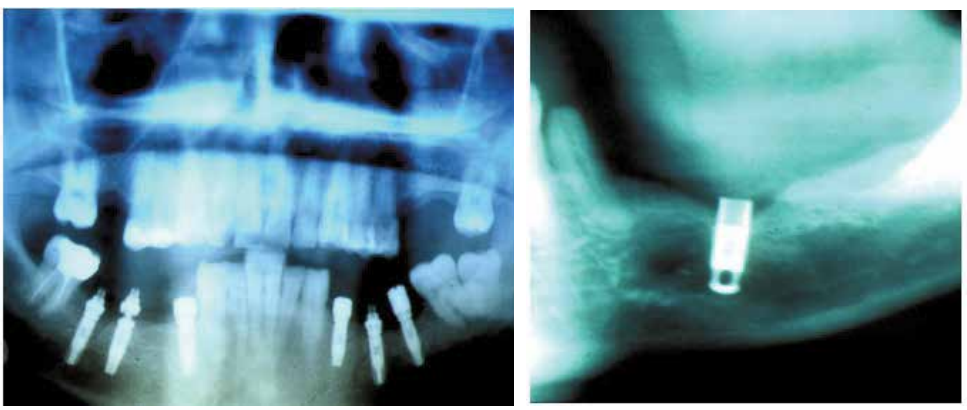


Fig. 8. Perforation of the lingual cortical during drilling.

It is an infrequent complication that could start during surgery and subsequently evolve to a serious complication if, as Oh et al. report (Oh et al., 2010), the implant causes an infection when it becomes displaced from the fracture line to a sub-mandibular space.
The main treatment consists in the reduction and stabilization of the fracture with titanium miniplates (Oh et al., 2010; Tiwana et al., 2009), or resorbable miniplates. Nevertheless, other authors report less atraumatic techniques such as splinting implants with a Dolder bar to reduce and immobilize the fracture (Romanos, 2009).
Chrcanovic and Custodio (Chrcanovic & Custodio, 2009) suggest, in order to prevent this complication in the management of thin mandibular alveolar crests, to increase width by performing bone grafts beforehand and through an accurate tomography imaging study to calculate these parameters with precision.
A case of a fracture and displacement of part of the lingual mandible cortical was reported, as a result of withdrawing a mono-cortical bone graft from the chin. The surgical procurement of a mono-cortical bone graft from the chin resulted in the fracture and displacement of part of the lingual mandibular cortical bone (Cordaro et al., 2004); it was decided not to perform any re-intervention due the lack of patient symptomatology.

2.9 Neurosensory impairment

Nerve lesions are both an intraoperative accident and a postoperative complication that can affect the infra-orbitary nerve, the inferior alveolar nerve, or its menton branch and the lingual nerve. Neurosensory impairment may occur at any time during implant surgery, including anesthesia administration, incision, raising a flap, as well separating it too tightly, during osteotomy preparation, bone augmentation, implant placement, suturing or any soft tissue swelling after surgery (Misch & Resnik, 2010) (Fig. 9). These complications have a low incidence (reported between 0%-44%) (Misch & Resnik, 2010). Symptomatology is of a large variety and depends on the severity of the axonal damage.



a) (b)

Fig. 9. (a, b). Several implants in contact to the Inferior Alveolar nerve in patients with postoperative paresthesia.

The complication could manifest as a paresthesia when the lesion is due to a nerve compression, or a minor stiffening of nerve fibers, without sectioning any of them (neuropraxia). Dysesthesia may occur in cases of nerve compression, traction, partial crush

or stretching (axonotmesis) of nerve fibers with different intensities. Hypoesthesia or hyperesthesia may be caused by extreme stretching, complete crush and direct trauma on nerve fibers (neurotmesis); anesthesia and pain appear as consequence of a complete nerve section.

A very useful review about lesions and treatment algorithms may be found in a report published by Misch and Resnik (Misch & Resnik, 2010). Treatments depend on the type of injury. The implant should be removed as soon as possible to avoid compression when radiological imaging reveals there is too much proximity between the implant and a nerve. Treatment with corticosteroids and non-steroidal anti-inflammatory drugs are indicated to control inflammatory reactions that provoke nervous compression. It seems that the topical application of dexamethasone (4 mg/ml) for 1 or 2 minutes enhances recovery, and when it is administered orally and in high doses within one week of injury, it has have shown to inhibit axon sprouting centrally and ectopic discharges from injured axons, and prevention of neuroma formation (Misch & Resnik, 2010).

An intraoperative nerve section requires microsurgery techniques to reestablish nerve continuity. Nerves can be partially or completely damaged, and this aspect determines the prognostic of recovering the sensibility completely. Clinically, the evaluation of the neurosensorial loss should be checked at different moments to determine with precision the evolution of the lesion and, if necessary, refer the patient to a neurosurgery specialist (Misch & Resnik, 2010; Greenstein et al., 2008). Hegedus et al. (Hegedus et al., 2006) propose an evaluation of the subjective symptoms and carry out an exhaustive neurosensory exploration of the patient's affected area to document all the written data referring this complication and thus evaluate patient's evolution. It is recommendable to resort to microsurgery if, after four months, patient's situation has not improved, pain persists and there is a remarkable loss of sensitivity.

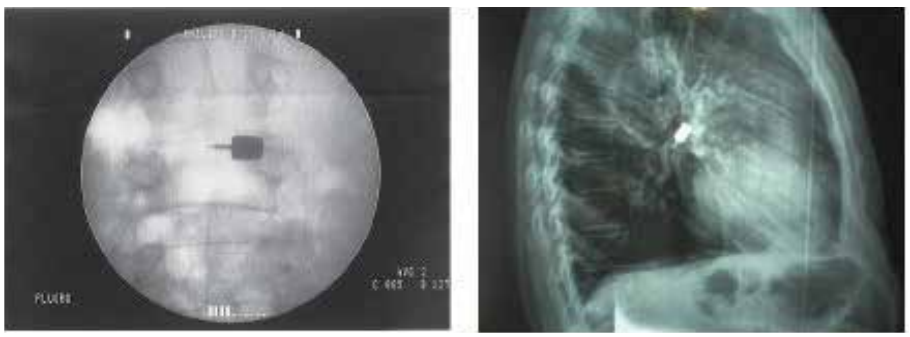
Another nervous lesion could manifest in the form of an idiopathic facial pain, as the case reported by Queral-Godoy et al. (Queral-Godoy et al., 2006), on a lesion subsequent to the placement of two implants in the anterior region of a mandible. The prevalence in implantology is unknown, although it is estimated to be between 3-6% of all the endodontic cases. The treatment suggested by these authors should basically comprise the administration of tricyclic antidepressant drugs, amitriptyline and benzodiazepines on a chronic basis. Rodriguez-Lozano et al. (Rodriguez-Lozano et al., 2010) describe another peculiar case of neuropathic pain that showed up eight months later after the surgical placement of eight dental fixtures.

Nahlieli et al (Nahlieli et al., 2011) propose to use a mini-endoscopic device to check the bottom of the surgical bed to detect any possible osseous defect; this technique could be interesting to prevent nervous lesions.

2.10 Aspiration and swallowing of instruments

When an accident involves the aspiration (Pingarron et al., 2010) or swallowing (Welcker et al., 2005; Worthington,. 1996) of an instrument (burs, screwdrivers, covering screws or healing abutments), it can become a vital emergency if the instrument has entered the airways. If the object has not been discharged from the respiratory track, it should then be necessary to perform a bronchoscopy. A special case describes the need to even perform a tracheotomy in a similar situation; the patient subsequently suffered a pneumothorax and a pleural effusion. Authors recommend general anesthesia in those cases in which there is the suspicion of any difficulty in the course of the procedure.

For daily surgeries, it is strongly recommended to tie all tiny and slippery instruments regularly used during the procedure with silk ligatures so as to quickly recover them if they fall from the operator's hands, or else use a rubber dam (Bergermann et al., 1992).
In the event an object entered the digestive system, it would require carrying out a series of post-operative controls with the help of radiological imaging. A rich diet in fiber will also help solve this problem sooner. A gastroscopy or colonoscopy could also be mandatory in those cases in which the object remains stationery in the intestinal tract, while a proper medical follow-up will help see any progress of the object, or objects, through the digestive tract (Fig. 10). This type of accidents can occur in any of the sequences of the implant treatment.



a) (b)

Fig. 10. (a) Images of a screw driver in the digestive tract. (b) Screw driver into pulmonary tissue.

3. Early and late complications

Early complications include: implant fracture, infection, edema, ecchymosis and hematoma, emphysema, bleeding, soft tissue dehiscence of the area and sensitive alterations. As to late complications we can distinguish: mucoperiosteal flap perforations, maxillary sinusitis, mandibular fractures, loss of osseointegration, periimplantitis (Annibali et al., 2009). Most of the complications described in this chapter, though, may occur early, or lately, or even be the consequence of a surgical accident or an iatrogenic act.

3.1 Edema

Swelling can appear after a surgical intervention, although it is more noticeable 24 hours after performing it. It can lead to trismus, lack of hygiene in the wound and discomfort to the patient. It normally decreases with time, and can easily vanish after a few days. Wide flaps, bone regenerating techniques, and surgery time are factors that trigger the occurrence of edemas and patient's susceptibility. The occurrence of edemas, and the need of analgesic and anti-inflammatory drugs is, in a statistically significant way, lower in guided flapless surgery, as can be seen in under-control studies (Arisan et al., 2010; Cannizzaro et ala., 2008), even in immediate implants after exodontic procedures (Cannizzaro et al., 2007) and immediately loaded implants (Cannizzaro et al., 2007; Cannizzaro et al., 2008; Merli et al., 2008; Arisan et al., 2010); however, a careful management of tissues, using non-excessive tension and bone-supported retrievers can minimize this effect along with the use of a cold pack, and non-steroid anti-inflammatories drugs; although in the event of a complex post-

op, the administration of corticosteroids during a short period of time can be very helpful. Before the suspicion that the inflammation could compress any nervous structure, corticosteroids are crucial to minimize the risk of lesions (Misch & Resnik, 2010).

3.2 Bleeding, ecchymosis and hematoma

Low-intensity bleeding is a frequent complication that must be solved as soon as possible, and it generally occurs on the basis of the surgical extension of the surgical procedure and the general condition of the patient. We recommend suturing all surgical wounds and compressing the zone with gauzes, as well as apply a cold pack, have physical rest, and the intake of a soft and cold diet to prevent an excessive bleeding after surgery. Nevertheless, the best method to avoid this problem is to write up a complete medical report from data provided by the patient's physician to collect any information on blood disorders. Sometimes we have to request a complete hemogram with coagulation tests to make clear the situation of a specific patient.

There are three main anticoagulants: coumarin, heparin and aspirin. Usually, they are prescribed to treat a number of cardiac or vascular disorders that include atrial fibrillation, ischemic cardiac disease, cardiac valvular disease, prosthetic cardiac valves, post-MI, deep venous thrombosis, pulmonary embolism, cerebrovascular accident, and many other disorders. Therefore, antiaggregant medication could be discontinued if the physician thinks it is appropriate to do so or even continue with the medication if the physician thinks that bleeding could be handled with local hemostatic measures, as in the case when the patient is taking aspirins.

Hematomas and ecchymosis are the result of a surgery procedure and are usually proportional to the magnitude of the intervention. Blood collected under the mucosa and skin are known as bruises and generally cause a feeling of esthetical discomfort to patients, but in some days reabsorption occurs and solves the disgusting aspect of the zone and surroundings (because coagulated blood frequently moves through the anatomy in a descendent trajectory)(fig. 11).

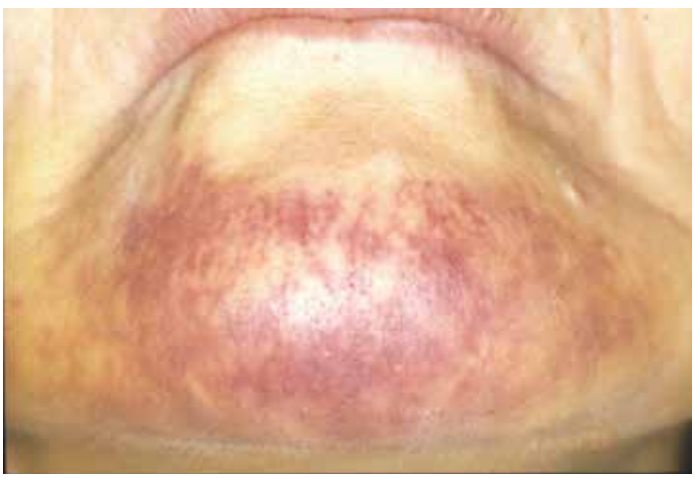


Fig. 11. Ecchymosis are the result of a intermental surgery procedure.

3.3 Emphysema

Tissue emphysema is one of these early complications caused by inadvertent insufflation propulsion of air into tissues under skin or mucous membranes, air from a high-speed handpiece, an air/water syringe, an air polishing unit or an air abrasive device can be projected into a sulcus, surgical wound, or a laceration in the mouth (Liebenberg & Crawford, 1997); it is a rare complication, though it can lead to severe consequences (McKenzie & Rosenberg, 2009). The use of abrasive air in implantology, or a laser device as a way to remove residues from an implant surface, has been suggested in the treatment of periimplantitis to facilitate decontamination. The chance for the occurrence of emphysema process during this cleansing maneuver is higher than that of the surgical process since the device uses mechanical motors. This complication occurs when pressurized-air devises are used to remove periodontal and peri-implantation calculi in the maintenance period, as Bergendal et al. (Bergendal et al., 1990) report in a case concerning an acute subcutaneous emphysema.

The air can follow facial planes and create a unilateral enlargement of the fascial and/or sub-mandibular regions. The region with a swelling does normally produce a crackling sensation (crepitation on palpation) as the gas is pushed through the tissue. The crackling sound is pathognomonic of tissue emphysemas, even when discomfort is not reported.

These complications could be avoided by using a sterile water jet handpiece with retrograde air insufflation when, besides an associated implantology surgical process, it is necessary to dry a surface (periodontal treatment, laser, shutting, periapical surgery on an adjacent tooth). The use of air abrasives directed on the sulcus should be avoided as well as its use on inflamed tissues with friable margins, so manual curettage is the best option.

Treatment usually consists on prescribing antibiotics and a mild analgesic therapy, close observation, saline rinses, light massage, apply heat packs and provide mental comfort to patients. The problem does normally solve in 3-10 days.

3.4 Mucosal dehiscence

This is a soft tissue complication that can develop infections in the surgical area and implant and/or graft failures that can lead to unfortunate esthetic results. As a rule, surgical wound dehiscences are associated with patients that have scarring problems due to a poor-quality mucosa (thin biotype, traumatized or cicatricial type), heavy smokers, patients treated with corticosteroids, diabetics, or irradiated patients (Lee & Thiele, 2010) (fig. 12). Another factor leading to surgical wound dehiscence is flap closure under tension, for it has been established that a higher tension causes a more frequent onset of these complications (Lee & Thiele, 2010).

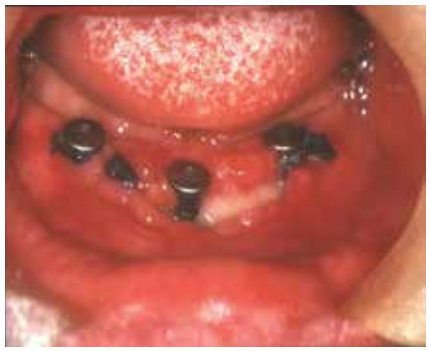


Fig. 12. Wound dehiscence at one week post surgery in a diabetic patient with oral candidiasis.

Many different solutions have been suggested to prevent dehiscence, in addition to flap management, to guarantee the success of bone regeneration (Park & Wang, 2007)(fig. 13). A study by Torres et al. established the efficacy of using platelet-rich plasma (PRP) on titanium meshes to prevent the occurrence dehiscences in all the cases described (Torres et al., 2010).

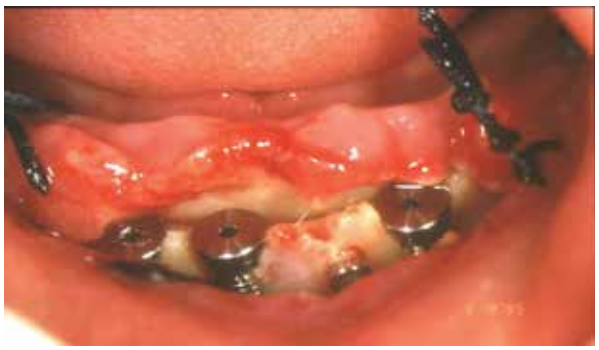


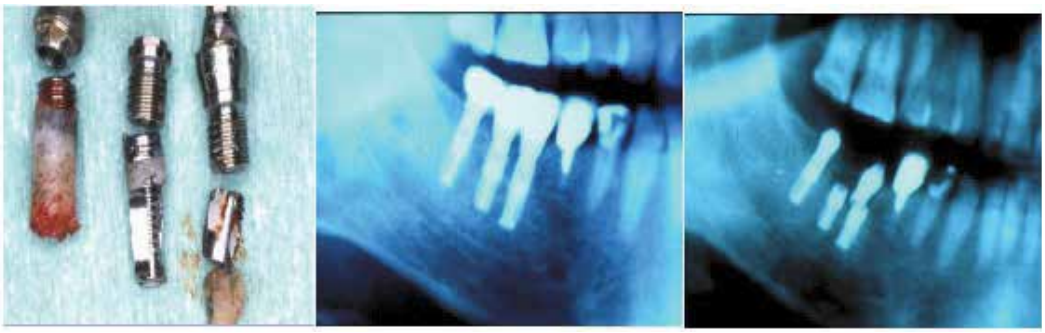
Fig. 13. Wound dehiscence in a case of bone regeneration with collagen membrane (one week control).

The use of free connective tissue grafts may be highly useful for both guaranteeing the closure of the wound and the enlargement of the mucosa thickness around implants, all of which allows better esthetical results in the long run as well as the maintenance of periimplant health (Speroni et al., 2010; Stimmelmayr et al., 2010). El Chaar describes the good results observed in the use of palatal pedicle connective tissue grafts to facilitate the primary closure of a post-exodontic alveolus (El Chaar, 2010), or an acellular dermal matrix interposed between the lap and the subjacent bone (Taylor et al., 2010; Park, 2006).
The use of dense politetraphluoroethilene (d-PTFE) membranes has been suggested to cover the regeneration zones; the benefit of this material resides in that it does not need to be completely covered by the flap since its internal surface does not become contaminated, which is an advantage over other type of membranes (Barber et al., 2007).
A mucosal dehiscence associated with a sinus lift technique represents an aggravating factor that can lead to an infection of the graft material, an acute sinusitis, or an oroantral fistula (Watzak et al., 2005). The management of these abnormal tracts is, depending on the size, laborious and requires well-designed flaps. The use of mono-cortical grafts of intraoral origin (Watzak et al., 2005) or PRP associated with a mucogingival plastia has also been suggested (DePoi et al., 2007).

3.5 Implant fracture

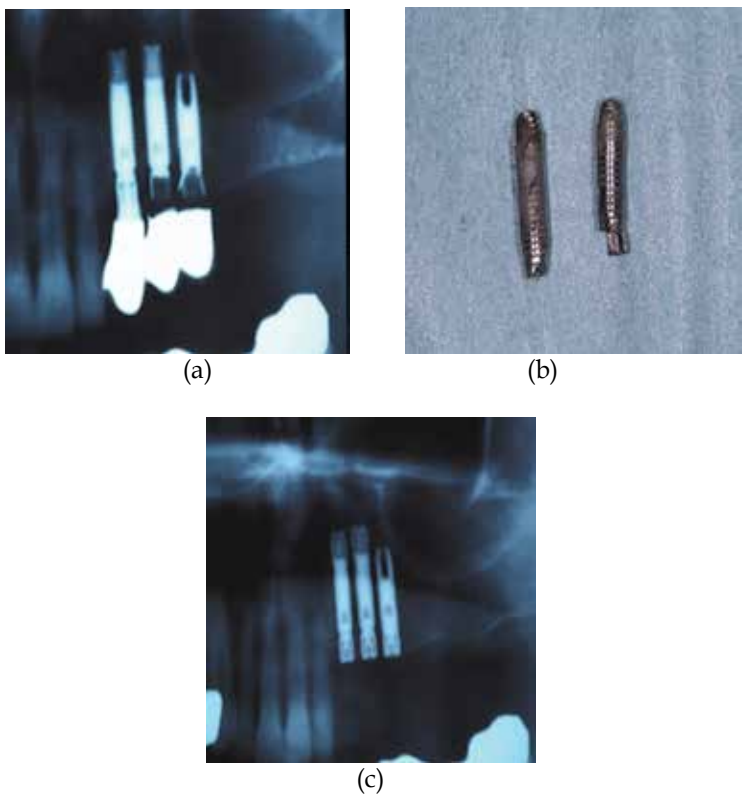
Fracture of prosthetic retaining screws is more common than implant fracture and it is normally due to a metal fatigue following an overload of materials (Al Jabbari et al, 2008). An implant fracture seems to be an infrequent complication (among 0,2 y 1,5% of cases) (Eckert et al., 2000) that could be ascribed to different reasons: defects in the implant design or materials used in their construction, a non-passive union between the implant and the prosthesis or by mechanical overload, specially cantilevers in fixed prostheses, occlusal overload or/ and parafunctional habits (Al Quran et al., 2009; Mendonca et al., 2009) (fig. 14). The incidence of these complications is higher in implants supporting fixed partial prosthesis than in complete edentulous patients.

This complication is frequently managed by the removal of the implant and its replacement by another one (Cardoso Lde et al., 2010; Gargallo-Albiol et al., 2008) (fig. 15); nevertheless, some cases describe the use of the remnant part of the dental fixture to rebuild a new prosthesis when is possible (Mendonca et al., 2009).



(a) (b) (c)

Fig. 14. (a) Implants retrieved in molar region in a patient with parafunctional habit. (b) Implant rehabilitation). (c) Implants fractured.



(a) (b)

(c)

Fig. 15. (a) Implant fractured in maxillary posterior region. (b) Implants retrieved. (c) Substitution for a wider diameter in the same surgery.

More than 80% of factures are located in the molar and premolar regions, and most of them occur 3-4 years after being loaded (Kohal et al., 2010; Gargallo-Albiol et al., 2008) (fig. 16).

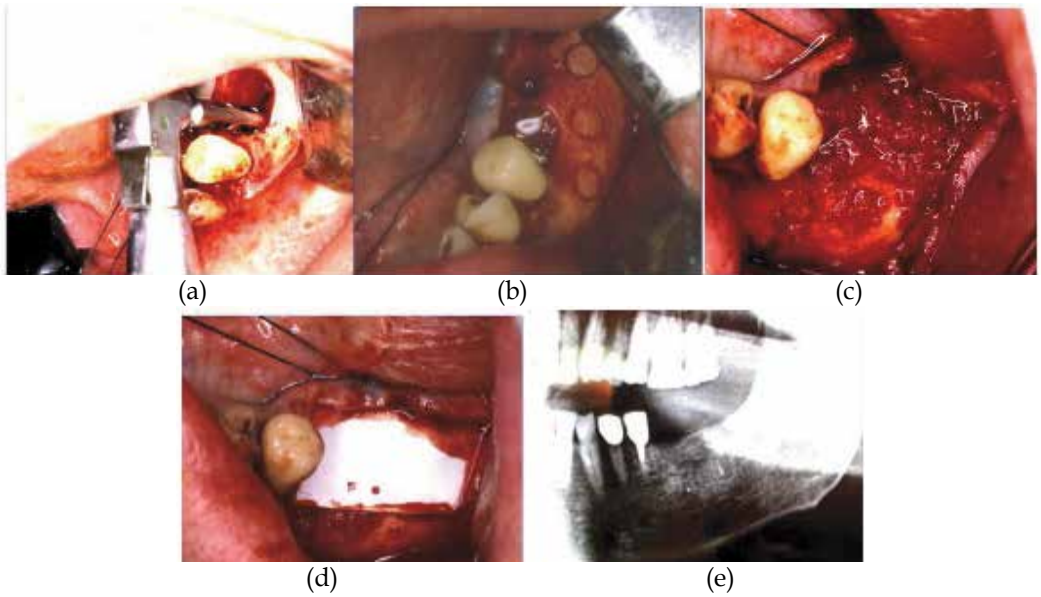


Fig. 16. Sequence of treatment of an implant fractured. (a) Retrieval with a trephine. (b) Bone harvesting for osseous regeneration. (c) Filling of the osseous defect. (d) Collagen membrane. (e) Image of the control 6 months later.

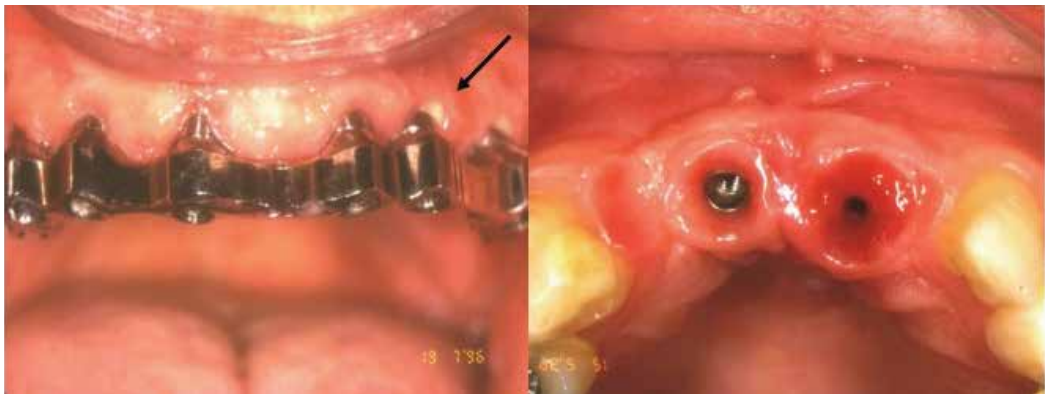
3.6 Infection

Infection of the implant is a common cause of failure but the efficacy of prophylactic antibiotics for dental implant placement seems not influence in risk of implant failure and other complications (Esposito et al., 2010a; 180 Esposito et al., 2010b; Anitua et al., 2009).

Microorganisms found in a periimplantitis are very similar to those found in chronic periodontitis, and the most outstanding are as follows: anaerobic Gram negative bacillus (Porphyromonas gingivalis and Prevotella intermedia), anaerobic Gram negative coccus (Veillonella spp) and the spiroquettes, such as Treponema denticola (Sanchez-Garces & Gay-Escoda, 2004). Nowadays, antibiotics are not accepted as the main treatment for periimplantitis, as it is considered a disease with a bad prognosis and predictability that formerly required implant removal (Pye et al., 2009) (fig. 17). This condition requires, on account of its importance and frequency, it own a chapter, so it will not be extensively developed here.

A recent complication detected in the last years is the use of oral bisphosphonates. This medication is broadly used to treat osteoporosis, bones diseases, such as Paget's disease, and osseous metastasis in malign neoplasias. The most common presentations of oral bisphosphonates are Alendronate and Risedronate. On the one hand, they are mainly used for treating osteoporosis and, whereas they curiously are helpful for the long-term stability of the implant, they generate a higher amount of quality bone around the implant surface. This has already been shown in several clinical trials, although a larger amount of patients is

needed to assure these results (Aspenberg, 2009). In other hand Zahid et al. observed in retrospective radiographic study those patients who take BP are at greater risk of implant failure or thread exposure (Zahid et al., 2010).



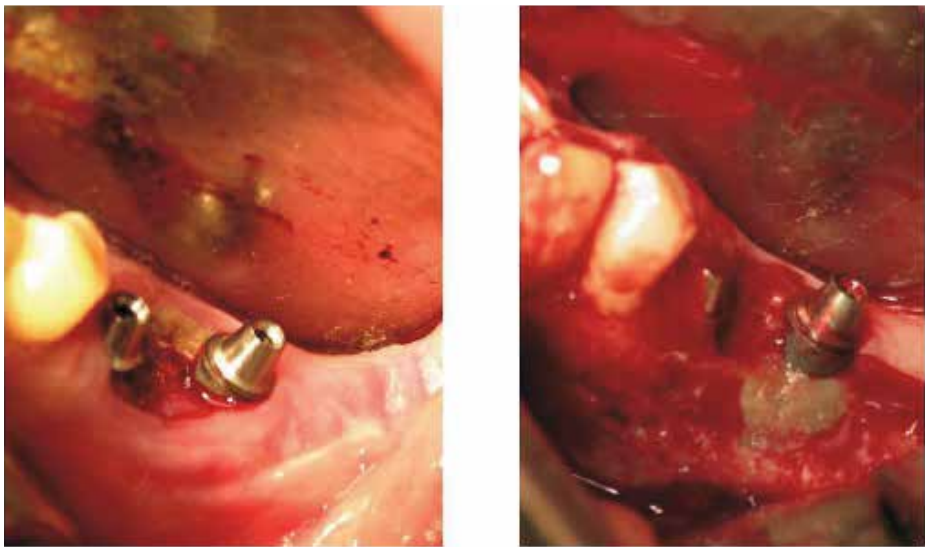
a) (b)

Fig. 17. (a) Image of periimplantitis and suppuration (arrow). (b) Image of mucositis.

On the other hand, this benefit soon disappears because these drugs can cause osteochemonecrosis of the jaws during the postoperative healing period once the implant has been placed. It has been shown that the length of the medication period is related to the onset and severity of this complication, although more investigation is needed to understand the exact mechanism, while a worldwide consensus is also necessary to prevent the risk posed by osteochemonecrosis after a surgical implant placement. The use of a biochemical marker of bone metabolism as CTX could help to predict the risk in some cases (Lee & Suzuki, 2009). An article on seven osteochemonecrosis cases treated with low and medium doses of bisphosphonates (oral and venous administration) concluded that it is necessary a strict treatment protocol to obtain good results. The treatment suggested consists in a deep surgical debridement of all the necrotic tissues before performing a regularization procedure on the edge of the bone crest, as well as a primary closure of the wound, avoiding dead spaces (fig. 18). An intravenous antimicrobial therapy should be established for a week to be followed by the oral intake of the drug for another three weeks (Alons et al., 2009). There are at present ten cases in the literature on the use of oral bisphosphonates that caused osteonecrosis. Symptomatology is not well defined at the early stages of this condition, so gammagraphy, using Technetium-99 methylene diphosphonate, ((99)Tc(m)-MDP) could be helpful to diagnose it. In those patients treated with bisphosphonates, controls must be frequent and rigorous and periimplantary hygiene must be carefully performed.

Osteomyelitis (OM) is another scarcely frequent osseous complication of the maxillae. OM is currently related to maxillary bone infections mainly caused by: odontogenic primary infections, after surgical oral procedures (removal of tooth and implant placement); when they are secondary to osteoradionecrosis, or any other osteochemonecrosis processes, or in a postoperative/posttraumatic process.

A retrospective study on 46 patients with OM of the mandible assessed the type of antimicrobial treatments used and the level of resistance against them. Streptococcus viridans



a) (b)

Fig. 18. (a) Osteonecrosis in a patient treated with biphosphonates i.v. clinical image. (b) Surgical debridement.

was the most frequent pathogen, 81% were sensible to penicillin and 96% to fluoroquinolone; however, only 11.5% were sensitive to clindamycin. Therefore, it was suggested that the drug of choice against maxillae OM should be antimicrobials of the beta-lactam family. As to patients allergic to penicillin, the best option is the combination of fluoroquinolones and rifampicin, or clindamycin, for a better anaerobic coverage (Pigrau et al., 2009).

4. Conclusions

The vast majority of complications in implant surgery can be prevented by correctly selecting patients and treating difficult cases in the most adequate way, while knowing the risks, trying to avoid them with the necessary information and having carefully devised a specific plan for every patient.

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Part 4

Computer-Aided Implant Dentistry and Imaging

18

Virtual Planning for Dental Implant Placement Using Guided Surgery

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1. Introduction

Technical and scientific advances in diagnostic and therapeutic methods in the biomedical field have substantially increased the predictability and longevity of the outcomes of restorative surgical procedures. In contemporary oral implantology, bioprototyping techniques based on computer-aided design and computer-aided manufacturing (CAD/CAM) technology have proven to be valuable aids in diagnosis, treatment planning, and surgical intervention. Among other aspects, use of these technologies improves preoperative planning, makes it easier for patients to understand proposed procedures, increases the predictability of treatment, and may reduce surgical morbidity.

The preliminary stages that precede dental implant surgery are as important as the actual operative intervention itself, or even more so. Inadequate placement of implants may injure important anatomical structures or lead to aesthetic or functional compromise of the prosthetic stage of oral rehabilitation.

Implant planning and placement can be based solely on computed tomography data and surgical guides fashioned after diagnostic models. However, direct three-dimensional visualization of the surgical site and real-model simulation of the intended surgical procedure can play a significant role in obtaining outcomes that are more consistent with the original treatment plan. Biomedical prototypes, or biomodels, provide faithful reproductions of the patient's bone condition, thus enabling this type of assessment.

Likewise, the surgery itself can be made safer and less invasive with use of individualized surgical guides for implant placement. The development of software programs for implant placement planning and the integration of these software packages with rapid prototyping methods have made fabrication of such guides possible.

The process of obtaining and reformatting computed tomography (CT) data, planning implant placement over a virtual model, fabricating a surgical guide with prototyping methods, and using this guide or template during dental implant surgery with specifically designed systems may be referred to as guided implant surgery.

2. Principles of guided surgery

Guided implant surgery is based on the combination of virtual surgical planning and prototyping techniques. Rapid prototyping can be defined as a set of technology-based

processes that enable fabrication of three-dimensional, concrete objects from a computer-aided design (CAD) project. The objective of rapid prototyping is to create a real model with the same geometric features of the virtual model, which can then undergo real-world manipulation for a variety of purposes (James et al., 1998; Peltola et al., 2008).

The greatest challenges in the surgical and prosthetic stages of dental implant surgery are due to diagnostic failure and poor case planning (Lal et al., 2006).

The American Academy of Oral and Maxillofacial Radiology (AAOMR) recommends that any evaluation of the available bony architecture for diagnosis and planning of extensive implant-based oral rehabilitation include CT imaging (Almog et al., 2006).

In this context, Jacobs et al. (1999) assessed the reliability of two-dimensional CT images for implant placement planning. The Jacobs study was performed on 100 partially or fully edentulous patients who underwent CT scanning and subsequent implant placement. Available bone height was measured below the maxillary sinus and nasal fossa and above the mandibular canal, and a 1.5 mm safety margin was respected. Planning took into account implant number, location, and size, available bone height, and possible anatomic complications, such as bony dehiscences and fenestrations, maxillary sinus perforation, absence of primary stability, and poor implant positioning in light of the required biomechanical and cosmetic outcome. The authors found that, in some cases, placement of implants according to plan was impossible, mostly due to intraoperative findings that had been missed or underestimated on CT images. Agreement between planned and intraoperative implant number and sites was satisfactory. Of the 416 planned implants, 395 were successfully placed. However, the authors found weak agreement between the size of the planned implant and that of the implant actually placed (44%). Implants were shorter or longer than planned in 110 and 74 of the 395 successful cases respectively.

According to the authors, CT images can be used for preoperative planning of the number of implants, as well as for choice of implant sites. However, CT scans are not sufficiently reliable for precise determination of implant size or for prevention of intraoperative anatomic complications, and can thus reduce the predictability of the implant placement procedure (Jacobs et al., 1999).

Thus, in light of the need for implementation of available treatment planning resources in implantology, virtual planning and rapid prototyping techniques have come to provide an excellent choice in the search for satisfactory outcomes with lower intra- and postoperative complication rates (Choi et al., 2002; Almog et al., 2006).

Biomedical prototypes and virtual evaluation provide the ability to visualize and manipulate the surgical sites, measure the patient's bony structures, and simulate the surgical intervention itself. These preoperative procedures increase predictability of the chosen technique, improve applicability of the surgical plan, and can shorten operative time (Choi et al., 2002; Sarment et al., 2003a; Sarment et al., 2003b; Meurer et al., 2008).

Erickson et al. (1999) surveyed the opinions of oral and maxillofacial surgeons about the use of stereolithographic models for diagnosis, treatment planning, preoperative surgical simulation, and construction of alloplastic implants for anatomical reconstruction. Seventy-six models were constructed in the study period. Prototypes were used by 69% of respondents as a diagnostic aid during the planning phase, and played a role in treatment planning in 92% of cases. Seventy-three percent of respondents used the models to instruct patients as to the planned procedure. Preoperative surgical simulation was employed in 38% of cases. Seventy-seven percent of surgeons reported reductions in operative time when

prototypes were used, 38% reported improvement in surgical access, and 46% noted that more complex procedures were made safer and more predictable by preoperative study of prototype models. Despite the wide applicability of biomodels (in 96% of treatment phases) only 15% of surgeons that responded to the survey believed that rapid prototyping had played an essential role in proper treatment. Nevertheless, the authors stressed the validity of prototypes as a tool for obtaining more predictable and lasting results.

In a similar study, Erben et al. (2002) interviewed 38 oral and maxillofacial surgeons to determine the main indications for biomodels and the key benefits provided by their use in preoperative planning. Models were used in planning placement of osseointegrated implants and other procedures. Surgeons noted that use of biomedical prototypes improved the quality of diagnosis, enabled preoperative simulation of planned interventions, made it easier for patients to understand the procedure and provided intraoperative guidance.

Wulf et al. (2003) published a study conducted between September 1999 and April 2002 in order to assess the relevance of biomedical prototypes in the preoperative, intraoperative, and postoperative stages of maxillofacial surgery. Fifty-four surgeons described their experiences on a total of 466 cases. This study corroborated the advantages of biomodel use described in the aforementioned investigations.

In dental implantology, bioprototyping techniques are not limited to model construction and analysis. With the advent of sophisticated data acquisition methods and image manipulation and virtual planning software, fabrication of rapid-prototyped surgical guides and templates have made it possible to achieve more reliable agreement between the planned and final position of dental implants. This improved predictability of implant and prosthesis positions is based on the concepts of guided surgery (Garg, 2006; Rosenfeld et al., 2006).

3. Guided surgery in dental implantology

Fabrication of a surgical guide that faithfully reproduces planned implant positions can play an essential role in the success of implant-based oral rehabilitation (Ganz, 2003).

Surgical templates fashioned using conventional methods have some drawbacks that can make successful aesthetic and functional outcomes more difficult to achieve. The diagnostic models on which these guides are based provide a rigid, non-functional representation of the soft tissues that cover the alveolar ridge, which prevents visualization of the underlying bone anatomy of the region of interest and consequently makes it impossible to decide on a definitive orientation for implants (Lal et al., 2006).

Guided surgery can be used in rehabilitation of fully or partially edentulous patients, or even for single teeth. Likewise, guides can be mucosa-supported (tissue-borne), tooth-supported (tooth-borne), or bone-supported (bone-borne).

In guided surgery for rehabilitation of fully edentulous arches, certain steps must be followed prior to implant position planning:

a. placement of diagnostic models on a semi-adjustable articulator;
b. diagnostic wax-up;
c. duplicate of wax-up for fabrication of acrylic-resin CT guide;
d. placement of at least six 1.5 to 2 mm-diameter holes on the vestibular region of the guide and filling with radiopaque material;

e. interocclusal registration with addition or condensation silicone for guidance during CT scanning;
f. occlusal CT view of guide and interocclusal record;
g. isolated scan of guide (for flapless procedures) (Parel & Triplett, 2004; Balshi et al., 2006; Marchack, 2007; Sanna et al., 2007; Van Assche et al., 2007).

CT slices acquired in the DICOM file format must be converted with the aid of a specific software package prior to manipulation and reformatting into 3D images. During this process, the isolated radiographic guide scan is superimposed onto its occlusal view, using the radiopaque points as landmarks (Parel & Triplett, 2004; Balshi et al., 2006; Marchack, 2007; Sanna et al., 2007; Van Assche et al., 2007). The threshold for the case is also defined during this stage (Souza et al., 2003).

Files obtained during this step can then be opened in implant position planning software. These applications provide axial, panoramic, and three-dimensional reconstructions of CT slices (Marchack, 2007; Sanna et al., 2007; Van Assche et al., 2007). They also enable manipulation of the panoramic reconstruction curve on the axial plane, providing orthogonal slices for assessment of alveolar ridge thickness in the vestibulolingual or vestibulopalatal direction (Parel & Triplett, 2004; Sanna et al., 2007; Van Assche et al., 2007).

Virtual planning gives implantologists the ability to choose the precise location, orientation, and dimensions of implants, abutments and other fixtures. Implant emergence and the relationship of the implant to the future prosthesis can also be visualized and adjusted as convenient. In short, virtual planning can overcome the limitations of conventional surgical guides (Lal et al., 2006).

Several virtual planning and guided surgery systems are available on the market. Each has its own particularities that should be taken into account by the implantologist, according to therapeutic indication, cost-benefit ratio, and learning curve for clinical use.

The final stage of planning consists of defining the sites for placement of two or more anchor pins for the surgical guide, which are inserted through the vestibular aspect of the guide, between the implants (Balshi et al., 2006; Marchack, 2007; Sanna et al., 2007).

After planning is complete, files are sent to a medical prototyping facility, where they will be converted into an appropriate format for prototype fabrication (Parel & Triplett, 2004; Marchack, 2007; Sanna et al., 2007; Van Assche et al., 2007).

With implant positions established, a surgical guide is designed on the basis of the virtual model and sent to the prototyping station, where it is then fabricated (Lal et al., 2006).

Three types of surgical guides or templates can be manufactured with rapid prototyping methods to improve the predictability of osseointegrated implant-based oral rehabilitation. Bone-supported (or bone-borne) guides are placed after the mucoperiosteal flap has been raised. Their advantages include positional stability during implant placement and the possibility of direct visualization of anatomical structures. The need for broader surgical exploration, however, may be regarded as a drawback of this type of guide, which is usually reserved for partially or fully edentulous arches (Tardieu et al., 2003; Garg, 2006; Lal et al., 2006).

In mucosa-supported (or tissue-borne) surgical guides, templates, anchor pins are placed transmucosally. The drilling sequence for implant placement begins with a tissue punch or extractor, with the guide in place (Parel & Triplett, 2004; Marchack, 2007; Sanna et al., 2007). These guides are indicated in fully edentulous patients. Use of the double-scan technique, in

which two independent scans of the radiographic guide—individually and in occlusion—are obtained, is mandatory in these cases. The key advantage of this method is the possibility of performing allows minimally invasive flapless procedures, which make for an easier intraoperative and postoperative period (Garg, 2006).

Tooth-supported (or tooth-borne) surgical guides, as the name implies, are supported by the patient's remaining teeth. They are indicated in rehabilitation of single teeth or partially partially edentulous regions, and provide good outcomes when used for minimally invasive flapless surgery (Garg, 2006).

Once the prototyped guide is ready, implant placement may proceed as recommended for each system (Figures 1 and 2).

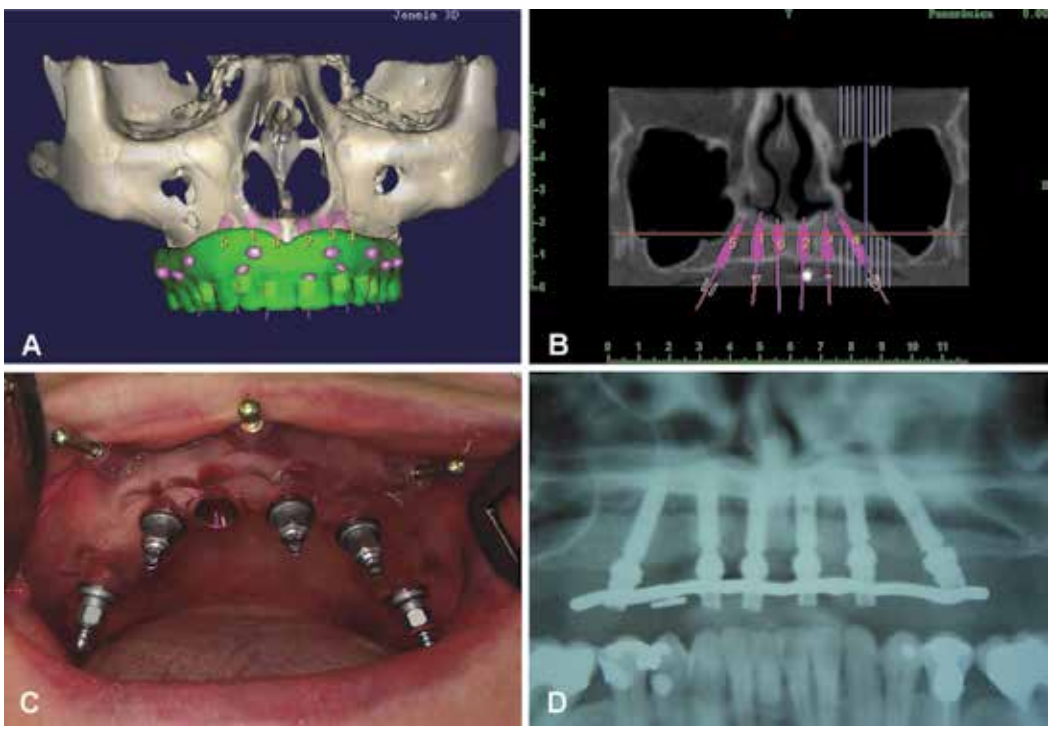

Fig. 1. Guided surgery for complete upper arch rehabilitation. a) Three-dimensional view of preoperative planning. Note radiopaque markers used to superimpose scans of the guide (isolated and in occlusion). Dentaslice® software package (Bioparts®, Brazil). b) Preoperative implant positioning. c) Five implants in place. Note insertion of anchor pins into stereolithographic guide. Neoguide® system (Neodent®, Brazil). d) Postoperative panoramic radiograph. Note similarity to virtual planning.

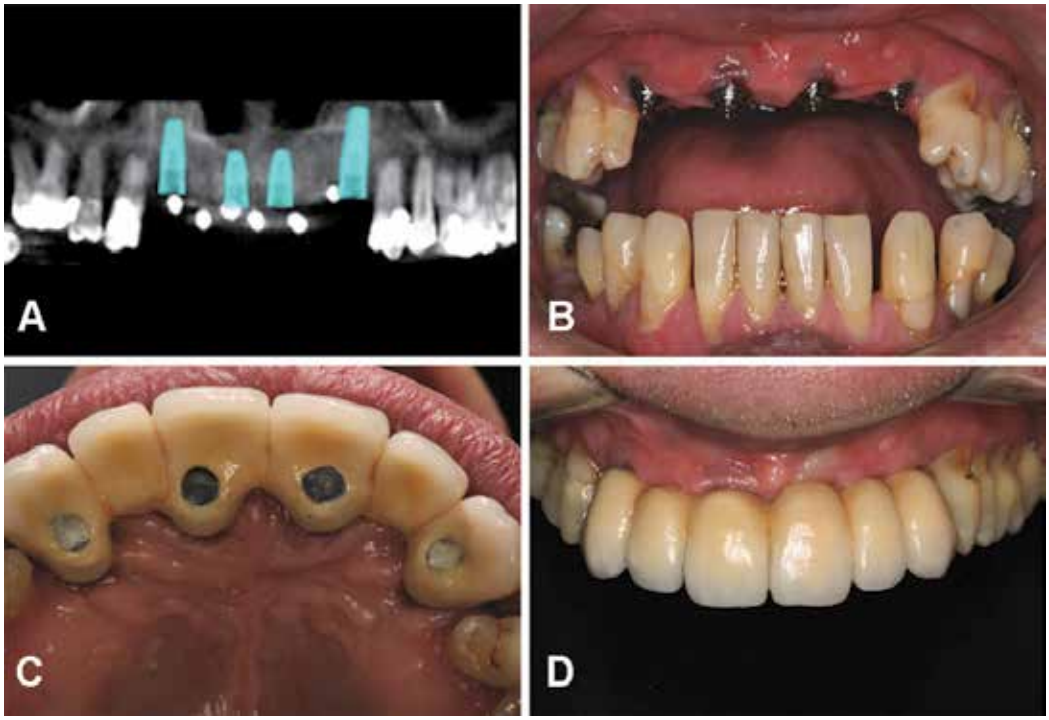

Fig. 2. Guided surgery for partial anterior-superior rehabilitation. a) Preoperative planning. Nobelguide® software system (Nobel Biocare®, Sweden) b) Implant position, immediately after surgery. c) Palatal emergence of implants, in accordance with virtual planning. d) Final result: porcelain-fused-to-metal restoration.

4. Final remarks

Implant planning has always been a major challenge of dental practice. CT is often unable to provide precise definitions of implant size and will not always be useful in preventing anatomical complications during surgery. Loubele et al (2007) found that bony dimensions were underestimated on the basis of measurements taken from several CT scans. Likewise, Suomalainen et al. (2008) reported errors in measurement of bone dimensions on CT images. These setbacks are even more critical when planning implants for patients with little available bone tissue.

The advent of virtual planning and rapid prototyping techniques has led to a shift in this paradigm. Biomedical prototypes have made it possible for the implantologist to visualize and directly manipulate areas of interest, thus narrowing the gap between pre- and intraoperative findings (Choi et al., 2002; Sarment et al., 2003a; Meurer et al., 2008). Virtual planning permits three-dimensional analysis of implant position. Its clinical application—guided surgery—optimizes intra- and postoperative outcomes, as it can reduce surgical morbidity and improve the predictability of the proposed treatment plan.

Studies on guided surgery have gained prominence in the dentistry literature since the early 2000s. Gateno et al. (2003) studied the fit precision of stereolithographic surgical splints and that of conventional acrylic resin splints. Their results indicated that stereolithographic

splints had a high degree of accuracy of seating, thus enabling transfer of virtual treatment plans directly to the patient during surgery.

Van Assche et al. (2007) evaluated the precision of transfer of computer-based planning for implant placement in four partially edentulous human jaws with soft tissues present. All stages of guided surgery were performed on the anatomical specimens, from molding and diagnostic wax-up to placement of implants (a total of 12).

Postoperative cone-beam CT scans were obtained and superimposed onto preoperative scans in a virtual environment. Placed implants had a mean angular deviation of 2 degrees (range, 0.7 - 4 degrees) from the planned position, whereas mean linear deviation was 1 mm (0.3 – 2.3 mm) at the hex and 2 mm (0.7 – 2.4 mm) at the apical implant tip.

The authors conclude that cone-beam CT images can be used in computer-based implant planning for guided implant surgery with rapid-prototyped guides (Van Assche et al., 2007).

Viegas et al. (2010) conducted an experiment on 11 dry human mandibles to assess transfer of virtual implant position planning to surgery. Implant positions were standardized and simulated in an appropriate software package, using preoperative cone-beam CT scans as a basis. Models and surgical guides for each mandible were fabricated using stereolithography methods. Simulated surgeries, including implant placement and removal, were first performed on each prototype. Implants were then placed into the mandible replicas. Analysis of results was based on standardized postoperative CT scanning of prototypes and replicas for visualization of the implant beds. Superposition of pre- and post-operative three-dimensional models enabled assessment of the linear distance and angle between the long axes of planned and placed implants. Linear measurements made directly on models were used to assess the validity of prototype-simulated procedures as a tool for treatment planning in oral implantology. The variations in distance between the coronal, central, and apical regions of the 22 implants evaluated in this study were, on average, <0.41 mm. Mean deviation in angle was 1.45 ± 0.89 degrees for implants placed on the right and 0.70 ± 0.30 degrees for implants placed on the left. Comparison of samples by means of Student's *t* test, using a reference value of zero, showed statistically significant differences for all measurements ($p < 0.05$). The correlation between implant bed positions in each mandible was fair to very good, depending on the distance analyzed and on the method used for interpretation of measurements. This study found that real-life deviation from planned dental implant positions determined by means of virtual planning and simulated surgery on a biomedical prototype were within a range that indicates the reliability of guided surgery as a method of dental implant placement.

In a clinical study, Nickenig and Eitner (2007) evaluated transfer of virtual implant plans to the operative field by means of the guided surgery technique. A total of 102 patients took part in the study (250 implants). Implants were placed into the posterior area of the mandible in 55.4% of cases. Eighteen cases (7.2%) involved rehabilitation of fully edentulous arches.

Computer-based virtual planning based on cone-beam CT scans was used in all cases. Flapless surgery was used for placement of 147 implants (58.8%) and all patients had uneventful intraoperative courses. In 98.4% of patients, there were no issues with surgical guide fit or instrumentation. Reduced interocclusal distance limited applicability of the technique in four patients.

Initial plans were changed in eight cases, when raising the mucoperiosteal flap revealed a need for modifications in manipulation of the available bone tissue and simultaneous

implant placement. Regarding predictability of implant size, only one implant had to be replaced with a smaller-diameter equivalent due to insufficient bone thickness.

Postoperative panoramic radiographs showed that anatomical structures such as the maxillary sinus, mandibular canal, mental foramen, and neighboring teeth were spared as planned. Comparison of postoperative radiographs and panoramic reconstructions created by the planning software revealed substantial differences in implant angle in nine cases.

The authors concluded that virtual plans based on cone-beam CT scans can be reproduced during implant placement surgery, and constitute a reliable method for defining implant position and size, preventing anatomical complications, and enabling wider use of flapless surgery (Nickenig & Eitner, 2007).

The minor variations between planned and actual implant placement in the aforementioned studies show that computer-based treatment plans can be reliably transferred to the operative environment by means of the guided surgery technique.

Furthermore, another key advantage of this procedure is the possibility of immediate loading of functional prostheses. Implant planning and positioning should not only take into account successful intraosseous implant placement, but also ensure that the future prosthesis is passively and securely seated on its fixtures. Prosthetic options for offsetting deviations in implant position vary according to the chosen guided surgery system. Even so, the current literature reveals a concern with the longevity of these implants.

Sanna, Molly, and Van Steenberghe (2007) evaluated the survival of 183 implants (30 patients) placed for rehabilitation of fully edentulous arches with the guided surgery technique, using cone-beam CT scanning, tissue-borne guides, and immediate loading. The results showed that guided placement of dental implants followed by immediate loading of fixed prostheses was associated with survival rates similar to those obtained with the same mode of rehabilitation on implants reopened after the osseointegration period.

Schneider et al. (2009) conducted a systematic review to assess the accuracy and clinical applications of guided implant surgery. A literature search yielded 3120 titles, eight of which met the inclusion criteria in terms of accuracy and 10 in terms the clinical performance. Meta-regression analysis revealed a mean deviation of 1.07 mm at the entry point (95% CI, 0.76 – 1.22 mm) and 1.63 mm at the apex (95% CI, 1.26 – 2 mm). No significant differences between the studies were found regarding method of template production or template support and stabilization. Early surgical complications occurred in 9.1% of cases. Limited access for instrumentation and fracture of the surgical guide were the sole technique-related complications observed—all others were equally possible during conventional implant placement procedures. Early prosthetic complications occurred in 18.8% and late prosthetic complications in 12% of cases. Noteworthy prosthesis-related complications included misfit of abutment to bridge, extensive adjustments of the occlusion, and incomplete seating of the prosthesis. The most common delayed complications were screw loosening and prosthesis fracture.

Implant survival rates, assessed in six clinical studies with a total of 537 implants—most loaded immediately after flapless implantation procedures—ranged between 91 and 100%.

Studies such as these related justify the relevance of investigations that seek improvement toward increasingly accurate and safe methods that are beneficial to patients and dentists alike, such as virtual planning and guided surgery.

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Computer Aided Techniques Developed for Diagnosis and Treatment Planning in Implantology

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1. Introduction

The wide spread application of dental implants with high success rates has made them a common treatment modality in the past two decades. Despite of the predictability of the osseointegration of dental implants, the surgeon has to overcome anatomic limitations as well as restorative demands to achieve precision in planning and surgical positioning of implants. It is essential that the implants are placed properly to ensure a successful operation outcome.

Dental implants are an expected treatment modality for either the restoration of partially and completely edentulous patients or other applications such as anchorage tool in orthodontics (Moslehifard et al, 2011). Successful provision of dental implants to patients lost their teeth and in most occasions their surrounding bone relies on careful gathering of clinical and radiological information achieved with an interdisciplinary communication and detailed planning. Treatment planning is crucial for the success of dental implants.

In osseointegration of dental implants, planning and surgical positioning of implants must be carried out accurately in spite of several inherent limitations. Capabilities of the clinicians, i.e. the surgeon and prosthodontics, play a crucial rule in achieving optimum surgical and prosthetic procedures. The predictability of success is increased when the implants are placed properly. The optimum placement makes it possible to have implants and prosthetic components under establishment of favorable forces. At the early stages of evolution of the implant surgery, clinicians would place implants in regions of maximum bone volume without fully considering where the crown eventually would be placed. Biomechanics, esthetics and maintenance can be compromised if the implant location does not align properly with the crown, ultimately resulting in substandard outcomes, including failure (Worthington et al, 2010).

Several techniques have been developed in years and are currently available in the planning procedure of implant placement during the clinical settings. Palpation of the ridge, use of an osteometer, diagnostic casts and evaluation of the relationship between the jaws, and the use of simple radiographs are the most well established methods beside other advanced methods (Kopp et al, 2003; Fortin et al, 2003).

2. Importance of pre-surgical assessment

The success of any therapeutic treatment depends upon a variety of parameters including careful selection of suitable patients for each treatment, formation of a treatment plan and careful implementation of a gentle treatment procedure. If operator strictly adheres to these principles, any operator-induced damage can be significantly prevented. For instance, the selection of patient and treatment planning is the critical step in the prevention of iatrogenically induced damage during implant treatment. Therefore, a great amount of careful attention should be taken at the pre-surgical era before going through the implementation of the treatment/patient selected.

At the preliminary stages of pre-surgical examinations, the anatomical structures such as vertical height of bone, ridge width, bone quality, vital structures and any bony defects or pathologies are important to learn. It would be also necessary to estimate the number of implants to be placed and their angulations using surgical templates as a useful aid in the orientation of the implant. Bone sites are considered as another issue to address in the presurgical study and should be determined reasonably in quality and quantity. In the other hand, the anatomical limitations for the placement of implants may propagate some damages to the maxillary sinus, mental nerve and inferior alveolar nerve, if not properly introduced in the therapeutic treatment process. Once the anatomical purview of the patient/treatment is established, it is generally recommended to perform the treatment planning in a partially edentulous patient in a manner which does not cause any damage to the adjacent teeth. This may be, for example, obtained when a minimum specified edentulous space retains between the adjacent tooth and fixture. However, there should be a minimum of one millimeter clearance between the apex of the fixture and the superior wall of the inferior alveolar canal. Nevertheless, those cases that are planned with allograft and membranes are less predictable compared to straight forward cases.

During the surgery, vital structures like maxillary sinus, mental nerve and inferior alveolar nerves should not be encroached. The damage occurred in nerves can cause numbness and tingling sensation. The encroachment into maxillary sinus can cause development of oroantral communication. If implants are inserted closely to the maxillary sinus after a poor oral hygiene, it may provide a route to spread infection from the mouth. Once the maxillary dental implant is infected, sinusitis occurs readily due to the spread of inflammation. The perforation of the membranous lining of maxillary sinus occurs commonly during sinus lift operations. The floor of mouth contains branches of submental and sublingual and mylohyoid arteries that may lead to life threatening complications. Damage occurrence to arteries, especially in the floor of the mouth has been reported in the literature (Shenoy et al,. 2006). It can result in most cases in fatal hemorrhage. The branch of the sublingual or facial artery may be situated in immediate contact with the lingual periosteum. This close proximity can explain why even a small perforation could cause severe bleeding (Givol et al,. 2000). Mason et al. (Mason & Triplett, 1990) reported a case of fetal hemorrhage arising from the dental implant placement in the mandible due to damage to sublingual artery. Injury to the mandibular canal results in paralysis or numbness of the chin and corner of the mouth. Furthermore, sudden loss of tooth vitality within a whole quadrant can occur, since the neurovascular bundle within the mandibular canal also supplies the teeth. The damage to the mandibular canal during the placement of implants (or extraction of third molars), therefore, develops one of the major issues for which legal actions may be taken against dentists. The major cause of this pitfall is submandibular fossa, which is a depression on the

medial surface of the body of the mandible inferior to the mylohyoid line. Submandibular gland is lodged in this fossa.

Studies by Bavitz et al. (Bavitz et al,. 1994) and Hofchneider et al (Hofchneider et al, 1999) indicated that submental and sublingual arteries may locate closely to the lingual cortical plate, from the floor of the mouth. It should be pointed out that the edentulous mandible is even shorter and, therefore, the perforations occurred are deeper in the floor of the mouth. Most of the reports of the floor of the mouth and sublingual hemorrhage have been iatrogenic in nature. Sharp instruments such as rotating dental disks and burs have slipped off mandibular teeth causing injury to the floor of the mouth and even laceration of the sublingual artery. Niamtu (Niamtu &Richmond, 2001) reported a case of near fatal airway obstruction after routine implant placement, secondary to sublingual bleeding and hematoma. The submental artery may be located against the usually concave medial body of the mandible in older edentulous patients. The horizontal wall of the submandibular fossa considered in this study has been above the mandibular nerve in all the subjects. Considering the anatomic variations, the risk of perforation is high, especially when the fossa is very deep. When delayed severe bleeding can be taking place, it is prudent to closely follow any patient with a significant penetrating trauma to the floor of the mouth. This includes injuries from iatrogenic dental treatment(s) (Niamtu &Richmond, 2001). In one study (Parnia et al,. 2010) the depth of submandibular fossa was more than 2 mm in 80% of the subjects. This situation raises the risk of perforation of lingual cortical plate, injury to the terminal branches of sublingual artery during implant placement and hemorrhagic accidents, which are critical and life-threatening. Therefore, precise evaluation of morphology and dimensions of submandibular fossa is of utmost importance before implant surgery in the posterior areas of mandible.

The mandibular inter-mental area is assumed to be a safe area for implant insertion and is considered in many other surgical procedures. It is essential to understand the anatomy of the region to avoid occurring injuries to the neurovascular bundles. Provided that the inferior alveolar or mental nerve is damaged during the preparation of an osteotomy, sensory dysfunction may appear due to nerve damage in the foraminal area. All-on-four procedure which has been recently developed, permits a quick placement of 4 dental implants in the interforaminal area of the lower jaw associated with a fixed bridge during only one appointment. The mental foramen is a strategically important landmark during osteotomy procedures. The inferior alveolar nerve may extend beyond the mental foramen as an intraosseous anterior loop. The location of foramen, as well as, the possibility that an anterior loop of the mental nerve may be present mesial to the mental foramen need to be considered before implant surgery to prevent mental nerve injury. The mandibular canal contains the inferior alveolar nerve and blood vessels and is divided into mental and incisive segments between the roots of premolars. The mental canal deviates toward the mental foramen. However, the incisive canal continues below the incisor teeth where it is generally divided into a plexus of nerve branches until its main trunk is lost (Greenstein& Tarnow, 2000). In this area lingual foramen is also present that is located in the midline, leveled with or superior to the genial tubercles. The foramen has a branch of the incisive artery that anastomosis with the lingual artery (McDonnell et al,. 1994).

In the establishment of a zone of safety (in millimeters) in implant placement, both the available bones coronal and anterior to the foramen are quite important. Any improper implant insertion anterior to the mental canal can cause oedema of the epineurium. This

may spread to the main mental branch, leading to neurosensory disturbances. Some authors have reported the evidences of discomfort, pain, and disturbance of sensation after implant insertion into the inter-mental area (Abarca et al,. 2006). Rosenquist assumed that implant failure and neurosensory dysfunction were partly due to the large diameter of the mandibular incisive canal. In this report, it was found that incisive bundle causes implant failure, by migrating of soft tissue around the implant, thus preventing osteointegration (Rosenquist, 1996). In addition, sensory disturbances of the mental nerve region may arise after endosseous implants are installed in the mandibular interforaminal region because of damage to anatomical structures in this area (Wismeijer et al,. 1997).

Other landmarks are anterior loop and incisive canal. The possibility of the presence of the anterior loop does not necessarily mean that implants can be safely placed close to the mental foramen. The sensory disturbances of the lower lip have been reported due to the direct trauma to the anterior looping of the mental nerve. Incisive canal is a continuation of the mandibular canal anterior to the mental foramen. The canal contains the incisive bundle that innervates the teeth in the anterior segment. Its precise anatomy and intermedullary content is important during implant insertion. One possible result from traumatizing the neurovascular bundle is sensory disturbance. Kohavi and Bar-Ziv (Kohavi& Bar-Ziv, 1996) described a case where pain and discomfort resulted from implants placed in the intermental area. CT images revealed that the implants were placed through a large lumen of the incisive canal (Bavitz et al,. 1993). Another factor might also be related to indirect trauma to the incisive canal bundle, causing hematoma in the closed chamber that may produce pressure on the mental nerve. Thus the canal could play an important role in successful osseointegration and prevention of postoperative sensory disturbances.

The lingual foramen is situated in the midline, leveled with or superior to the genial tubercles. A pilot study by Mc Donnell (Mc Donnell et al,. 1994) revealed an incidence of 49% of the lingual foramen on periapical radiographs of the mandibular incisor region in adult population. The study suggested that the lingual foramen was a consistent finding to the lingual side of the mandible in the midline, being present in over 99% of the dried specimen examined. The foramen was definitely visible in 81% and 82% in Makris (Makris et al, 2010) based on CBCT and Jacobs (Jacobs et al,. 2002) based on CT studies, respectively. The lingual artery has sufficient size to present difficulty in control of hemorrhage intraosseously or in soft tissue. Also in view of its position it could be a factor in implant placement in the midline.

Sufficient knowledge of performing implant surgeries should be available to manage arterial hemorrhage and other medical emergencies. Accurate radiographic imaging is indispensable for the selection of appropriate implant size and placement and is an invaluable guide for surgery.

3. Imaging objectives

Over the past decade, there has been a diverse progress in using noninvasive technology to visualize human internal structures in their true form and shape. The discovery of X-ray gave birth to radiology in years. Its outcome developed in dentistry is that the pre-surgical radiographic examinations for the treatment with osseointegrated implant give detailed information on the potential area for implantation. The presence of lesions and anatomical landmarks is known as the confining conditions and structures that may influence the placement of osseous implants. The radiographic examinations should also provide

evaluation of morphology, angulations of the alveolar ridge, and quantity and quality of the alveolar bone (Tyndall & Brooks, 2000). The global objective of this phase of treatment is to develop and to implement a treatment plan for the patient that enables restoration of the patient's function and esthetics by the application of accurate and strategic requirements being transformed physically into a three-dimensional diagnostic template. This enables the implant team to identify the specific sites of prospective implant surgery in the imaging examinations. The specific objectives of pre-prosthetic imaging are to (1) identify disease, (2) determine bone quantity, (3) determine bone density, (4) identify critical structures at the proposed implant regions, and (5) determine the optimum position of implant placement relative to occlusal loads (Misch, 2005).

Initially, implants were placed by use of simple periapical and panoramic radiographs (Sarment et al,. 2003). Pre-surgical imaging can provide important information in osseointegrated dental implant treatment. Placement of implants has been performed by use of simple periapical and panoramic radiographs for years (Kopp et al,. 2003). However, surgical templates have attracted much interest in obtaining more precise placement of implant on the surgical site. Because both the final restorative prosthesis, i.e. the biomechanical and aesthetic requirements, and the internal anatomy, i.e. bone volume and position of vital structure, are to be taken into account (Fortin et al,. 2003 ; Misch, 2005).

Proper radiographic assessment is an essential aspect of dental implant planning. Different imaging methods have been used to evaluate bone quantity and anatomic structures in relation to the proposed implant site, ranging from conventional 2-dimentional radiographs (panoramic and periapical radiographs) to more complex 3-dimentional modalities, such as computerized tomography (CT) or cone beam computerized tomography (CBCT).

Some clinicians may solely evaluate the edentulous site on periapical and/or panoramic radiographs. The results of these radiographic evaluations can be enhanced at the time of scanning by the application of a radiographic template with radiopaque markers or materials. Different radiopaque markers, including metal balls and tubes, gutta-percha, and barium sulfate, have been used to delineate the position and/ or the contour of the proposed restoration, but little or no information exist about the restoration contour of the proposed restoration, or it can decrease the quality of the image by increasing the scatter artifact (Zahran & Fenton, 2010). The main drawback of both techniques for implant treatment is that the images are two-dimensional. Studies have shown that only 17% of panoramic radiographs reveal true osseous height on dried specimens (Richtert, 1989). In the posterior mandibular region the buccolingual location of the mandibular canal and submandibular fossa are of utmost importance because there is a potential risk of causing damage to the inferior alveolar nerve and submental and sublingual arteries during surgery. The mental foramen is an important landmark where implant placing must be performed in the foraminal region of the mandibular arch. To prevent the mental nerve injury during implant surgery, it is necessary to define the location and also whether an anterior loop of the mental nerve may become mesial to the mental foramen. Sensory dysfunction due to nerve damage in the foraminal area could occur provided that the inferior alveolar or mental nerve is damaged during osteotomy. Since improper placement of implant in the intermental area causes pain and discomfort, reported in several cases, a zone of safety (in millimeter) for implant placement must be determined. However, neither the panoramic nor the periapical films can provide correct information (Parnia et al,. 2010).

4. Pre- prosthetic imaging

4.1 Intraoral radiography

4.1.1 Periapical radiography

Periapical radiographs are the images of a limited region of mandibular or maxillary alveolus. Periapical radiographs may suffer from distortion and magnification. The long cone paralleling technique eliminates distortion as long as it limits the magnification to less than 10%. The opposing landmark of available bone in implant dentistry is beyond lingual muscle attachments in the mandible or beyond the palatal vault in the maxilla. As such, the image most often must be foreshortened to visualize the opposing cortical plate. As a result, the actual available bone height may be difficult to determine. In terms of the objectives of preprostetic imaging, periapical radiography is (1) a useful high-yield modality for ruling out local bone or dental disease; (2) of limited value in determining quantity because the image is magnified, may be distorted, and does not depict the third dimension of bone width; (3) of limited value in determining bone density or mineralization (the lateral cortical plates prevent accurate interpretation and cannot differentiate subtle trabecular bone changes); and (4) of value in identifying critical structures but of little use in depicting the spatial relationship between the structures and the proposed implant sit (Misch,2005; Goaze &White, 1992).

4.1.2 Occlusal radiography

Occlusal radiographs have minimal application in implant dentistry. Cross-sectional occlusal radiographs of the mandible give some information about the buccolingual dimension of the mandible, but this information is only accurate in comparison to the inferior aspect of the body, not the width of the alveolar ridge where the implant is to be placed. The use of cross-sectional occlusal radiographs can be helpful when evaluating the position of the implant within the jaw following placement; this applies to both the mandible and maxilla (Mansour &Dudhia, 2008).

4.2 Extraoral radiography

4.2.1 Cephalometeric radiography

Lateral cephalometric radiographs provide accurate information about the available bone in the mid-sagittal region of the maxilla and mandible. Because of the long film-focal distances used in cephalometric radiography the resultant image has minimal magnification. The cross-sectional dimensions and morphology of the ridge are shown accurately in the midsagittal plane of the anterior maxilla and mandible.

4.2.2 Panoramic radiography

Panoramic radiography is a curved plane tomographic radiographic technique used to depict the body of the mandible, maxilla, and the lower one half of the maxillary sinuses in a single time. This modality is the most useful diagnostic modality properly utilized in implant dentistry. However, for quantitative pre-prosthetic implant imaging, panoramic radiography is not the most diagnosing way (Grondahl K, 1996; Dove& McDavid, 1993). When using panoramic imaging for diagnosis, one of the most frequent problems in the panoramic radiography is the loss of definition that happens when either the patient is improperly positioned in the machine or the curve of the mandible does not match the focal trough predetermined by the manufacturer (Truhlar et al,. 1993). One early study has

shown that only 17% of panoramic radiographs represent true osseous height on dried specimens (Klinge et al,. 1989). Accordingly, due to inexorable changes in the magnification on horizontal dimensions, panoramic image does not match the real dimension. The vertical magnification is approximately 10% and horizontal magnification is approximately 20% and varies depending on the anatomical location, the position of the patient and the focus object distance, and the relative location of the rotation center of the X-ray system. Traditional panoramic radiography is a high-yield technique for demonstrating dental and bone disease. However, panoramic radiography (1) does not demonstrate bone quality/mineralization, (2) is quantitatively misleading due to lack of high magnification and third dimension cross-sectional view, and (3) is of some use in demonstrating critical structures but of little use in depicting the spatial relationship between the structures and dimensional quantitation of the implant site. For the evaluation of the recipient sites and the determination of optimum implant dimension, diagnostic templates having 5-mm ball bearing and being worn by the patient during the panoramic x-ray examination enable the dentist to determine the amount of magnification in the radiograph (Misch, 2005). The development of computer technology applied to radiology has allowed the manipulation of image such as converting the conventional radiographs into scanned images to display readily on a monitor. The scanned images can enhance interpretation, for resources such as brightness and contrast control, colorization, and inversion effects which may be applied to radiographic images (Sakakura et al,. 2004).

4.2.3 Computed tomography

Three-dimensional radiographic imaging was first conceived in the early 20th century and was proved by calculating an infinite number of projections of image of a three-dimensional object. The original purpose of the use of CT scanner was to examine the human cranium (Hounsfield, 1973). Early devices provided 1 cm thick axial cross-sectional images. Until 1980s technical developments resulted in obtaining 1.5 to 2 mm thick images. For several years, the technique was used to diagnose the lesions of the head and neck and for the evaluation of the anatomic structures of patients who were to undergo craniofacial surgery (Iplikcioglu et al,. 2002). CT scans have become one of the most frequently used imaging techniques for pre-operative evaluation of the jaws before implant treatment. The first commercially developed program was DentaScan (General Electric,Milwaukee, Wis), which produced "dentistry-friendly" images (Schwarz et al,. 1987).

CT produces axial images of a patient's anatomy. Axial images are produced perpendicular to the long axis of the body. CT images are inherently three-dimensional. The original imaging computer can create secondary images from almost any perspective by reprojecting or reformatting the original three-dimensional voxel data. These images can be used for diagnostic imaging (Kawamata et al,. 2000). A patient's slice data from CT scan images is analyzed via automatic contouring algorithms to provide a representation of the surface of the studied structures (Woolsen et al,. 1986). These structures can be displayed on the screen, compared against normative references for surgical planning, and modified to represent a simulated surgical operation. Implant design and bony augmentation can be simulated on-screen as well (Benjamin, 2002).

Computer tomography (CT) assisted implant planning systems can considerably enhance the quality of implant therapy by improving the transfer from planning to surgery and the ability to convey the treatment plan to the patient. The significance of proper prosthetically

driven treatment planning was reorganized first with the aim of the optimal crown position used as a guide to implant position. CT is considered a valuable tool towards achieving this goal and enables the use of a three-dimensional image for the diagnosis of osseous features, including its density, volume and architecture, and the identification of vital anatomic structures (such as the maxillary sinus and the mandibular canal) in the radiographic image. Prosthetic and radiographic implant planning can be further enhanced with computer software, using template-guided surgery enabling the clinician to optimize implant position, angle, diameter and length (Horwitz et al,. 2009).

In 1986, Fellingham first demonstrated the use of interactive graphics and 3D modeling for surgical planning, prosthesis, and implant design. 3D images were transmitted to a computer-controlled milling machine for reproduction of the anatomical structure. This technology was used to reproduce atrophic mandibular and maxillary jawbones for full arch subperiosteal implants (Fellingham et al,. 1986). After that, multiplanar reformatted CT had become the most comprehensive and accurate aid for endosseous implant treatment planning. Advancement in diagnostic technology, namely helical or spiral CT and stereolithography, have allowed the development of a CAD-CAM processed surgical guide to be placed directly on the bony site. The surgical guide facilitates the predictable transfer of the analysis of the bony morphology and accurate positioning of the endosseous implant (Cucchiara et al,. 2001).

Limitation of this technique includes the determination of bone quality by use of the imaging computer; hard copy images that only include a limited range of the diagnostic gray scale of the study; and the tilt of the patient's head during the examination, which is critical because all the cross-sectional images are perpendicular to the axial imaging plane. CT examinations are expensive and deliver a relatively high radiation dose to the patient. During dental radiographic procedures, organs most susceptible to the side effects of radiation are the hyoid gland, brain, active bone marrow, lymphatics, and salivary glands. However, although the biologic effects of radiation are known, the risk associated with CT is assumed to be low (Iplikcioglu et al,. 2002). Usually a diagnostic template is necessary to take full advantage of the technique. The diagnostic template enables the dentist to incorporate the three-dimensional treatment plan of the final prosthetic result into the imaging examination; evaluate the patient's anatomy relative to the proposed implant sites; esthetics; and occlusion; and record and transfer these finding to the patient at the time of surgery. CT enables identification of disease, determination of bone quantity, determination of bone quality, identification of critical structures at the proposed regions, and determination of the position and orientation of the dental implants (Misch, 2005).

4.2.4 Cone beam computed tomography

Cone beam volumetric tomography was pioneered at the Nihon University School of Dentistry during the 1990s, and the first machines became commercially available in 2000 (Terakado et al,. 2000; Ito et al,. 2001). Since then, numerous machines have been commercialized and much research on the usefulness of the technology in dentistry have been performed. Cone-beam technology is progressing rapidly and scanners are constantly being refined and upgraded. A reasonable number of scanners have already been installed in dental practices and radiology practices, and this number is sure to grow in the future. While CBCT permits three-dimensional visualization of the dental hard tissues in a similar manner to multislice CT, there are some fundamental differences. With the majority of cone-

beam machines, the patient is seated or standing rather than being supine (Vannier, 2003; Monsour &Dudhia, 2008). This imaging modality is very promising with regard to pre-implant imaging. CBCT generally delivers a lower dose to the patient compared to CT and provides reasonably sharp images with three-dimensional information. While CBVT permits three-dimensional visualization of dental hard tissues in a similar manner to multi-slice CT (Vannier, 2003), there are some fundamental differences. Image acquisition times vary and are specific to particular models, but typically range between 10 to 70 seconds (Scarfe et al,. 2006; Pinsky, 2006). Acquisition time with a 16-slice CT scanner is shorter than the fastest CBCT scan, and newer 64-slice CT units reduce the scan time even further. This effectively minimizes the risk of patient movement. The theoretical resolution of CBCT is higher than CT (Sato et al,. 2004), but the difference may not be as significant as once through due to the impact of patient movement resulting from the increased scan times. Longer scanning times utilizing an increased number of images permits increased resolution or a decrease in image noise but with a significantly higher radiation dose and an increased risk of patient movement (Scarfe et al,. 2006). As cone-beam technology was built on the platform of complex-motion tomography, the radiation dose is typically lower than a multi-slice CT scan of the jaws (Terakado et al,. 2000; Hashimoto, 2003).

Recent studies indicate that CBCT images are of sufficient accuracy for use in pre-implant assessments (Pinsky, 2006; Ludlow et al,. 2007). It has been demonstrated that the error in measurements obtained from CBCT scans is less than 0.5 mm (Marmulla et al,. 2005). Volume-rendered images obtained from CT data have been found to be superior to those from CBVT. The low exposure parameters of CBVT result in poor soft-tissue contrast compared with CT, (Scarfe et al,. 2006; Katsumata et al,. 2006) and the inability to alter the exposure parameters in most machines means that image quality is compromised in larger patients. Furthermore, CBVT suffers from the same beam-hardening artifact that CT does, limiting the usefulness of the exam in patients with metallic restorations, posts or surgical plates (Guerrero et al, 2006).

While it is recognized that multi-slice CT is a higher dose examination way compared to CBVT, some reports indicate that low-dose CT protocols result in significantly less exposure than previously thought, without compromising image quality significantly (Ekestubbe, 1999). A consequence of the lower dose of CBVT is the reduced contrast and subsequent image quality. It is important to note that while the radiation dose from a CBVT scan may be less than that from low-dose CT, the dose is still significantly higher than that from other forms of dental radiographic examinations (Scarfe et al,. 2006).

4.2.5 Magnetic resonance imaging

Magnetic resonance imaging (MRI) has become a powerful imaging tool in medicine. This technique was announced first by Lauterbur in 1972.Useful medical images were produced in the early 1980s (Lauterbur, 1973).Using the magnetic properties of the hydrogen atom, MRI units are capable of producing images of the human body. As the technology is dependent upon the presence of hydrogen atoms, MRI is particularly suitable to imaging soft tissues. Using various radiofrequency pulse sequences and relaxation times, images may be produced to better demonstrate anatomy or pathology in the body. Since MRI relies on the use of a strong magnetic field, MRI examinations are contraindicated in patients with metal foreign bodies in the eyes, ferromagnetic intracranial aneurysm clips, cardiac

pacemakers, cochlear implants and patients in the first trimester of pregnancy. The presence of certain metals such as amalgam and non-precious alloys will result in considerable artifact on the images and often render the examination useless (Hubalkova et al,. 2002; Hubalkova et al,. 2006). Pure titanium implants show no artifact with MRI, but if there are any impurities in the titanium artifact will appear. Other considerations include the significant cost to the patient for MRI examinations and claustrophobia is a real concern as the examinations are generally performed with the patient in a very confining tunnel. MRI is used in implant imaging as a secondary imaging technique when primary imaging techniques fail (Monsour &Dudhia, 2008).

Most studies using MRI for pre-implant imaging have focused on the ability of MRI units to locate the inferior dental canal (Eggers, 2005). With MRI the inferior dental canal appears as a black void within the high-signal cancellous bone. If the inferior dental canal is surrounded by sclerotic bone, visualization of the canal is more difficult with MRI as the presence of sclerotic bone results in a low bone marrow signal. The contrary is true for CT, as the presence of sclerotic bone in the mandibular body makes the inferior dental canal more obvious. Magnetic resonance imaging has a significant potential for pre-implant imaging due to the lack of ionizing radiation, but acquisition times can be as long as 30 minutes and there is limited bone information available (Monsour &Dudhia, 2008).

5. Surgical guides

In spite of significant advances in devices and techniques, placement of dental implants in correct position still remains a challenge. Diagnostic casts, probing depths and panoramic radiography can lead to unpredictable results as they do not give three-dimensional (3-D) radiographic information required for correct positioning and orientation of implant (Almong et al,. 2001). Moreover, predictable implant supported prosthesis also requires a determination of final prosthesis in treatment planning stage. Thus for a successful implant supported prosthesis, the prosthodontist should plan the implant positioning in accordance with accurate mesiodistal and buccolingual location, angulation with residual bone and correct implant orientation. To achieve these objectives, surgical guide (stent) with radiopaque marker in conjunction with dental CT scan imaging should be used.

The purpose of stent is to purview the definitive restoration and its relationship to adjacent structures, to communicate the restoration planned by the prosthodontist to the surgeon, to reduce osteotomy and to locate healing screws at the time of second stage surgery (Garber, 1995). For a successful implant supported definitive restoration the implant must be placed at a correct and pre-planned position and angulation. The mesiodistal placement of the implant should aid in preservation of papilla and provide an esthetic implant restoration profile (Lazzara, 1993). The implant should be placed at least 1.5 mm from the adjacent teeth with a minimum 3 mm interimplant distance. The distance of implant from buccal and lingual cortical plates should be greater than 0.5 mm. In the buccolingual plane the angle between the implant trajectory and residual bone trajectory should be less than 20 degrees to prevent unfavorable bending moment (Horiuchi et al,. 1995; Almong et al,. 1995). In multiple implant situations, non parallel implant placement is the primary cause of non axial loading and subsequent failure (Taylor et al,. 2000). It has been suggested to use stents to achieve the above mentioned objectives. Information present in the literature implies when the implants are placed using stents, the outcome of the positioning is more accurate than those placed without stent (Talwar et al,. 2011).

5.1 Diagnostic template

Evaluation of bone for implant placement may be provided through the use of radiographic or dual-purpose stents. Since the ultimate objective of implant placement is a functional, aesthetic, and durable restoration, the imaging of potential recipient sites should provide accurate information that facilitates precise placement of implants in a correct three-dimensional position (Cehreli & Sahin, 2000). Placement of implants in the anterior maxillary region requires special attention. localized soft tissue and bone contour may affect the emergence profile and the final appearance of the prosthesis. Implants over-angulated toward the labial can lead to aesthetic disharmony. For extremely malaligned implants, an opening for screw access on the facial surface of the prosthesis or its complete removal may be indicated. Implants placed in the inter-proximally areas of a prosthesis may cause aesthetic and hygiene problems, and implants placed too lingually generally result in a bulky prosthesis with an unfavorable lingual contour that may also interfere with speech (Iplikcioglu, 2002).

The goals of presurgical prosthodontics that converts to a radiographic stent include: (i) establishment of vertical dimension of occlusion and adjustment of sufficient interocclusal gap space, (ii) determination of centric occlusion at centric relation position (centric occlusion position is used to mount the casts of patients who are only partially edentulous and have enough remaining teeth), (iii) creation of a harmonious plane of occlusion, (iv) achievement of optimum esthetics, phonetics, and (v) incisal guidance so as not to alter the patient's speech patterns, and (vi) definition of the end result of treatment and its appearance.

Prior to the CT scan, a scan prosthesis that plays several critical roles in obtaining accurate roles in obtaining accurate input data is developed. The scan prosthesis stabilizes the opposing jawbones to prevent jaw movement during the CT scan. Movement is minimized with the helical CT, even slight jaw movement needs to be prevented. Second, occlusion and future tooth set-up can be simulated and represented in 2D and 3D reconstruction. The base of the scan prosthesis should be constructed to represent a radiolucent appearance on the transaxial images. Porous maxillary bone and knife-edge ridges on the mandibular bone must be distinguished from the CT scan prosthesis. Prosthetic teeth are represented as radio-opaque images clearly distinguishable in form and shape. Simulation of the prosthetic tooth position can be accomplished using opaque dental teeth, or coating acrylic teeth with barium sulfate, or using gutta percha markers or a similar radio-opaque material. In addition, the drilling hole must represent the drilling direction for implant insertion (Benjamin, 2002).

There are different surgical templates. Clear vaccuform stents are simple and quick to fabricate but the stents bear too much flexibility in the positioning of implant and are less accurate. Self cure acrylic stents with channels filled with gutta percha provide acceptable accuracy and are easy to fabricate and inexpensive. Self cure acrylic stents with metal sleeves are the most accurate tools in this respect.

Templates are usually fabricated in the laboratory with heat-cured acrylic resin. To indicate screw-access channels, 2-mm-diameter holes should be drilled in the cingulum area of the anterior teeth and the central fossa of the posterior area so that the channels do not interfere with the interproximal papillae or the opposing dentition. After fabrication of the surgical template, it will be drilled by the clinician forming pilot holes or grooves as indicators to implant positions or planned osteotomies, respectively (Zinner et al,. 2004). Figure 1

illustrates an image of milling machine used for the preparation of the access holes in the surgical template. During the surgery, these stents dictate the implant's position and angle. The stents can combine with CT scan to increase accuracy of the stent. Tomography and CT have been used to provide a three-dimensional picture of the region to be implanted (Ames et al,. 1980; Hollender &Rockler, 1980). Reformatted computed tomography (CT) has been proven to be an effective technique in planning complex cases in oral implantology (Besimo et al,. 2000). Pre –surgical imaging can provide important information in osseointegrated dental implant treatment. The use of cross-sectional images in the bucco-lingual direction, which can be delivered by computed tomography (CT), allows us to plan a more accurate design of implant placement before surgery (Naitoh et al,. 2000).

Fig. 1. A scheme of the preparation of implant access holes along the long axis of teeth using milling machine.

This template is the key to the system, since it allows the transfer of the predetermined prosthetic setup to the actual implant planning. The template is an exact replica of the desired prosthetic end result. Incorporation of the scanning template into the computerized tomography (CT) scanning data allows the surgeon to base implant planning on the desired prosthetic outcome. The treatment plan is thus driven by the prosthetic final result, not vice versa (Tardieu & Philippe, 2001). According to the literature, scannographic template can be made of 15wt% of barium sulfate in resin powder, or by means of other radiopaque substances (Wouteres et al,. 2000; Tardieu & Philippe, 2001). However, a variety of mixtures of barium sulfate ranging between 5 and 35wt% have been used. It has been experimentally revealed that the percentage should vary with respect to the surrounding elements (metal crowns or natural teeth) and also as a function of the opacity gradient that one wants to obtain within the same template containing several different radiopaque layers (Tardieu et al,. 2003). In figure 2, radiopaque templates are shown.

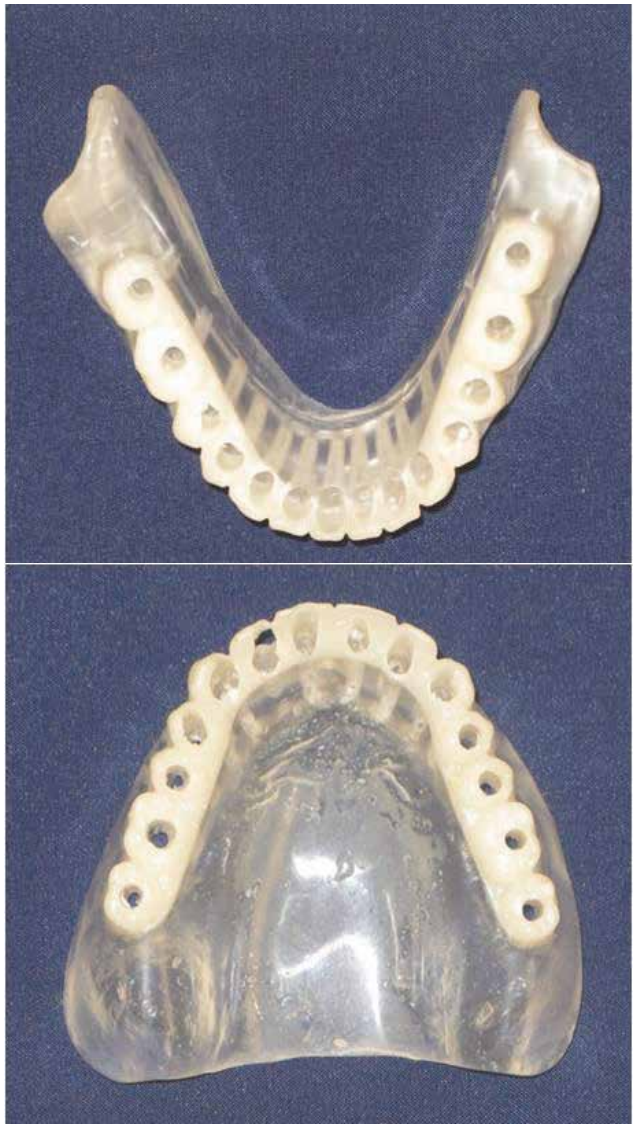

Fig. 2. Radiopaque templates.

In comparison to conventional radiographic stents, radiographic or dual-purpose stents with radiopaque markers offer the advantage of transferring the CT data onto the same stent for surgery. However, since errors which appear in the conversion of the stent may lead to malalignment of the implants, the angle of the radiopaque markers should facilitate reorientating the surveying table if guide channel preparation must be performed in a different angle.

The incorporation of treatment planning modified by CAD-CAM offers significant advantages, including the evaluation of the 3-D anatomy and the fabrication of anatomic site models and osseous-supported surgical templates. Other advantages include shorter surgery durations, shorter treatment times, minimizing of intraoperative radiographs

during implant placement, less invasive surgical technique (flapless surgery, with less change of swelling, less pain, and faster initial healing times), prefabricated definitive prosthesis and immediate use of a fixed prosthesis (Saement et al,. 2003; Machrack, 2005). Furthermore, the surgical guides are useful to determine and insert the dental implant in appropriate position and length (Nokar et al,. 2011).

5.2 Scanning

The patient must be instructed on how to position the guide in the mouth and how to stabilize the guide by biting on two cotton rolls or on a bite template during CT scan (Fig. 3). The radiographic template can be visualized in both the 2D and 3D reformatted CT images. The concentration of barium sulfate used in creation of the ScannoGuide determines the level of visibility. It is crucial to remember that very low concentrations may result in a lack of visibility of the template in the CT images. And, concentrations of barium which are too high can cause artifacts and minimize proper visualization. It is possible to vary the concentration of barium between the teeth and the base plate of the ScannoGuide, so that both parts can be visualized separately. A hole can be drilled along the longitudinal axis of the tooth. This hole will appear black in the CT images and is clearly visible in 2D cross-sectional reconstructions. The following pictures illustrate what can be seen using a proper ScannoGuide in 2D and in 3D. Having the patient bite on cotton rolls has several advantages, it allows arches stabilization during CT scan, it gives a better comfort to the patient who can bite on something solid and it separates jaws avoiding artifacts from the opposite arch. The axial view clearly shows the image of teeth with open hole through. 3D images of the ScannoGuide can be combined with the bone information. The 3D reconstruction of the CT images of a ScannoGuide can show the teeth set-up for this partially edentulous case.

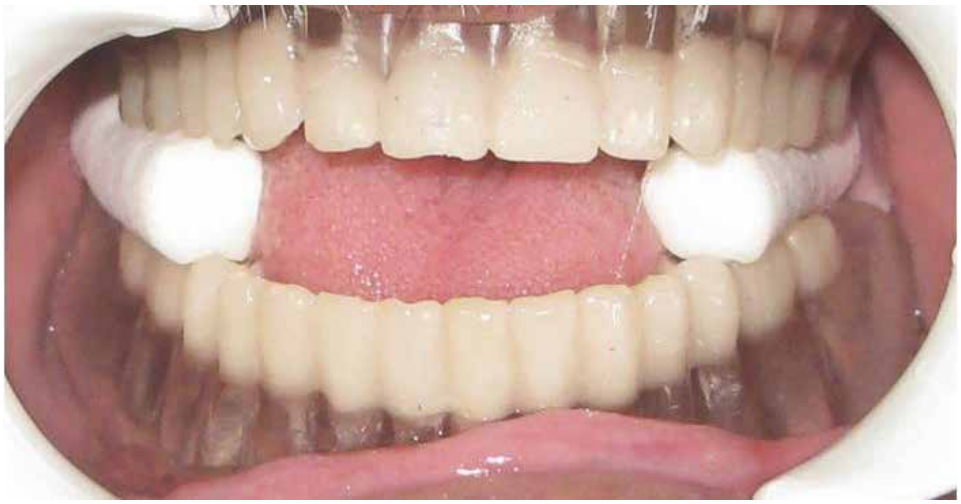

Fig. 3. Stabilizing the templates during CT scan

5.3 Process of the images and conversion of imaging data

The CT scan image data must be produced according to a specific scan protocol to obtain the data compatible with the imaging software. The imaging software (Mimics,

Materialise/Columbia Scientific, Glen Burnie, Md) converts slice image data into 2D and 3D computer models for analysis. The imaging software performs 2D image processing by reformatting the data along planes and/or curves. The simplest reformatting is the construction of images that show slices oriented orthogonally to the original plane of section. These are called planar reconstruction: sagittal if they pass through the midline and are oriented front to rear; coronal if they pass through the midline and are oriented left to right; parasagittal or pericoronal if they are parallel to the sagittal or coronal plane and do not pass through the midline; and paraxial if they are obliquely oriented (Herman &Liu, 1977) (Fig 4).

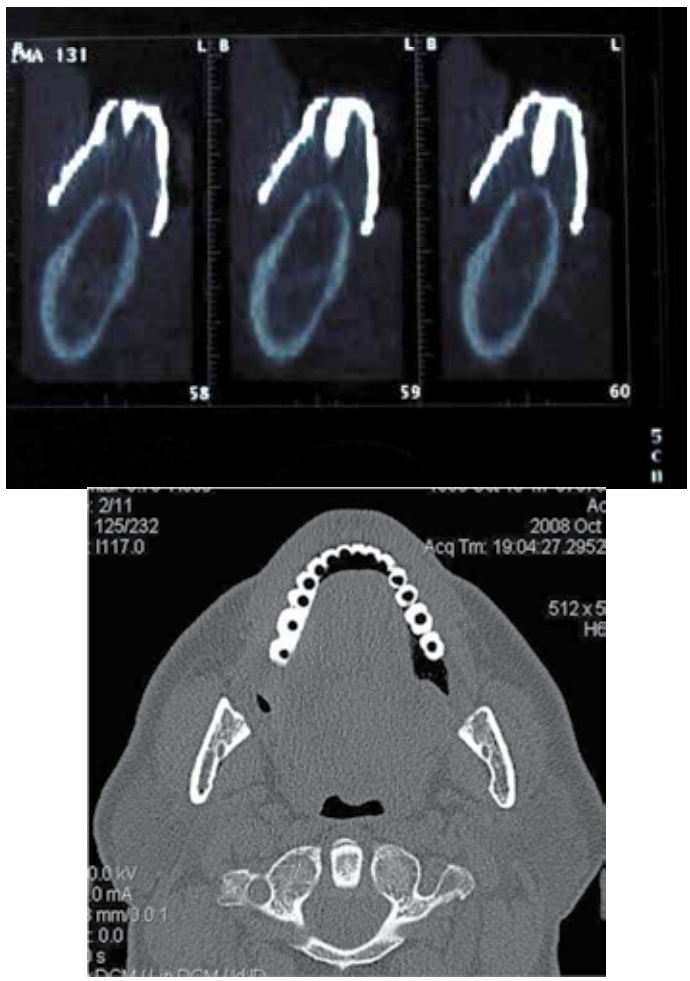

Fig. 4. CT scan images in two views, representing the access holes of radiographic template

In order to make 3D images of the anatomy, a 3D coordinate system must be established. Each slice of data has a location relative to the other slices given by the extent of the table indexed between acquisitions. The distance between slices develops the third dimension rather than the other 2 dimensions including right/left and up/down on each slice (Woolson et al,. 1986). The imaging software can interpret slices taken in a sequence in form

of a "volume" of data, while the overlap of slices must be inhibited. In order to create a 3D image of bone, for example, the slices are individually processed to extract only the tissue of bone density range. Where necessary, slices are interpolated between the original slices to give a more realistic 3D image. The edges (or contours) of the anatomy of interest, i.e. bone, are stored for the entire set of slices (including the interpolated ones) as a "contour file". The processed contour file is used to create the 3D images (Herman & Liu, 1977; Liu, 1977). In addition, the imaging software provides the capacity to alter or to edit the contour files themselves. This is useful for editing out obvious metal artifacts, doing disarticulation, or for designing implants. Since the contour file itself is being modified, subsequent 3D images generated from that file will show the results of the editing operation (Woolson et al, 1986).
After completion of the scan, the data are sent for data processing. A computer engineer edits the images by removing the scattered and useless parasite images including spinal column, antagonist teeth, and projection of the upper extremities of the mandible for maxillary analysis). The scanning template can be easily identified in axial sections. Different anatomic structures such as maxilla and mandible and the template are separated in different masks. Each of these masks can be toggled on or off to allow separate visualization and interpretation. After calculation of the corresponding 3D models, the patient data were sent back to the surgeon and prosthodontist so that the implant treatment plan could be started (Tardiue et al,. 2003).

5.4 Treatment planning

Advances in implant dentistry over the past 40 years facilitate the predictable replacement of missing teeth. It is now common to treat edentulous areas in the mouth partially and fully with implant-supported restorations. In the standard planning for a dental implant case, the doctor procures mounted dental models and designs a surgical guide based on the model. Conventional dental radiographs and CT scans help the practitioner determine where the underlying bone relates to the final prosthesis. But in surgery, a crestal incision with subperiosteal reflection of the gingival, a method that can inadvertently displace the model-based surgical guide, is in most cases required to determine the anatomy of the alveolar ridge. Implants can only be placed in a flapless manner in cases associated with excess amounts of alveolar bone. Even with a model-based surgical guide, placing the implant in the preplanned position depends on the skill of the operator. The use of model-based guides to position dental implants becomes even more demanding especially in placing multiple implants, as in an edentulous arch with limited bone available (Spector L, 2008).
The incorporation of treatment planning modified by CAD-CAM offers significant advantages, including the evaluation of the 3-D anatomy and the fabrication of anatomic site models and osseous-supported surgical templates. Other advantages include: shorter surgery times, shorter treatment times, minimizing of intraoperative radiographs during implant placement, less invasive surgical technique (flapless surgery, with less change of swelling, less pain, and faster initial healing times), prefabricated definitive prosthesis and immediate use of a fixed prosthesis (Sarment et al,. 2003). Furthermore, the surgical guides are useful to determine and insert the dental implant in appropriate position and length.
The software allows the practitioner to view the axial images and also the 3D image. In this stage the length, diameter and position of the implant body can be determined. Using the method, the flapless surgical technique for oral implant placement may be promoted and the invasiveness of surgical techniques may be reduced. The system under test was

observed to be reliable for the preoperative assessment of both the number and location of implants and implant size(s) needed, as well as potential anatomic complications. These procedures can be done using specialized software and also can be made using simple software. This method is very useful for fabrication of template in dentistry science field because it can eliminate rigid dependency on commercial companies and it can be used widely in clinical situation.

5.5 CAD/CAM surgical guide

CAD/CAM (computed-aided design/computed aided manufacturing) systems have evolved over the last two decades and have been used by dental health professionals for over twenty years. In 1971, Francois Duret introduced CAD/CAM in restorative dentistry and, in 1983; the first dental CAD/CAM restoration was manufactured (Priest, 2005). This system can be used in an intraoperative use for dental restoration and fabrication of surgical guides (Fuster-Torres et al,. 2009). Treatment planning decisions made with computer-aided design (CAD) can be easily transferred to the surgical treatment phase, be performed, in turn, with computer-aided manufacturing (CAM). It has been mentioned that CAD can be conducted through reading and interpreting multiplanar computerized tomography (CT) scans, performing measurements, and evaluating anatomic relationship by placing virtual images on the screen. In the CAM process, stereolithography method can be used for the fabrication of three dimensional surgical templates. The method includes a laser beam travelling above the photosensitive liquid acrylic, allowing the surgical template to be polymerized in the layers according to the design. Then, stainless steel tubes are inserted in the spaces that represent implant location. After insertion the tubes, the surgical template is ready for use. Thus, CAD/CAM surgical templates allow the software based planning to transfer to the surgical field. Template-guided implant surgery requires preoperative steps, starting with the fabrication of a radiographic template, CT acquisition with the template in position, computer-assisted implant planning and ending in fabrication and use of a surgical guide for drilling and implants insertion. Template-guided implant surgery requires pre-operative steps, starting with fabrication of a radiographic template, CT acquisition with the template in position, computer assisted implant planning and ending in fabrication and use of a surgical guide for drilling and implant insertion (Horwitz et al,. 2009).

5.6 Custom-made surgical guide

CT scans, when properly utilized, will help remove the limitations associated with two-dimensional imaging modalities for planning implants, and will empower clinicians with more diagnostic information to make informed decisions for their patients, especially when considering immediate-load protocols. The ability to visualize root, tooth-to-implant and implant-to-implant proximity through 3D technologies is an important step in the advancement of the science of dental implantology. Incorporating the restorative goal with interactive 3D planning tools for the creation of CT-derived surgical templates allows for the highest degree of accuracy and consistency in transferring the treatment plan to the patient at the time of surgical intervention. High-speed spiral CT image files can now be transferred to personal computers for further analysis and being used as reference, and computer programs are available to provide 3D jaw reconstruction and virtual surgical planning. With the advent of rapid prototyping techniques, virtual computer planning can be linked to the physical production of stereo models and surgical drill guides, which can then ensure an

accurate transfer of data from the planning phase to the clinical setting. Van Steenberghe et al developed a procedure for immediate implant loading in the maxilla using 3D planning and a custom drill template (Van steenberghe et al, 2002). Their preliminary results indicated that this procedure is effective, precise, and safe (Chow, 2005). Furthermore, horizontal stabilization pins (a minimum of one in partially edentulous cases and up to four in edentulous cases) are virtually placed through the labial denture flange to anchor the surgical template during surgery. After the restorative dentist and surgeon approve the virtual treatment plan, the three-dimensional computer planning files are digitally sent through for fabrication of the stereolithically constructed surgical template (Spector L, 2008).

5.6.1 Computer simulation and virtual implant planning

Computer simulation and virtual implant surgery are valuable tools developed for treatment planning. Three dimensional images allow clinicians a direct visualization of the jawbone morphology, bone volume, level of the maxillary sinus floor, and the location and course of the inferior dental nerve. Computer simulation also allows clinicians to place dental implants according to the future prosthetic tooth position. Furthermore, clinicians can study their cases alternatively and modify the virtual surgical implant placement until they are fully satisfied with the plan. Such surgical rehearsal is a kind of mental navigation system allowing further improvement of clinical performance. Computer simulation and virtual planning are, however, associated with some disadvantages including radiation hazards, and greater requirements for resources and time.

Originally, surgical drill guides were used only for the preparation of implant osteotomy. In 2003, Tardieu and Vrielinck introduced the SAFE System for dental implant placement using a single surgical guide for both the drilling and implant placement. When it is possible to control the position, angulation, and the depth needed for drilling and implant placement, this kind of template-navigated procedure can be safe and accurate (Tardieu & Vrielinck, 2003). Although the precision of the surgical drill guides is high, the accuracy of actual implant placement can be affected by the following factors: (1) correct positioning of the guide; (2) fitting the guide to the underlying structures (bone, mucosa, or tooth); (3) stabilization mechanisms (bony irregularities, pins, or screws); and (4) discrepancies between the diameter of the implant drill and the corresponding metal drill sleeve (Chow, 2005).

SurgiCase software allows for the regeneration of 3-dimensional images of the jaw anatomy, which can simulate the insertion of the fixtures. Even bone density in the area of the future implant location can be taken into account. This system contains cylindrical representations of oral implants, and their lengths and diameters can be modified according to the available product sizes of the implant manufacturer so as to obtain the best use of the available bone, and when possible, the contact with the cortical parts of the jaw bone (Sarment et al,. 2003). The magnitude of bone density allows developing a bone map, where it is possible to identify the sites that could assure the fixtures' major primary stability and offer better resistance to the biting forces (Vrielinck et al,. 2003).

The effectiveness of CAD/CAM surgical guides has been studied. For instance, Van Steenberghe et al (Van Steenberghe et al,. 2003) examined the accuracy of a CAD/CAM surgical guide in cadavers or Vrielinck et al (Vrielinck et al,. 2003) performed a similar study in human subjects. Vercruyssen (Vercruyssen & Quirynen, 2010) has discussed possible errors of CAD/CAM surgical guides, which may occur at any of the following stages: CT-scan data collection, positioning of the radiographic guide, segmentation of bone, teeth,

and/or tissue from the complete image, stereolithographic or CAD/CAM modeling, fixation of the surgical guide to the jaw bone, and use of precision sleeves. This type of precise surgical guide has also presented some challenges to clinicians. Yong and Moy studied early complications using CAD/CAM-guided implant placement with the NobelGuide system (Nobel Biocare AB, Goteborg, Sweden) claiming that the most common early surgical complication includes incomplete seating of the prosthesis due to bony interferences (Yong &Moy, 2008).

6. Stereolithography

Once CT scan data are segmented, the software interpolates the data on all 3 planes to form a smooth 3D model. A computer file of this model can be alternatively transferred to stereolithography equipment where a physical model of the patient's bone structure is created. The finalized treatment plan is thus used for fabrication of a surgical template using this technique.

The stereolithography process is a rapid prototyping method that produces physical models by selectively solidifying an ultraviolet sensitive liquid resin using a laser beam. It represents a relatively new technology in the diagnostic area. The status of this new methodology in clinical and surgical medicine is still at the prototype stage. It can be used for surgical simulation to assure predictable results and to diminish operation time (Choy JY, 2002). In oral implantology, this technology develops a precise evaluation of anatomic points such as the size of the maxillary sinus in the upper jaw and the location of the alveolar nerve in the lower jaw. It also provides information about size, direction, and bone location for accurate positioning of implants. In one study, the possible benefits in planning implant surgery using stereolithographic models and surgical guides relating to clinical guidance methodology have been indicated in detail (Sammartino G et al,. 2004).

From a detailed technical point of view, as point out earlier, stereolithography is a computer-driven process that creates precise models using laser and epoxy resin. A computer controlled HeCd laser generates an ultraviolet beam, which travels across the surface of a vat of photocurable liquid polymer. The laser draws each cross section of the anatomy one layer at a time. The photovoltaic energy from the laser polymerizes the epoxy immediately. The cured cross-sectional layer is lowered into the vat of resin and the next layer is processed. Successive layers of the anatomy are built in 0.15 mm increments until the model is completed (Herman GT& Liu HK, 1977).

Using this technique, surgical procedure would be simplified, reliable, and easily reproducible. Wound size and bone surface exposure would be minimal and no soft tissue trauma would be derived by use of burrs during bone drilling. Furthermore, during the operative procedure, there will no major problems of bleeding or nerve lesion. The postoperative time would be without complications.

This technique offers many advantages which include: correct management of the tissues with minimal trauma and a superior planned treatment. In cases of severe atrophy, this methodology allows fixture measurements by the indication of exact surgical limitations, and prevents complications related to poor stability of a denture. This technologic improvement can simplify oral management in implant dentistry and avoid complications related to the surgery (Sammartino G et al,. 2004).

The accuracy of anatomical models generated by this method depends on the quality of the CT scanners and the thresholding method (the computer process that determines what is

soft tissue), but studies have shown a dimensional stability in general in the range of 0.6 mm (Choi et al,. 2002). The accuracy of steriolithographically made surgical guides have been studied by different groups. Sarment (Sarment et al,. 2003) used SIMplant software for fabricating template. A significant statistical improvement was found in all measurements when stereolithographic surgical guide was used compared to conventional guides. The average distance between the planned implant and the actual osteotomy was 1.5±0.7mm in conventional group and 0.9± 0.5 in stereolithographic group. Measurement of the angle formed in conventional group was 8± 4.5 degrees with an accuracy of 4.5±2 degrees in the stereolithographic group. In the other in-vitro study reported by Van steenbergehe and coworkers (Van Steenberghe et al,. 2002), LITORIM software was used for the fabrication of the guide and the match between planned implant and actual implants in the maxilla was on average within 0.8± 0.3mm and the match between planned and actual implant axis was on average within 1.8±1 degrees. Naitoh and colleagues (Naitoh et al,. 2000) suggested that angulation diverges by 5 degrees on average when utilizing a template based on the CT images. Application of stereolithographic surgical guides presented by Giacomo (Giacomo et al,. 2005) gave rise the match between the planned and the placed implant axis within 7.25±2.67 degrees. The differences in distance between the planned and placed position at the implant shoulder were 1.45±1.42mm. Besimo and associates (Besimo et al,. 2000) utilized a modified scannographic template for surgical guidance. Placement was evaluated by measurement on the casts of more than 70 clinical cases. They found 1.5mm differences at entrance point when used control guide and 0.9mm when the stereolithographic surgical guide was used. Van sttenberghe (Van Steenberghe et al,.2002) evaluated placement of 45mm long zygoma implants on human cadavers. They reported less than 3 degrees of deviation. Fortin (Fortin et al,. 2003) found that transfer error was less than 0.2 mm and 1.1 degrees.

Tardieu (Tardieu et al,. 2003) presented a case of implant placement using a drill guide created by stereolithography. They suggested that the scannographic template could be designed for proper transfer into a temporary fixed prosthesis which can be used in immediate loading. Mesiodistal accuracy influences implants from esthetic point of view especially in anterior region because the proper embrasures and golden proportional are not established yet. Length difference may also cause complication. A maximum length of 2 mm above the mandibular canal is generally recommended. If the length exceeds this limitation, it may cause parasthesia and pain leading finally to the removal of implant. Otherwise, if the length is very short, crown to implant ratio may be improper and biomechanical factors compromise the implant integrity. The accuracy of angle causes the forces directed towards the implant long axis.

The main drawback of the surgical template can be seen in the possible movement of the template during surgery and reproducibility of the splint position between the CT exam and surgical procedure. The template is supported on the remaining dentition or stabilized by a specific form of either the hard palate or the mandible. Sicilia (Sicilia et al,. 2000) reached a considerably higher precision using a fixed surgical template in the placement of implants in patients with edentulous maxilla. In a large edentulous area or completely edentulous jaw, the stereolithographic guide is advantageous since it is osseous-supported. The degree of the difference between the proposed and actual implant direction may be influenced by various factors, such as the construction accuracy of the template, the surgical accuracy using these templates, the accuracy of the study model, the accuracy of the stereolithographic machines and the measurement accuracy. The CT scan involves a higher dose/higher cost method. But the CT scan is less time consuming when multiple implants

are required, and it allows imaging of the entire jaw, making it possible to use software for virtual implant placement (Nokar et al,. 2012). As long as radiographic imaging has been enhanced by the development of various techniques , multi-slice and spiral CT are being replaced by cone beam CT systems (CBCT) for oral and maxillofacial imaging, enabling a significant reduction dose (Loubele et al,. 2008).

7. Simplified CAD/CAM technique

This method has been recently developed to generate surgical guide by a much simpler method based on CAD/CAM. For making CAD/ CAM template, anatomic guide was digitized by ATOSII camera (optical measuring technique).The ATOS system was used to digitize the objects and process the data into an STL data set. Some reference points were applied onto the model. By using the photogrammetry camera and its supplied software, coordinates of the points were measured with high accuracy. Three reference markers were applied to each part added. At this stage, a second series of images were taken and the visible reference point coordinates were measured on these images. Using the points on the model, obtained with two sets of measurements, the second set of the points was transformed into the coordinate system that was previously defined after the first measurement. In the next step, all the measurement points were changed to IGES format with Rapid form software and consequently the IGES format was changed to SLD format using Solid work software. This model was assembled on the jaw model. The implant access holes were evaluated and the implant path and length in each tooth were determined. The length of implants was considered 2 mm above the mandibular canal and the diameter of implant holes was increased up to 4mm (Fig 5).

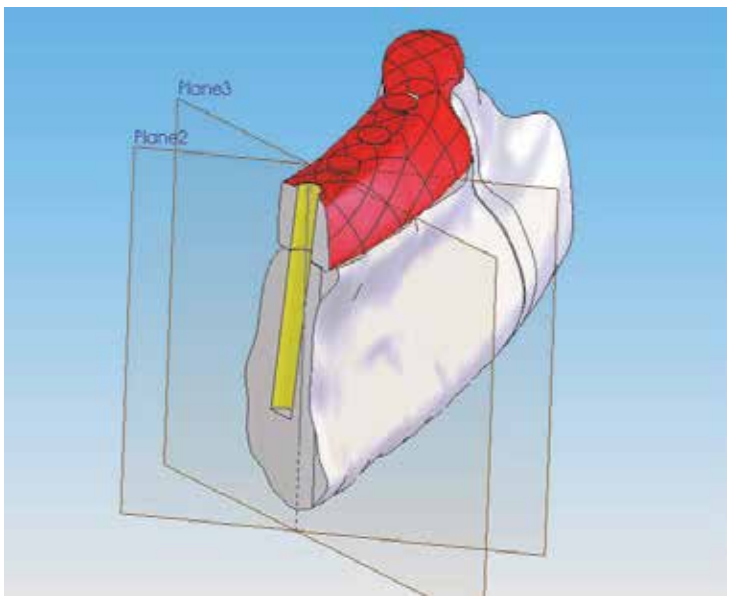

Fig. 5. Drilling direction and implant length determined in Solid work software.

This model, named gold standard model, was filed and applied for evaluating the results. After designing the implant path and length, the CAD-CAM surgical template was

fabricated using stereolithographic rapid prototyping machine. Then, the stainless steel sleeves were fabricated to be used as drills of implants in two different sizes. The sleeves were, then, inserted in the CAD-CAM template. The sleeves kept the planned implants length and diameter as designed. In the first group, concerning the anatomical surgical template, implant path was modified via measuring CT scan file. For the second group, there was stereolithographic template with incremental guiding tube diameters, fabricated by CAD-CAM techniques (Fig 6).

Fig. 6. Fabrication of the guide by stereolithography machine.

8. Conclusion

In this chapter an overview to different techniques of pre-surgical diagnosis and treatment planning with emphasis on computer assisted methods was given. At first the importance and all effective parameters in implantology were discussed. In the second section different imaging modalities including intra- oral and extra- oral were reviewed. The next parts described more recent pre-surgical diagnostic procedure which reveals that computer assisted methods enhance the capability of prosthodontists to diagnose and choose a convenient and easy to apply methods for implant placement.

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Bone Quality Assessment for Dental Implants

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1. Introduction

Dental implants have become a predictable treatment option for restoring missing teeth. The purpose of tooth replacement with implants is to restore adequate function and esthetics without affecting adjacent hard and/or soft tissue structures. The use of dental implants in oral rehabilitation has currently been increasing since clinical studies with dental implant treatment have revealed successful outcomes (Turkyilmaz et al., 2008a). The successful outcome of any implant procedure depends on a series of patient-related and procedure-dependent parameters, including general health conditions, biocompatability of the implant material, the feature of the implant surface, the surgical procedure, and the quality and quantity of the local bone. (Turkyilmaz et al., 2007)
Successfully providing dental implants to patients who have lost teeth and frequently the surrounding bone relies on the careful gathering of clinical and radiological information, on interdisciplinary communication and on detailed planning. One of the most important factors in determining implant success is proper treatment planning. In the past, periapical radiographs along with panoramic images were used as the sole determinants of implant diagnosis and treatment planning. With the advancement of radiographic technology, Computed tomography (CT), as well as cone-beam computed tomography (CBCT) is increasingly considered essential for optimal implant placement, especially in the case of complex reconstructions (Benson & Shetty, 2009; Chan et al., 2010; Resnik et al., 2008).

2. Radiologic examination

The objectives of diagnostic imaging depend on a number of factors, including the amount and type of information required and the period of the treatment rendered. The desicion to image the patient is based on the patient's clinical needs. After a desicion has been made to obtain images, the imaging modality is used that yields the necessary diagnostic information related to the patient's clinical needs and results in the least radiologic risk (Resnik et al., 2008).
The ideal imaging technique for dental implant care should have several essential characteristics, including the ability to visualize the implant site in the mesiodistal, bucco-lingual and superioinferior dimensions; the ability to allow reliable, accurate measurements; a capacity to evaluate trabecular bone density and cortical thickness; reasonable access and cost to the patient and minimal radiation risk (Benson & Shetty, 2009). Diagnostic imaging is an integral part of dental implant therapy for preoperative planning, intraoperative assessment, and postoperative assessment by use of a variety of imaging techniques.

2.1 Selecting imaging technique for preoperative implant planning

The objectives of the preoperative implant imaging include all necessary surgical and prosthetic information to determine the quantity, quality and angulations of bone; selection of the potential implant sites, and to verify absence of pathology. However, there is no ideal imaging technique in the field of oral implantology that would be acceptable for all patients. All imaging techniques have inherent advantages and disadvantages (Resnik et al. 2008).

In dental and medical radiology, a recommended principle when selecting the appropriate radiographic modality is based on radiologic dosage. Obviously, the goal is to choose a radiographic method providing sufficient diagnostic information for treatment planning with the least possible radiation dose (ALARA principle: as low as reasonably achievable) and costs for the patient. The preferred imaging procedure for this purpose seems to vary much among different parts of the world as well as among individual dentists.

2.1.1 Intraoral radiography

Traditionally, conventional radiographic images e.g., periapical and panoramic images have been used to assist practitioners in planning implant treatment. Periapical radiographs commonly are used to evaluate the status of adjacent teeth and remaining alveolar bone in the mesiodistal dimension. In addition they have been used for determining vertical height, architecture and bone quality (bone density, amount of cortical bone and amount of trabecular bone). Although readily available and relatively inexpensive, periapical radiography has geometric and anatomic limitations. If the paralleling technique is not used, periapical radiographs create an image with foreshortening and elongation (Benson & Shetty, 2009; Chan et al., 2010). When the x-ray beam is perpendicular to the film, but the object is not parallel to the film, foreshortening will occur. If the x-ray beam is oriented perpendicular to the object but not the film, elongation will occur. The most accurate intraoral radiographic technique used for implant planning is the paralleling technique. These principles in positioning will allow for an intraoral image with minimal distortion and magnification. Therefore, standardized periapical radiographs with bite-blocks by using paralling technique should be perform to the longitudinal studies (Benson & Shetty, 2009; Resnik et al., 2008).

Because the periapical radiographs are unable to provide any cross-sectional information, occlusal radiographs are used to determine bucco-lingual dimensions of the mandibular alveolar ridge. However, the occlusal image records only the widest portion of the mandible, which typically is located inferior to the alveolar ridge. This may give the clinician the impression that more bone is available in the cross-sectional dimension than actually exists. The occlusal technique is not useful for the maxillary arch because of the anatomic limitations (Benson & Shetty, 2009).

2.1.2 Panoramic radiography

Panoramic radiographs have been used frequently as a radiographic method for preimplant evaluation and the preparation of treatment protocols. Although the resolution and sharpness of panoramic radiographs are less than those of intraoral radiographs, panoramic radiographs is an excellent tool for the overview of the maxillofacial area, including many of the vital structures, such as maxillary sinus, inferior alveolar nerve and nasal fossa. Panoramic radiography units are widely available, making this imaging technique very useful and popular as a screening (Benson & Shetty, 2009; Chan et al., 2010). (Figure 1)

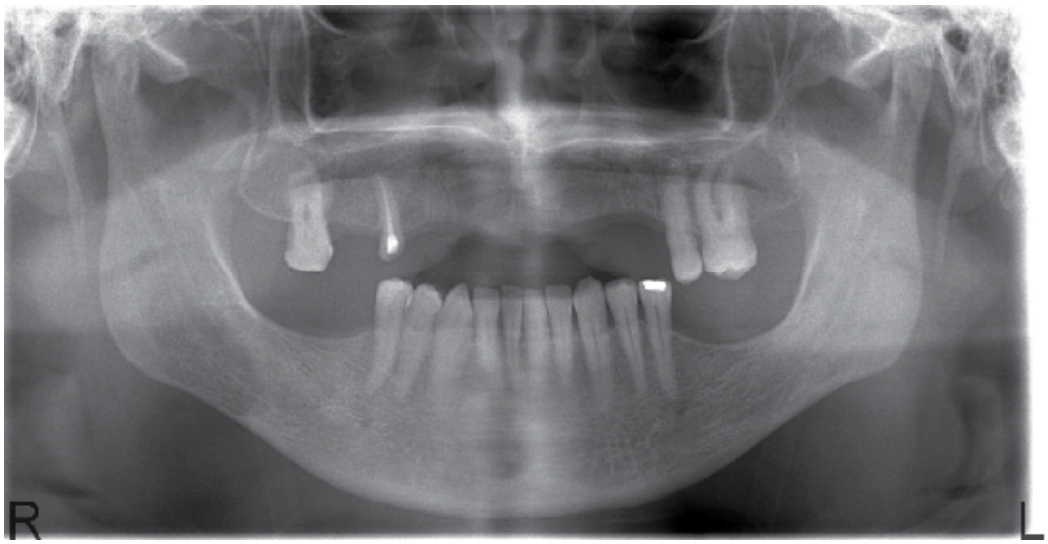

Fig. 1. Panoramic radiography before implant placement

Information acquired from panoramic radiographs must be applied judiciously because this technique has significant limitations as a definitive preoperative planning tool. With regard to panoramic radiographs, the lack of image sharpness and resolution, coupled with nonuniform distortion often leads to inaccurate interpretation and measurements (Benson & Shetty, 2009; Chan et al., 2010). The magnification of panoramic radiographs can be >30%, especially when patients are not in the optimal position. Angular measurements on panoramic radiographs tend to be accurate, but linear measurements are not. Vertical measurements are unreliable because of foreshortening and elongation of the anatomic structures because the x-ray beam is not perpendicular to the long axis of the anatomic structures or to plane of the image receptor. Similarly, dimensional accuracy in the horizontal plane of panoramic radiographs is highly dependent on the position of the structures of interest relative to the central plane of the image layer (Benson & Shetty, 2009). However, the magnification factor can be calculated at the given site by dividing the actual diameter of the object by the diameter measured on the radiographs. Diagnostic templates that have ball bearings or wires incorporated around the curvature of the dental arch and worn by the patient during the panoramic examination enable the dentist to determine the amounts of magnification in the radiograph (Resnik et al., 2008).

2.1.3 Computed tomography

Clinicians have been diagnosing, treatment planning, placing and restoring dental implants using periapical and panoramic radiographs to assess bone anatomy for several decades. Two dimensional images have been found to have limitations because of inherent distortion factors and the non-interactive nature of film itself provides. With the advent of technology, CT has lead to a new era of implant imaging.

CT enables the evaluation of proposed implant sites and provides diagnostic information that other imaging or combinations of imaging techniques cannot provide. CT has several advantages over conventional radiography. First, CT eliminates the superimposition of images of structures outside the area of interest. Second, because of the inherent high-

contrast resolution of CT, differences between tissues that differ in physical density bt less than 1% can be distinguished; conventional radiography requires a 10% difference in physical density to distinguish between tissues. Third, data from a single CT imaging procedure, consisting of either multiple contiguous or one helical scan, can be viewed as images in the axial, coronal or sagittal planes or in any arbitrary plane depending on the diagnostik task. This is referred to as multiplanar reformatted imaging (Frederiksen, 2009). (Figure 2) Direct images are problematic in the coronal plane because of difficulties in positioning the patient and metallic artifacts from dental materials. For this reason, special software programs have been developed to reformat the data from axial CT scans into the sagittal and coronal planes or any other arbitrary plane (Benson & Shetty, 2009; Chan et al., 2010; Resnik et al., 2008). DentaScan provides programmed reformation, organization and display of the imaging study. The radiologist simply indicates the curvature of the maxillary and mandibular arch, and the computer is programmed to generate referenced cross-sectional and tangential or panoramic images of the alveolus along with three-dimensional images of the arch. The cross-sectional and panoramic images are spaced 1 mm apart and enable accurate preoperative treatment planning (Resnik et al., 2008).

The individual element of the CT image is called a voxel, which has a value, referred to in Hounsfield units (HU), that describes the density of the CT image at that point. HU also known CT numbers, range from -1000 (air) to +3000 (enamel), each corresponding to a different level of beam attenuation (Benson & Shetty, 2009; Frederiksen, 2009; Resnik et al., 2008). The density of structures within the image is absolute and quantitative and can be used to differentiate tissues in the region (i.e., muscle, 35–70 HU; fibrous tissue, 60–90 HU, cartilage, 80–130 HU; bone 150–1800 HU) and characterize bone quality (D1 bone, >1250 HU; D2 bone, 750–1250 HU; D3 bone, 375–750 HU; D4 bone, <375 HU) (Misch, 2008).

The utility of CT for dental implant treatment planning was evident, but the access to these imaging techniques is limited. Nevertheless, CT scans are not without their limitations/concerns and radiation exposure and cost are the major two (Benson & Shetty, 2009; Chan et al., 2010, Scarfe & Farman, 2008).

2.1.4 Cone-beam computed tomography

Because of higher radiation exposure, higher cost, huge footprint, and difficulty in accessibility associated with CT, CBCT was developed. As the name implies, CBCT generates cone-shaped beams and the images are acquired in one rotation by an image intensifier of flat panel detector, resulting in reasonably low levels of radiation dosage (Arai et al., 1999; Chan et al., 2010; Scarfe & Farman, 2008). During the rotation, multiple (from 150 to more than 600) sequential planar projection images of the field of view (FOV) are acquired in a complete, or sometimes partial arch. Obvious advantages of such a system, which provides a shorter examination time, include the reduction of image unsharpness caused by the translation of the patient, reduced image distortion due to internal patient movements, and increased x-ray tube efficiency. However, its main disadvantage, especially with larger FOVs, is a limitation in image quality related to noise and contrast resolution because of the detection of large amounts of scattered radiation (Scarfe & Farman, 2008).

The resolution and therefore detail of CBCT imaging is determined by the individual volume elements or voxels produced from the volumetric data set. In CBCT imaging, voxel dimensions primarily depend on the pixel size on the area detector, unlike those in CT, which depend on slice thickness. The resolution of the area detector is submillimeter.

Therefore, the theoretical resolution of CBCT is higher than CT (Scarfe & Farman, 2008; 2009). In the literature, the accuracy of CT and CBCT in the assessment of implant site dimensions were compared and CBCT measurements found more accurate than CT measurements (Al-Ekrish & Ekram, 2011; Kobayashi et al., 2004; Loubele et al., 2008; Suomalainen et al., 2008).

The reformetted images of CBCT data result in three basic image types; axial images with a computer generated superimposed curve of the alveolar process and the associated reformatted alveolar cross-sectional images and panoramic-like images. Such reformatted images provide the clinician with accurate two dimensional diagnostic information in all three dimensions. Both CT and CBCT images provide information on the continuity of the cortical bone plates, residual bone in the mandible and maxilla, the relative location of adjoining vital structures and the contour of soft tissues covering the osseos structures (Benson & Shetty, 2009; Scarfe & Farman, 2008).

Voxel values obtained from CBCT images are not absolute values, like HU values obtained using CT, various methods have been proposed to evaluate the bone density (Naitoh et al. 2009; 2010; Mah et al., 2010). HU provide a quantitative assessment of bone density as measured by its ability to attenuate an x-ray beam. To date, there was not any standard system for scaling the grey levels representing the reconstructed values. In a study, (Katsumata et al., 2007), the authors found that calculated HU on a CBCT scan varied widely from a range of -1500 to over +3000 for different types of bone. However, after a correction has been applied to grey levels with the CBCT, the HU values are much similar to those one would expect in a medical CT device than to the original grey levels obtained from the CBCT scanners (Naitoh et al. 2009; 2010; Nomura et al., 2010, Mah et al., 2010).

The clinical utility of preoperative implant planning by use of in imaging stent that helps relate the radiographic image and its information to a precise anatomic location or a potential implant site. The intended implant sites are identified by radiopaque markers retained within an acrylic stent which the patient wears during the imaging procedure so that images of the markers will b created in the diagnostic images. The imaging stent subsequently may be used as a surgical guide to Orient the insertion angle of the guide bur and hence the angle of the implant. Generally, nonmetallic radiopaque markers are used in CT and CBCT imaging (Benson & Shetty, 2009).

The availability of CBCT is also expanding the use of additional diagnostic and treatment software applications. CBCT permits more than diagnosis, it facilitates image-guided surgery. Diagnostic and planning software are available to assist in implant planning to fabricate surgical models (eg, Biomedical Modeling Inc., USA); to facilitate virtual implant placement,; to create diagnostic and surgical implant guidance stents (eg, Virtual Implant Placement, Implant Logic Systems, Cedarhurst, USA; Simplant, Materialise, Belgium; Easy Guide, Keystone Dental, USA) and even to assist in the computer-aided design and manufacture of implant prosthetics (NobelGuide/Procera software, Nobel Care AG, Sweden) (Scarfe & Farman, 2008). When those programs are applied, different diameters and length of implants can be 'tried in' before the most optimal one is selected. Furthermore, the placed implant can be assessed from several different viewpoints as well as from three dimensional view. Moreover, once treatment planning is determined in the computer, it can be saved and applied to surgical sites by means of image-aided template production or image-aided navigation. It is important to note that although computer aided implant placement is a promising technique, the unexpected linear and anguler deviation can be a major concern (Chan et al., 2010; Ganz, 2008).

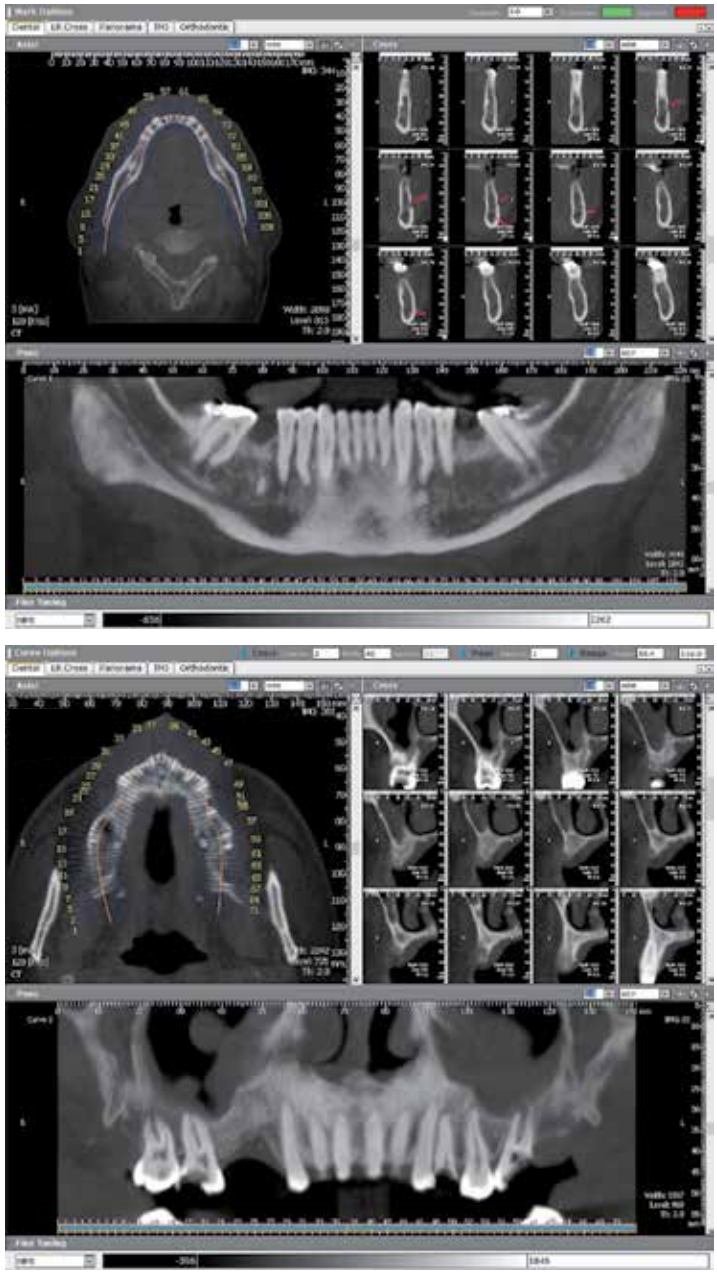

Fig. 2. CT images of preoperative implant site.

2.2 Bone quality assessment of implant sites

Several factors, such as implant geometry, preparation technique, and quality and quantity of local bone influence primary stability, and primary implant stability is one of the main factors influencing implant survival rates. (Friberg et al., 1991; Meredith, 1998, Turkyilmaz & McGlumphy, 2008a).

2.2.1 Implant stability measurements

Implant stability can be measured by non-invasive clinical test methods (i.e., insertion torque, the periotest, resonance frequency analysis). One of these quantitative methods is the *insertion torque* described by Johansson and Strid (1994). This method records the torque required to place the implant and provides valuable information about local bone quality.
Another method, named *Periotest*, has been developed to measure the degree of the periodontal integration of teeth and the stiffness of the bone/implant interface (Olive & Aparicio, 1990; Turkyilmaz & McGlumphy, 2008b). The Periotest instrument measures the deflection/deceleration of a tooth or implant that has been struck by a small pistil from inside the instrument's hand piece. The contact time of the accelerated pistil to the implant, which moves according to the strike, is calculated into a value called the Periotest value. However, Periotest values include only a narrow range over the scale of the instrument and thus, provide relatively less sensitive information about implant stability. Therefore, its benefit on detection of osseointegration is a matter of debate.
Another method, *resonance frequency analysis* (RFA) has been introduced by Meredith and coworkers (1996). In RFA, the stiffness of the bone/ implant interface is calculated from a resonance frequency as a reaction to oscillations exerted onto the implant/bone system. The implant is excited with an oscillating transducer screwed onto the implant and the resonance specific to the resonance system 'implant/bone' is captured electronically over a range of 5 to 15 kHz. RF values have clinically been correlated with changes in implant stability during osseous healing, failure of implants to integrate and the supracrestal dimensions of the implant. The results of a histomorphometric study suggested that RFA values correlated well with the amount of bone-to-implant contact. These findings support the use of RFA in evaluating changes in the bone healing and osseointegration process following implant placement (Turkyilmaz & McGlumphy, 2008b).

2.2.2 Bone quality and quantity

The term *bone quality* is commonly used in implant treatment and in reports on implant success and failure. Lindh et al. (2004) emphasized that *bone density (Bone Mineral Density, BMD)* and *bone quality* are not synonymous. Bone quality encompasses factors other than bone density such as skeletal size, the architecture and 3-dimensional orientation of the trabeculea, and matrix properties. Bone quality is not only a matter of mineral content, but also of structure. It has been shown that the quality and quantity of bone available at the implant site are very important local patient factors in determining the success of dental implants (Drage et al., 2007; Lindh et al., 2004).
The success rate obtained with dental implants depends to a great extent on the volume and quality of the surrounding bone. Therefore, it is important to know the bone quantity and quality of the jaws when planning implant treatment. Bone quantity of jawbone is broken down into five groups (from minimal to severe, A- E), based on residual jaw shape different rates of bone resorption following tooth extraction (Ribeiro-Rotta et al., 2010). During all stages of atrophy of the alveolar ridge, characteristic shapes result from the resorptive process. It is difficult to obtain implant anchorage in bone that is not very dense. Sufficient bone density and volume are therefore crucial factors for ensuring implant success (Lekholm & Zarb, 1985).
Bone quality is broken down into four groups according to the proportion and stucture of compact and trabecular bone tissue (Ribeiro-Rotta et al., 2010). Bone quality is categorized into four groups: groups 1-4 or type I to IV (Bone Quality Index-BQI) (Figure 3).

Type I: homogeneous cortical bone;
Type II: thick cortical bone with marrow cavity;
Type III: thin cortical bone with dense trabecular bone of good strength;
Type IV: very thin cortical bone with low density trabecular bone of poor strength.

Type I Type II Type III Type IV

Fig. 3. Bone Quality Index

In the jaws, an implant placed in poor-quality bone with thin cortex and low-density trabeculae (Type IV bone) has a higher chance of failure compared with the other types of bones. This low density bone is often found in the posterior maxilla and several studies report higher implant failure rates in this region (Bryant, 1998; Drage et al., 2007; Penarrocha et al., 2004). When compared to the maxilla, clinical reports have indicated a higher survival rate for dental implants in the mandible, particularly in the anterior region of the mandible, which has been associated with better volume and density of the bone (Turkyilmaz et al., 2008). Histomorphometric studies show that the posterior maxilla has a lower trabecular volume, with a reduction in the thickness and number of trabeculae (Drage et al., 2007). Regional differences in jaw anatomy and bone structure may explain some of the variation in clinical success rate of implant therapy in the maxilla and the increased rate of residual ridge resorption reported in the mandible. Surveys have shown that implant therapy in the maxilla has a significantly higher clinical failure rate than that in the mandible, and regional differences in maxillary BMD may be partly responsible (Devlin et al., 2008).
Mish (2008) defined four bone density groups (D1 to D4) in all regions of the jaws that vary in both macroscopic cortical and trabecular bone types. The homogeneous, dense D1 bone type presents several advantages for implant dentistry. The cortical lameller bone may heal with little interim woven bone formation, ensuring excellent bone strength while healing next to the implant. D1 bone is more often found in anterior mandibles with moderate to severe resorption. The percentages of light microscopic contact of bone at the implant interface is greatest in D1 bone type and greater than 80%. In addition, this bone density exhibits greater strength than any other type. The strongest bone also benefits from the greatest bone-implant contact. Less stresess are transmitted to the apical third of the implants than other bone types. D1 bone has fewer blood vessels than the other three types, and therefore it is more dependent on the periosteum for its nutrion. The cortical bone receives the outer one third of all its arterial and venous supply from the periosteum. This bone density is almost all cortical and the capacity of regeneration is impaired because of the poor blood circulation. Also, greater heat is often generated at the apical portion of the D1 bone. D2 is a combination of of dense-to-porous cortical bone on the crest and coarse trabecular bone on the inside. The D2 bone trabeculae are 40% to 60% stronger than D3

tarbeculae. This bone type occurs most frequently in the anterior mandible, followed by the posterior mandible. On occasion it is observed in the anterior maxilla, especially for a single missing tooth. D2 bone provides excellent implant interface healing, and osseointegration is very predictable. The intrabony blood supply allows bleeding during the osteotomy, which helps control overheating during preparation and is most beneficial for bone-implant interface healing. D3 is composed of thinner porous cortical bone on the crest and fine trabecular bone within the ridge. The trabecula are approximtely 50% weaker than those in D2 bone. D3 bone is found most often in the anterior maxilla and posterior regions of the mouth in either arch. The D3 anterior maxilla is usually of less width than its mandibular D3 counterpart. The D3 bone is not only 50% weaker than D2 bone, the bone-implant contact is also less favorable in D3 bone. The additive factors can increase the risk of implant failure. D4 bone has very little density and little or no cortical crestal bone. It is the opposite spectrum of D1 (dense cortical bone). The most commen locations for this type of bone are the posterior region of the maxilla. It is rarely observed in mandible. The bone trabeculae may be up to 10 times weaker than the cortical bone of D1. The bone-implant contact after initial loading is often less than 25%. Bone trabeculae are sparse and, as a result, initial fixation of any implant design presents a surgical challenge (Misch, 2008).

2.2.3 Bone mineral density measurements

BMD is the amount of bone tissue in a certain volume of bone. Assessment of jaw BMD may be considered useful in implant planning (Gulsahi et al., 2010). Several approaches have been introduced to measure jawbones and skeletal bones density. Densitometric measurements of panoramic and periapical radiographs have been used, as have more advanced methods such as Dual Energy X-Ray Absorptiometry (DEXA), CT and CBCT.

By including and referencing an aluminum step-wedge standard image with each exposure, densitometric evaluation of periapical or panoramic radiographs can be performed (Figure 4). Equal thicknesses of mineralized tissue and aluminum produce similar radiographic densities. The optical density of the jawbone site, and each step of the stepwedge is measured on the reference radiograph, and the values are plotted against the corresponding thickness of aluminum. The curve is obtained provided the corresponding aluminum equivalents in millimeters to the measured mean optical density of the jawbone (Gulsahi et al., 2007).

DEXA is a technique that enables fast, noninvasive, and highly precise measurement of BMD). In daily clinical practice, DEXA is the most useful method for BMD assessment in the vertebrae, femoral neck, and forearms. This technique was introduced in 1987. Its operation is based on the principle that bone and soft tissue exhibit different properties of attenuation as a function of photon energy. Therefore, DEXA uses an x-ray source to produce a beam of discrete energies that is attenuated as it travels through the patient. The radiation dose is low enough to allow BMD measurements in different skeletal sites and in longitudinal studies (Devlin et al. 1998; Hildebolt, 1997; Hildebolt et al., 1993; von Wovern, 2001). Most studies have examined mandibular or maxillary BMD by DEXA (Drage et al., 2007; Drozdzowska et al., 2002; Gulsahi et al., 2007; Gulsahi et al., 2010; Horner & Devlin, 1998a, 1998b; Pluskiewicz et al., 2000). Studies revaled that maxillary BMD is lower than mandibular BMD (Devlin et al., 1998; Drage et al., 2007; Gulsahi et al., 2010). However, the relation between the jawbone BMD and other skeletal sites BMD is still controversy (Figure 5).

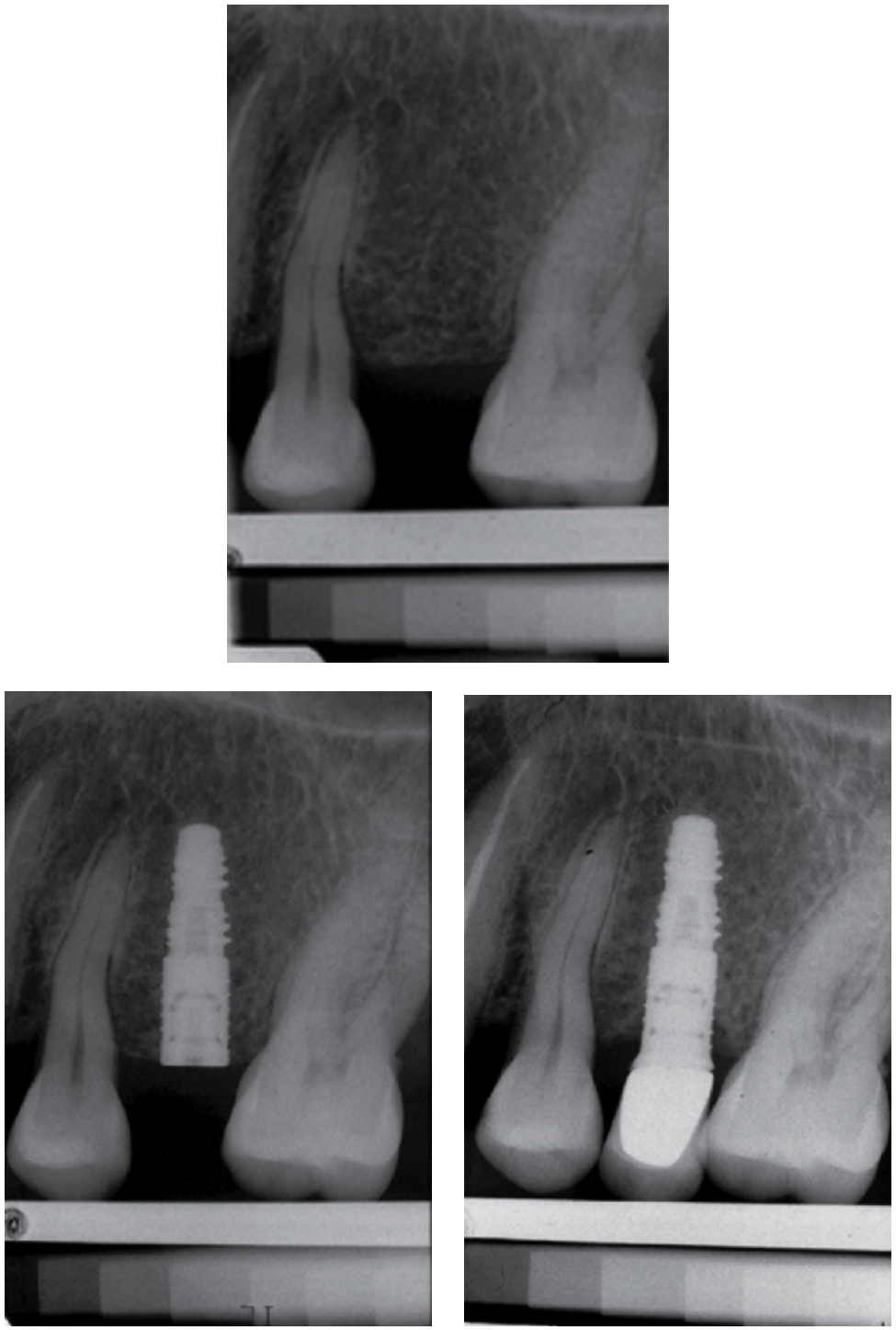

Fig. 4. Periapical radiographs obtained with aluminum stepwedge for densitometric evaluation before and after implant placement.

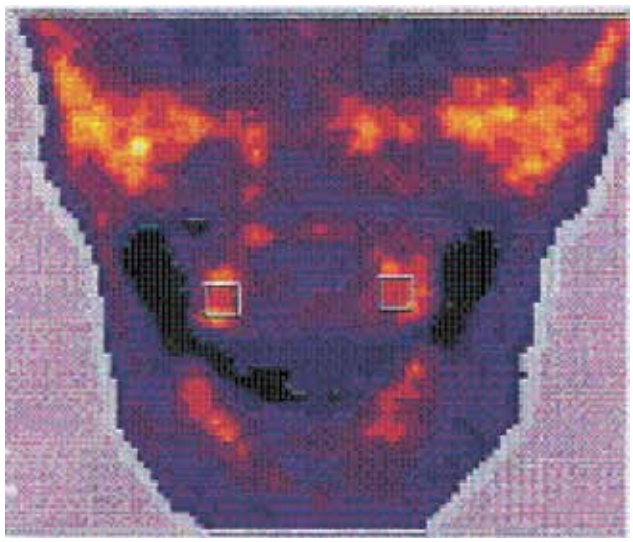

Fig. 5. DEXA measurement before implant placement.

Qualitative and quantitative indexes, including the mandibular cortical index (MCI), mental index (MI) or panoramic mandibular index (PMI) have also been used for panoramic radiographs to assess the bone quality. MCI is the appearance of the inferior mandibular cortical thickness as follows; C1; the endosteal margin of the cortex is even and sharp on both sides, C2; the endosteal margin shows semilunar defects (lacunar resorption) and/or seems to form endosteal cortical residues on one or both sides, C3; the cortical layer forms heavy endosteal cortical residues and clearly porous (Klemetti et al., 1994) (Figure 6).
MI is measurement of the cortical width at the mental foramen region (Ledgerton et al., 1999).

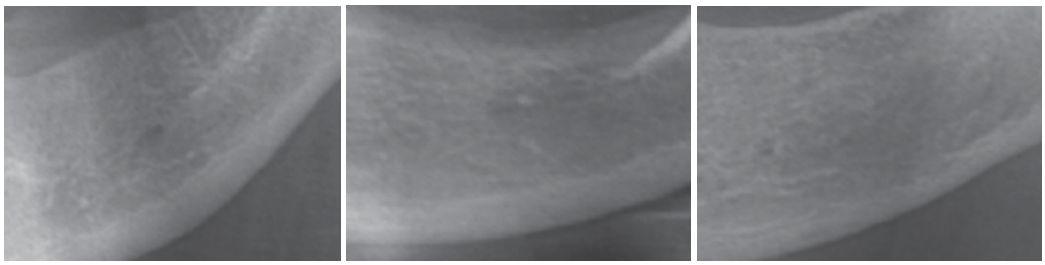

Fig. 6. Shows the C1, C2 and C3 classification of mandibular cortex index.

The inferior PMI is the ratio of the thickness of mandibular cortex to the distance between the inferior margin of mental foramen and the inferior mandibular cortex (Benson et al., 1991) (Figure 7). Some authors concluded that panoramic radiomorphometric indices significantly correlated with mandibular BMD (Horner & Devlin 1998a, 1998b; Drozdzowska et al. 2002). However in a study, there was no found such a correlation (Gulsahi et al. 2010).

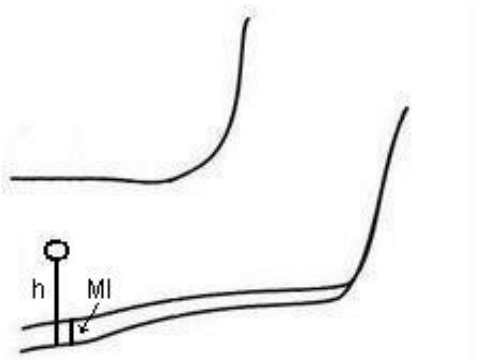

Fig. 7. Shows the MI and PMI (MI/h) measurements.

Assessments have primarily been made of the bone tissue status of the entire jaw, and site-specific variations have been ignored, as have the consequences of differences between the compact and trabecular parts of jawbone tissue. CT is the only method that allows the components of trabecular and compact bone tissue to be investigated separately (Lindh et al., 2004). With CT, it is possible to measure bone density that its effect on the survival of the implant can be estimated. Norton & Gamble (2001) suggested an objective scale of bone density that was besed on mean HU values taken from CT and could be used for bone tissue classification before implant treatment. They reported the mean bone density from CT was 682 HU for 139 sites. They recorded that the mean bone densities in the anterior mandible, the posterior mandible, the anterior maxilla, the posterior maxilla were 970, 669, 696, and 417 HU respectively. Shapurian et al. (2006) reported that the average bone density values in the anterior mandible, the anterior maxilla, the posterior maxilla, the posterior mandible were 559, 517, 333 and 321 HU for 219 implant sites. When considering all implant sites, the mean bone density was 887±180 HU in the other study (Turkyilmaz & McGlumphy, 2008a), which is higher than those reported earlier (Norton & Gamble, 2001; Shapurian et al., 2006). However, in the other study, variations in bone density between different regions of maxilla were found (Lindh et al., 2004). Within individuals, both total BMD and trabecular BMD values were higher in the cuspid-frontal regions than in the posterior region of maxilla. In addition, a significant correlation was found between the total BMD and trabecular BMD and between the mean BMD values and mean HU values. The large variations between the BMD of the different region in the maxilla or mandible emphasize the importance of the site-specific measurements of tissue before implant placement. In the study, the authors noted that it is important that an objective tool for the evaluation of bone tissue is found so that clinicians can more easily determine whether to load the implant immediately, earlier or later (Ericsson et al., 2002).

2.3 Intraoperative and postoperative assessments

Intraoral and panoramic radiographs usually are adequate for both intraoperative and postoperative assessments. Intraoperative imaging may be required to confirm correct placement of the implant or to locate a lost implant. The two aspects that are usually assessed with time after implant placement are the alveolar bone height around the implant and the appearance of the bone immediately adjacent to and surrounding the implant. In general, periapical radiographs are appropriate for longitudinal assessments. The

angulation of the x-ray beam must be within 9 degrees of the long axis of the fixture to open the threads on the image on most threaded fixtures (Benson & Shetty, 2009).
In evaluating the the bone height around an implant, an effort should be made to reproduce the vertical angulation of the central ray of the x-ray beam as closely as possible between radiographs. Distal and mesial marginal bone height is measured from a collar of the implant, or in tha case of threaded implants by use of known interthreaded measurements and compared with bone levels in previous periapical radiographs. The presence of relatively distinct bone margins with a constant height relative to the implant suggests successful osseos integration. Any resorptive changes, if present, are evidenced by apical migration of the alveolar bone or distinct osseos margins. Radiographic studies suggest that the rate of marginal bone loss after successful implantation is approximately 1.2 mm in the first year, subsequently tapering off to about 0.1 mm in succeeding years (Benson & Shetty, 2009).
The success of an implant can also be evaluated by the appearance of normal bone surrounding it and its apposition to the surface of the implant body. The development of a thin radiolucent area that closely follows the outline of the implant usually correlates to clinically detectable implant mobility, it is an important indicator of failed osseointegration. Changes in the periodontal ligament space of associated teeth are also useful in monitoring the functional competence of the implant-prostheses composite. Any widening of the periodontal ligament space compared with baseline radiographs indicates poor stress distribution and forecasts implant failure (Benson & Shetty, 2009).
After successful implantation, radiographs may be made at regular intervals to assess the success or failure of the implant fixture (Benson & Shetty, 2009).

3. Conclusion

In summary, diagnostic imaging is an integral part of dental implant therapy for preoperative planning, intraoperative and posoperative assessment by use of variety of techniques. In general, good starting point would be proceed with panoramic radiograph and possibly intraoral radiographs if greater image detail is required. If images are required of all of the maxilla and mandible to evaluate possible implant sites, cross-sectional images assists to clinician. Today, CBCT is the best modality for the ease of acquisition and relatively low radiation risk even for single implants.

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21

Current Concept of Densitometry in Dental Implantology

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1. Introduction

Bone density measurement have an important clincal role in the evaulation of bone quality and volume pre-operative and bone loss during dental implant treatment. It can be based on intra-oral and panoramic radiographs, cone beam and micro-computed tomography (CBCT and CT), dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MR), quantitative ultrasound and laser Doppler flowmetry. DEXA is recognized by some clinicians as the gold standard for bone density analysis. Bone densitometry performed by DEXA have provided the foundation for treatment of patients with osteoporosis. However, the equipment needed is usually not available in dental clinics and its units are quite expensive. A major challenge is to develop a widespread, low cost, user- and patient-friendly tool for bone density evaulation. The most widely used densitometric method in implantology is Computer Assisted Densitometric Image Analysis (CADIA). CADIA is computer program based on densitometric interpretion of digitalised radiografic images. CADIA is most commonly used for periapical and panoramic images. Due to inexpensive, non-invasive diagnostic method, CADIA is capable to detect minimal variations of the mineralized tissue density, such as bone remodeling after flap surgery, peri-implant tissue variations after flap surgery, the healing process in the furcation area after regenerative procedures. Before digital era was introduced in clinical diagnostic practise (where images are automatically digitalized), conventional radiographic images were digitalized mostly using scanner or video camera, which resulted in 10% reduced quality of images. CADIA analysis has been reported to be highly sensitive and specific, showing a diagnostic accurecy of 87%. Digitalized 2D images are presented in pixels and 3D images in voxels. A image quality (i.e. resolution) changes according to increasing or decreasing pixel/voxel size. The three parameters of image quality are contrast, sharpness and noise. The contrast describes differences in dose, brightness or intensity in an image, the sharpness refers to the transitions between the different densities. Since densitomeric evaluation is used for comparing images, in attempt to achieve more objective and precise interpretation, it is of utmost importance to standardize criteria in radiografic imaging. For the standardisation of the intraoral radiogfraphs following criteria should be considered:

- The procjection used should minimize distortion of the anatomic structures of interest
- The method should provide information about the degree of standardization achieved

- The ionizing radiation exposure should be the minimum necessary to provide diagnostic information
- The method should be flexible enought to allow monitoring of all sites in the mouth
- The method should not be uncomfortable to the patient
- The method should not require extensive training for use
- The method should use readly avaible meterials

The most common method in standardizing densitometric technique is by using a copper calibrating stepwedge which consists of 5 layers, with the first layer presented by 0,1 in width and visible on a particular and predetermined site of the image (Figure 1). Copper is chosen due to its effective atomic number which is similar to bone. In the past aluminium

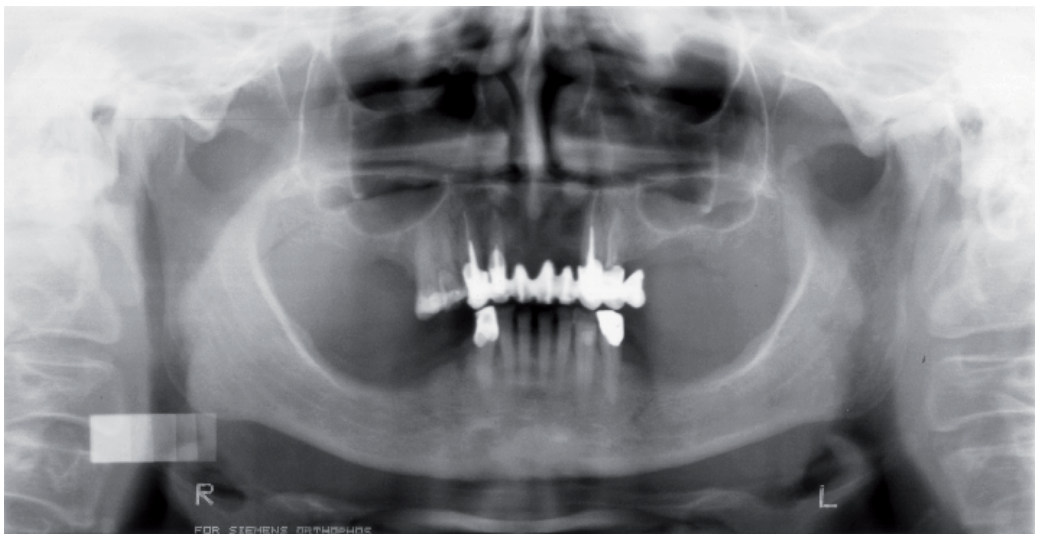

Fig. 1. Position of copper calibrating stepwedge on digital panoramic image.

was used instead, but was found to be too massive for positioning when used in retroalveolar images. In the manner of the easiest X-ray-film manipulation, many other materials such as a nickel in various thicknesses, hydroxyapatite, barium sulfate or some solutions such as CsCl or CaCl2 to simulate bone density, ethanol for fat and water for soft-tissue equivalent are in use. Stepwedge is used for linearisation, contrast-brightness adaptation and contrast optimisation for every measured image. Densitometric evaluation is based on intensity of gray shadows, which is predetermined on a scale varying from 0 (zero=black) to 255 (white) for intra-oral and panoramic radiographs. Recent CT scan devices can distinguish up to 4000 different gray shadows and therefore are far more precise, objective and reliable in comparison with periapical and panoramic images. Gray shadows determined by CT machines and its software programs are called Houndsfield Units (HU) representing a radiation attenuation for every pixel of the computer slice image. An HU value of 0 is equivalent to the radiation attenuation value of water, while an HU scale starts at value of -1000 corresponds to the value of air and generaly ends at around 3000 HU corresponding to the enamel. The density of structures within the images using CT scan is absolute and quantitative and can be used to differentiate tissuas in a region (i.e. muscle, 35-70 HU; fibrous tissue, 60-90 HU; cartilage, 80-130 and bone 150-1800 HU

depending of the gradation of the bone quality). CT enables the evaulation of proposed implant sites and provides diagnostic information that other imaging methods could not. Recent CBCT scans have few advantages in a comparasion to CT witch are lower effective dose of the radiation, better device avaibility for dentist (size and price), 3D view of images insted of 2D, simple computer software device and better tool for implant placement. A lack of CBCT ,due to lower effective dose, is resolution of images compared to CT device. MR is used in implant imaging as a secondary imaging technique when primary imaging techniques such as CT or CBCT fails. MR is a technique to image the protons of the body using magnetic fields. MR depicts trabecular bone as a negative image by virtue of the strong signal generated by the abundant fat and water protons in the sorrounding tissue, whereas bone mineral lacks free protons and generates no MR signal and its not useful in charecterizing bone density. It is reasonable to say that the preoperative densitometric evaulation of bone undergoing implant placement using CT scans are far more precise than any avaible devices. When it comes to postprosthetic imaging whose purpose is to evaulate the status and prognosis of dental implant, method of choice is CADIA. Periapical or panoramic radiography produces high resoultion images of the dental implant and sorounding alveolar bone (Figure 3). CADIA have limitations in determining buccal and linqual changes in alveolar bone and depiction of the 3D relationship between dental implant and soruonding bone. CT is able to determine that changes but it cannot match the resolution of periapical image due to artifacts which produse titanium implant (Figure2).

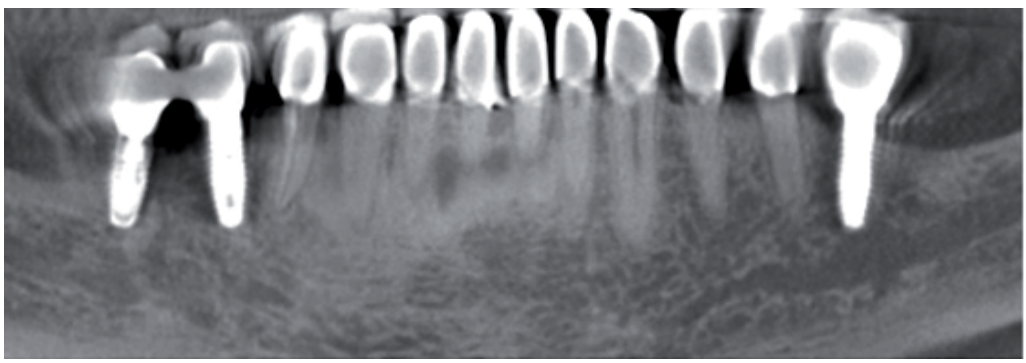

Fig. 2. CBCT scan used after implant placement shows lower resoultion of bone around dental implant due to interaction with titanium implant

2. CADIA modification

In this chapter densitometric measurement will be shown throught the modification of conventionally used CADIA and DIGORA software. Digital periapical and panoramic images were used, due to their minimal radioactive emission and high image quality that are not lost upon digitalization. Main task was to measure bone density around inserted dental implants using titanium implant itself as a stepwedge. This modification contains 12 measurement points for periapical and 10 points for panoramic images. They are preciselly located in positions in and around dental implants. The measurement of bone density is obtained automatically due to performed software package after entering the RVG image. Positions of the 12 points are specified in advance and inserted in the software database,

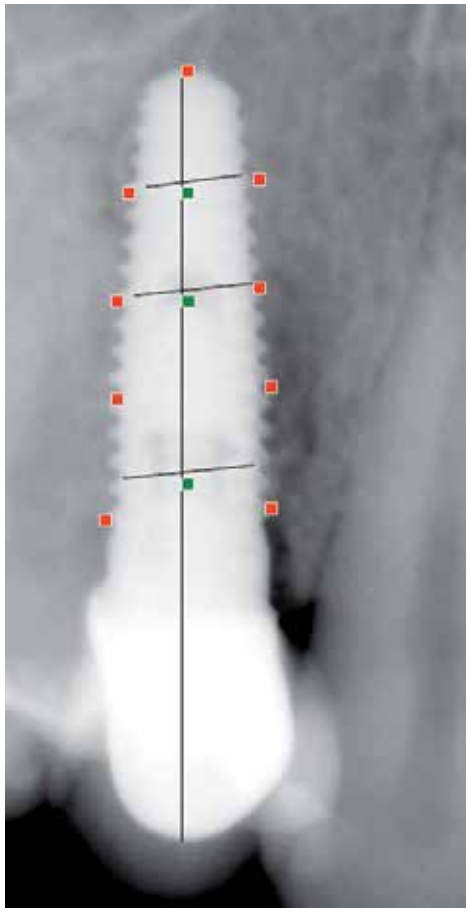

Fig. 3. Position of the correction (green) and measurement (red) points.

so the points remain in the same location for all evaluated images (Figure 3). The first 3 points are regarded as correction factors (modified stepwedge) which are situated on different parts of the implant. The first correction point is located in the apical part of the implant, where density of the gray shadows was the highest; the second correction point is located in the middle part of the implant where density of gray shadows have minimal intensity due to the perforated structure of the implant and the third correction point is located in the cervical part of the implant where density of gray shadows have midium intensity in the position where the crown screw is attached to the implant. Correction points served for revision of density change in measurements which occured due to discontinuity of the x-rays (i.e. distortion of x-rays present in each image in the series of follow-ups, as well as difference in exposition in the same series of images that were taken during a follow-up period). Measuring points are positioned as follows: the first point was placed in the middle line 1mm apically to the implant, and the remaining 8 points were placed in the bone surrounding the implant in precisely determined positions. This CADIA modification is designed to monitor changes in bone density around implants and to compare it with other images. If there is a need to precisely determine a densitometric value, original stepwedge is inevitable.

3. Usage of modificated CADIA in clinical purpose

Current modification of CADIA is in use since 2008. and there are few publications describing its use in clinical purpose about various tehniques of implant placement. In the first study complete densitometric measurement with images, grapfhs and tables will be shown while in the other cases only final images will be presented.

3.1 Comparison between flapless and two-stage techique of dental implant placement

Minimally invasive surgical techniques are a current trend, not only in dental implantology but in all surgical fields. It gives an atraumatic approach for the patients which results in better and easier accomplishment of treatment, not only for the patient but for the surgeon as well. Both of surgical techniques, two-stage and flapless, are safe methods with a long

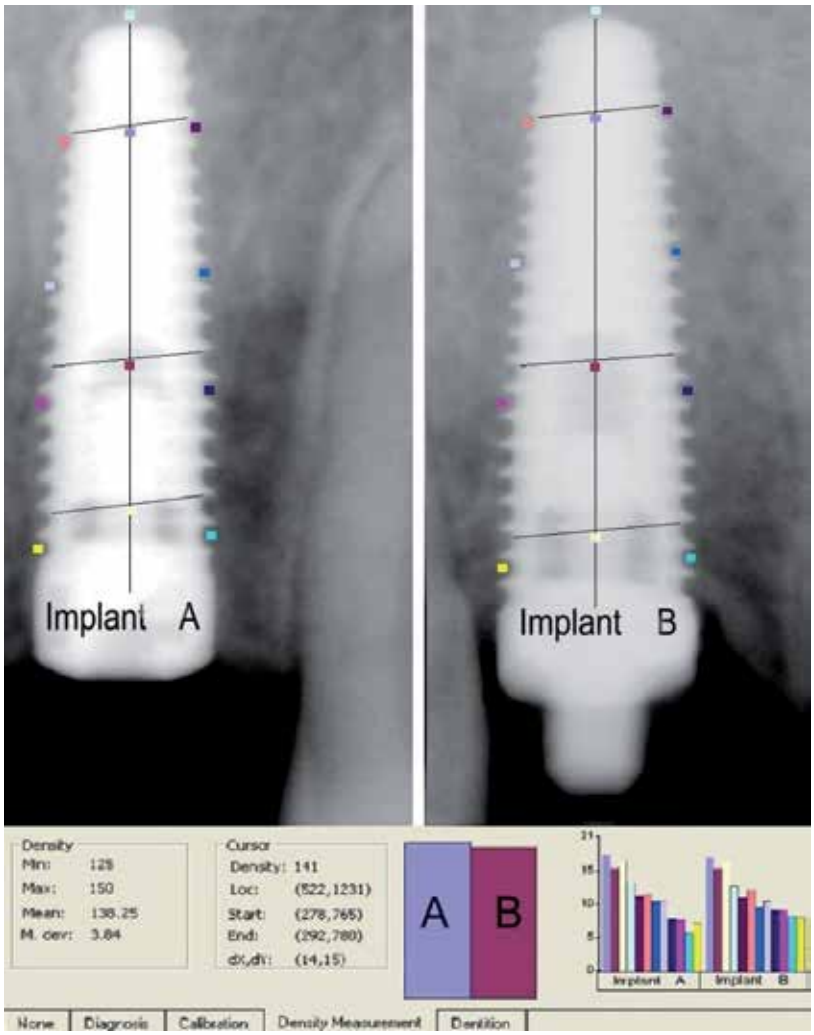

Fig. 4. CADIA comparison between two stage(left) and flapless (right) technique.

term success and satisfaction for the patients. Further cases describes radiographic assessment of flapless technique and determination of its clinical values in comparison with two-stage dental implant technique through computerized densitometric analysis. Values of densities were measured in all 10 patients through 3 months in certain time interval in 12 determined points. The first point was placed in the middle line 1mm apically to the implant, and the rest of 8 points were placed on the precise positions between 4. and 5., 9. and 10., 13. and 14., and between 18. and 19. of the screw thread, on each side of the dental implant (Figure 4).

The validaity of results in measured densities for all 5 patients, in which the implants were inserted using two-stage technique, throught all 3 measurements are shown in Table 1. The validaity of results in measured densities throught 3 measurements in all 5 patients, in which the implants were inserted using flapless technique are shown in Table 2. For easier analogy of measured densities, we used average densities for each technique according to stage of measurement.

Due to pilot study, the results were notstatistically analyzed, but compared through the values of average densities. Average value of density in period of 3 months (first measurement) in two-stage technique was 174.1, and in flapless technique were 158.8. Second measurements were done 12 months after the implants were inserted, and the results were: 172.18 in two-stage technique, and 158.47 in flapless technique. Average value of density after 18 months (third measurement) was for two-stage technique 170.86, and for flapless technique 157.57. All these results are shown in Figure 5. After mutual comparison of average densities, the results showed approximately the same decrease of density for both surgical techniques in the follow-up period of 18 months, conventional two-stage technique shown 3.24 and flapless technique 1.23. It shows minimal loss of density in both surgical techniques, as it is shown in the Figure 6.

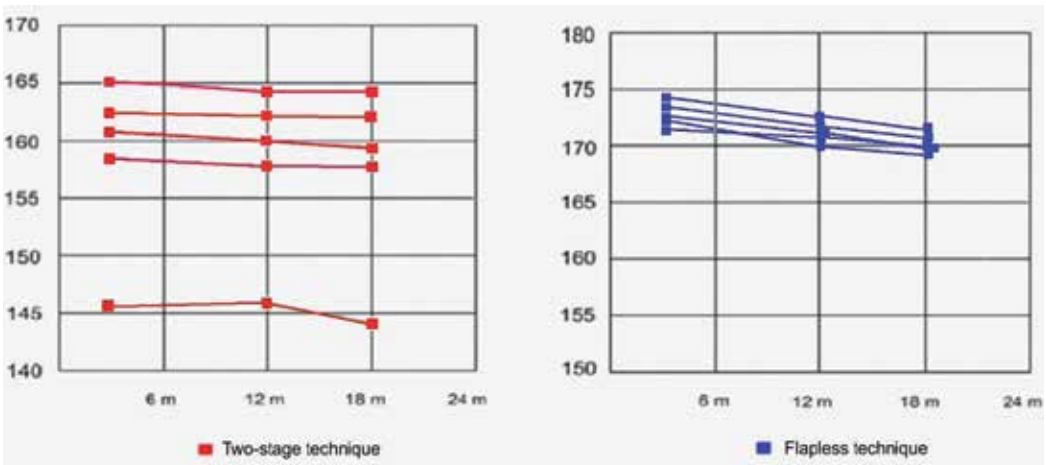

Fig. 5. Average values of bone density around inserted implants throught all 3 measurements.

After dental implant loading, values of density changes due to masticatory forces. Effect of masticatory forces can be enrolled in the changes of the bone around inserted implant with the help of densitometric analysis.

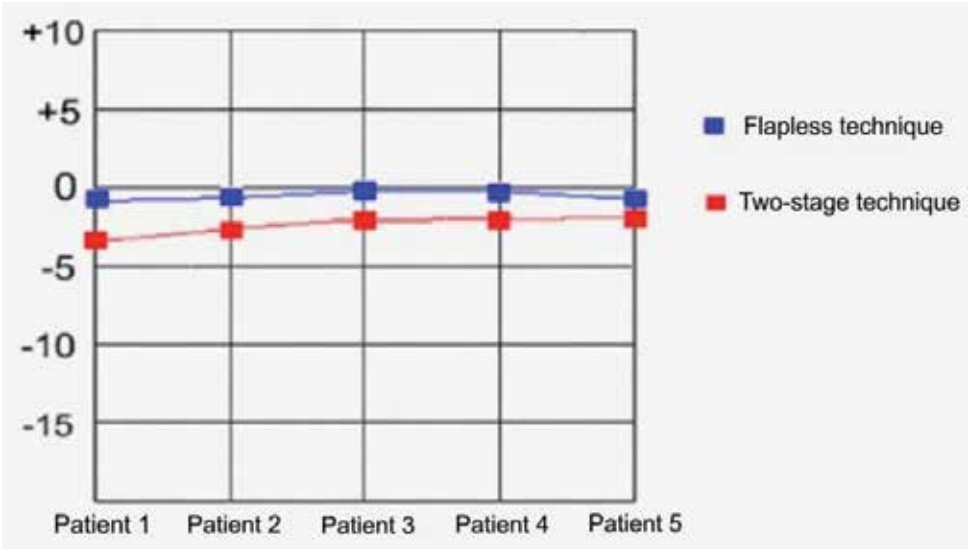

Fig. 6. Comparation of average bone densities showed approximately the same decrease of density for both surgical techiques in the follow-up period.

	Patient 1			Patient 2			Patient 3			Patient 4			Patient 5		
Point	3. month	12. month	18. month	3. month	12. month	18. month	3. month	12. month	18. month	3. month	12. month	18. month	3. month	12. month	18. month
1	254.96	255.14	254.19	254.52	255.41	254.99	254.42	254.85	254.12	253.99	254.12	254.01	253.84	254.04	253.94
2	248.23	244.21	245.74	247.91	244.82	245.83	248.35	247.68	246.07	244.25	244.58	244.56	242.38	243.15	242.67
3	247.43	251.25	250.69	249.95	251.36	251.44	250.81	251.04	251.83	248.08	247.84	247.92	247.09	246.44	246.89
4	204.16	200.31	198.22	204.16	203.54	202.38	194.69	192.42	192.1	200.74	199.86	197.94	198.53	197.69	196.99
5	190.12	187.21	183.72	191.29	189.36	188.45	193.53	191.34	189.79	185.65	186.41	184.27	184.33	183.04	182.85
6	192.82	216.23	205.14	190.21	191.24	190.41	193.74	190.95	190.41	193.54	192.85	192.41	186.37	184.65	184.22
7	177.2	179.43	179.12	172.28	174.67	173.56	177.6	177.52	177.08	174.91	175	173.57	180.7	178.31	177.68
8	180.74	176.78	172.1	181.84	180.05	179.68	184.9	182.37	181.69	184.67	180.49	179.62	182.57	179.91	179.56
9	171.4	155.62	151.12	170.69	167.66	166.95	168.67	167.44	166.55	168.55	167.21	165.74	174.39	171.24	170.77
10	166.53	162.23	169.72	169.88	163.59	160.06	174.18	175.73	174.28	178.79	177.12	174.83	174.95	172.58	171.72
11	144.71	132.87	134.21	141.41	138.48	134.59	144.69	140.25	134.52	134.07	133.43	132.07	140.55	138.69	137.41
12	143.42	137.44	140.59	140.97	137.26	133.08	141.64	138.92	136.95	144.61	140.81	138.4	139.84	138.29	137.59
Average	174.57	172.01	170.44	173.64	171.76	169.91	174.85	172.99	171.49	173.95	172.58	170.98	173.58	171.6	170.98

Table 1. Bone densities throught 3 measurements for 5 patients in the two-stage technique group.

Changes of the bone around inserted implant were mostly expressed on the points 7, 8, 9 and 10 which are located on the 9., 10., 13. and 14. thread of the implant. In the two-stage and flapless surgical technique, average values of bone density change (with the same indications) were approximately the same. Decrease of 3.24, and in flapless technique was 1.23. Due to our knowledge, there are no published results in the recent literature regarding densitometric comparison between these two surgical techniques. Most of the authors use the minimally invasive surgical techniques in everyday practice, including the flapless

	Patient 1			Patient 2			Patient 3			Patient 4			Patient 5		
Point	3. month	12. month	18. month	3. month	12. month	18. month	3. month	12. month	18. month	3. month	12. month	18. month	3. month	12. month	18. month
1	231.76	228.05	232.8	255	251.56	253.21	254.17	254.56	254.8	254.8	253.73	254.01	253.88	254.2	253.65
2	208.79	219.69	217.45	226.07	227.22	226.69	229.3	229.44	230.04	231.59	230.67	231.58	235.67	236.47	232.91
3	222.6	215.41	212.92	240.13	242.42	240.9	240.08	241.42	242.17	244.12	243.77	243.99	247.53	248.33	247.93
4	189.65	185.35	185.01	196.96	185.56	184.54	197.58	196.96	196.35	190.37	188.38	188.17	190.77	188.2	187.69
5	188.84	187.3	186.92	169.11	169.65	163.01	180.42	178.6	178.22	185.9	184.93	184.56	185.39	184.62	183.74
6	186.97	185.63	184.9	171.36	180.13	178.63	181.64	181.04	180.74	184.55	183.41	183.2	184.06	183.93	183.53
7	164.52	164.08	163.96	157.68	155.75	155.44	163.27	164.28	164.16	174.71	175.36	175.06	173.61	173.07	171.49
8	157.15	160.43	158.24	159.76	159.13	158.41	164.88	164.58	164.4	173.43	174.11	173.58	171.94	172.38	171.15
9	151.28	150.27	149.53	120.07	120.46	118.3	141.09	140.9	140.84	163.27	163.24	162.89	155.07	154.75	153.57
10	149.71	150.42	148.71	122.76	125.58	123.17	142.37	141.89	141.79	160.76	161.39	160.85	153.26	153.57	152.94
11	133.1	132.07	130.56	105.01	108.06	107.63	125.99	125.74	125.47	127.69	126.5	126.23	130.43	129.76	128.4
12	131.58	130.01	128.72	108.5	109.5	107.21	126.07	125.94	125.86	124.95	125.49	124.73	128.82	128.7	128.07
Average	161.42	160.62	159.62	145.69	145.98	144.04	158.15	157.77	157.54	165.07	164.76	164.36	163.71	163.22	162.29

Table 2. Bone densities throught 3 measurements for 5 patients in the flapless technique group.

approach in dental implantology. Becker et al. have found that implants placed without flap reflection remained stable and exhibited clinically relevant osseointegration similar to when implants were placed using conventional flap procedures. Campelo and Camara have published the most extensive study about using one-stage flapless surgical technique in dental implantology. In their 10-year retrospective study the cumulative success rate, for 770 implants using a flapless surgical technique, have varied from 74.1% to 100%, relative to the year of placement, which can be explained with a learning curve combining technology and material development in dental implantology. Survival rates in other reported studies, for flapless surgical approach, are between 91% and 98.7%5 which indicate successful results of this technique application. Based on our results, we can say that both of examined groups, and two different techniques in dental implantology show the same clinical values after 18 months of follow-up.

3.2 Comparation between two different techniques of sinus floor elevation

Prior to planning implant surgery and prosthetic reconstruction in the posterior maxillar region, it is not uncommon not to consider sinus floor elevation surgery first, which can be achieved using either open or closed technique approach, or minimal invasive baloon sinus lifting thechnique which has recently been in use. Two clinical cases presented in the literature, in which densitometric measurements were compared by both techniques of sinus elevation, the baloon sinus lifting with open and closed access. In the first case elavation of the right maxillary sinus was done by the balloon controled technique (transcrestal approach). The augmentation was done with alloplastic bone filler (tricalcium phosphate). Lifting of the left maxillary sinus was performed by forming lateral fenestration on the buccal cortical plate followed by augmentation with the mixture of xenogenic bone filler and autologous bone graft. After 6 months of augmentation 3 implants on each side were placed and prosthetic suprastructure was completed within next 4 mounths. Values of bone density were measured in 10 points around each inserted implant compared with RFA measurements of implant stability before loading and 3 and 12 mounths after prosthetic loading. After mutual comparison of average densities, the results showed approximately

the same decrease of density for both surgical techniques in the follow-up period of 12 mounths. It shows minimal loss of density around inserted implants in grafted maxillary sinus areas elevated by both surgical techniques. Gained data results are showing that sinus lifting method with enclosed balloon approach techique can result in gaining enough area for implant placement as well as with opened approach technique. Furthermore balloon technique is more over less traumatic experience for patient with a much fewer side effects and postoperational problems. In addition if there is a sufficient bone width for the purpose of sinus lifting in favour of placing of two up to 3 implants in that area it can equaly sufficient use enclosed balloon technique instead of open lateral approach which is causing much more traumatised experience for patient and much more postoperative problems (Figures 7, 8 and 9).

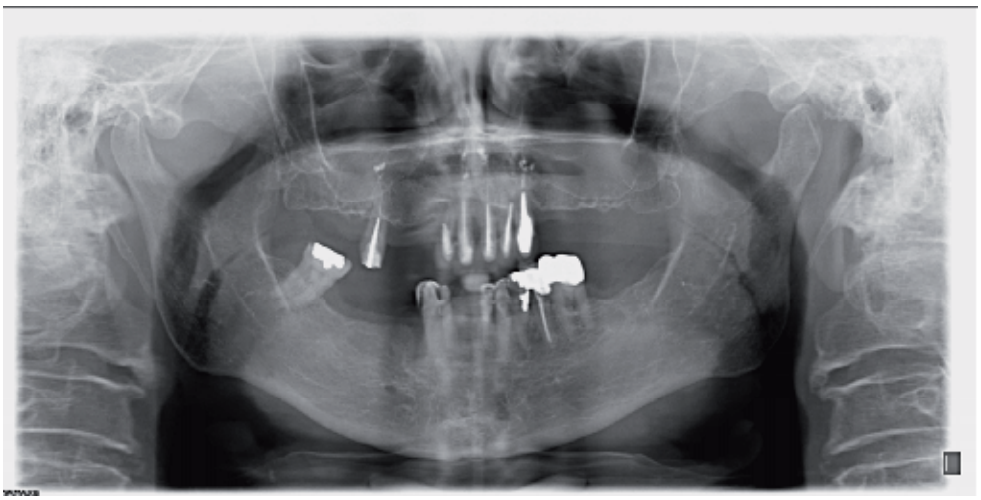

Fig. 7. Initial radiograph before surgical treatment

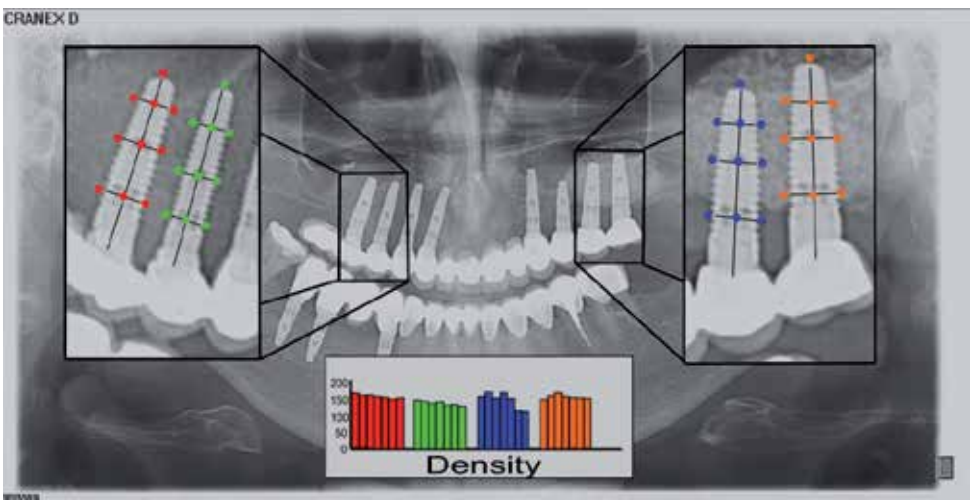

Fig. 8. Densitometric measurement of two different approaches, open sinus lift technique (red and green points) and ballon technique (blue and orange points).

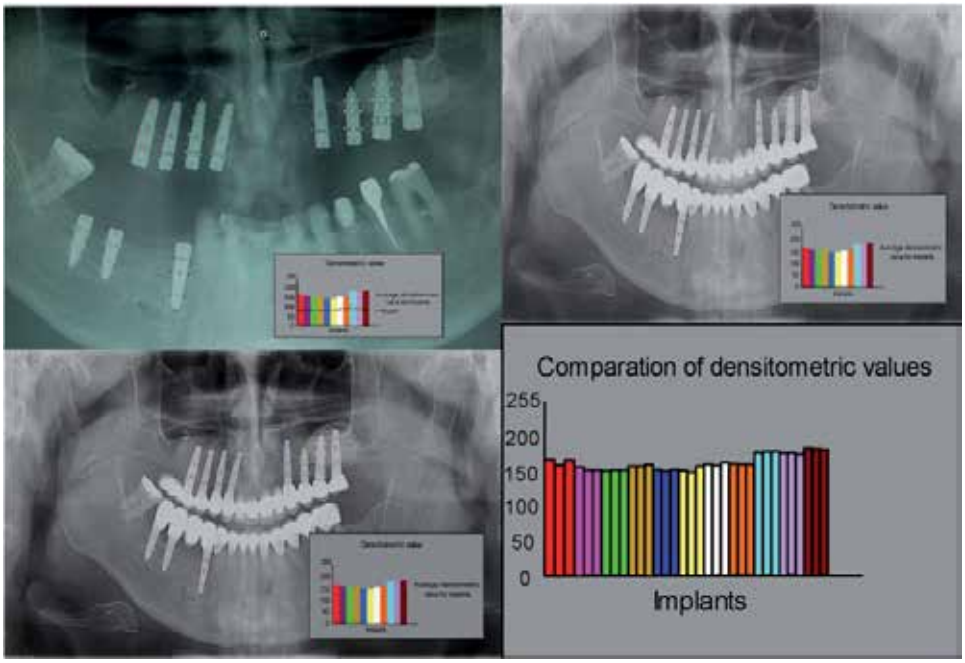

Fig. 9. Densitometric comparison between two different approaches, open sinus lift technique (left upper molar region) and ballon technique (right upper molar region). Lower right molar region was augmented using splitting technique (2 implants)

In the second case elevation of the right maxillary sinus was done by close sinus lift technique and on the left side by the ballon controled technique filled with alloplastic material (tricalcium phosphate). After 6 months of augmentation one implant on each side were placed and prosthetic suprastructure was completed within next 4 mounths. Values of bone density were measured in 10 points around each inserted implant compared with RFA measurements of implant stability before loading and 3 and 12 mounths after prosthetic loading, same as int he first case. First densitometric measurement showed, that the bone

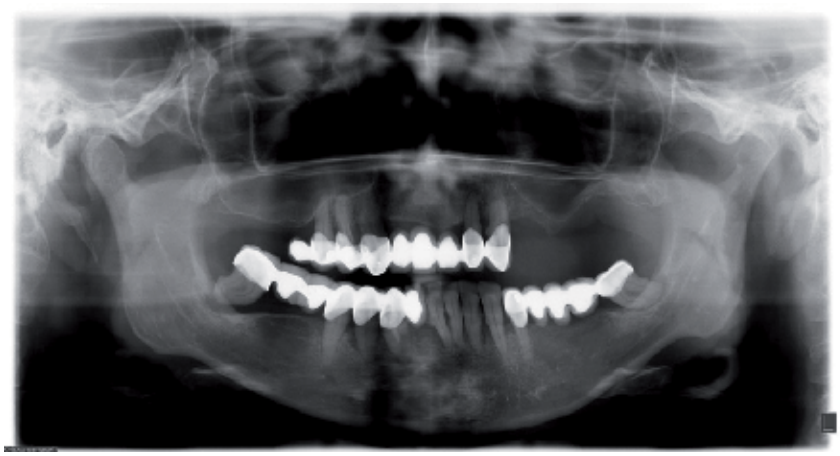

Fig. 10. Initial radiograph before surgical treatment

around dental implants augmented by ballon sinus lift technique, had twice more value in comparation with close sinus lift techinque due to bone filler. After follow-up period of 12 mounths, like in the first case, the same decrease of density for both surgical techniques were observed (Figures 10 and 11).

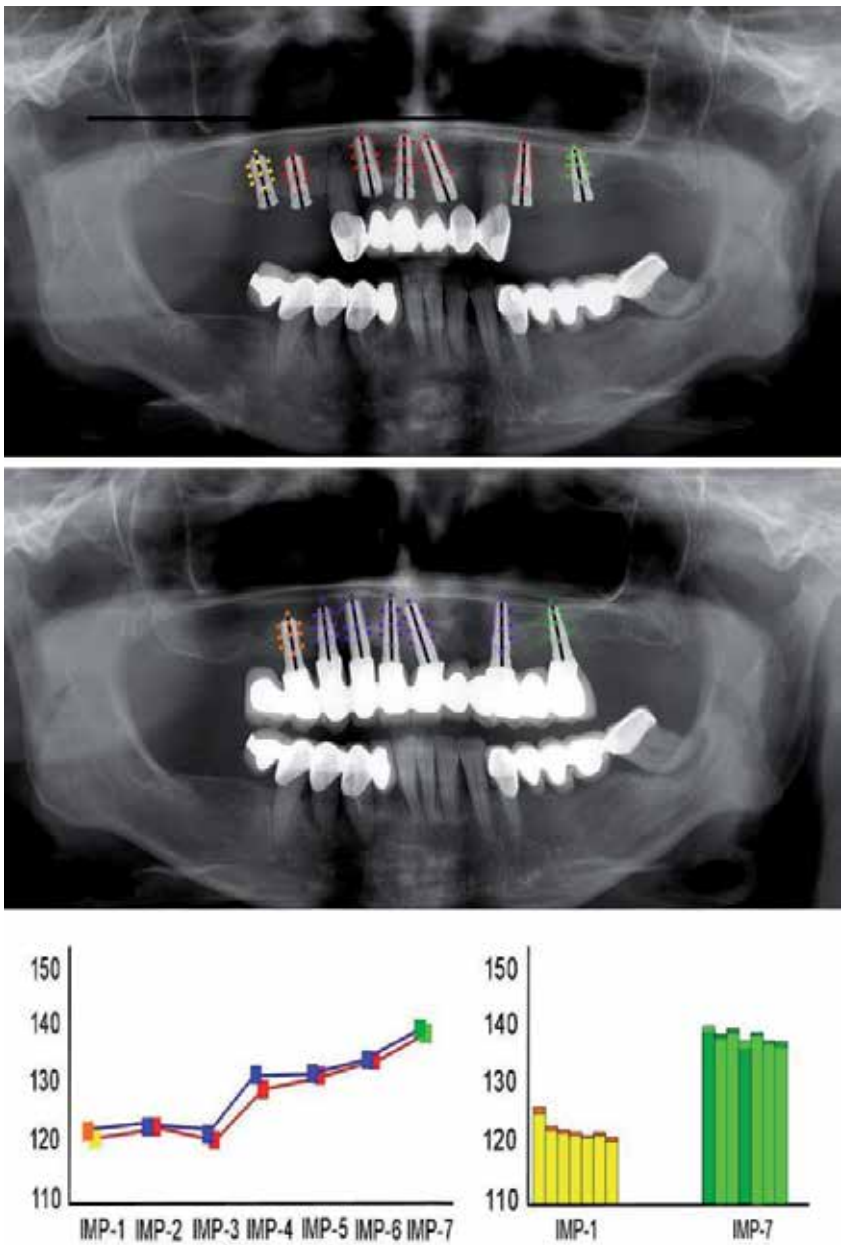

Fig. 11. Densitometric comparison between two different transcrestal approaches, close sinus lift technique (left implant and yellow graph) and ballon technique (right implant and green graph).

3.3 Alveolar ridge augmentation using splitting technique

In oral implant surgery, in order to widen the alveolar ridge and avoid horizonatal ridge augmentation by using autologous bone transplants, splitting and spreading techniques are indicated instead. These two methods are regarded as minimally invasive surgical techniques which reduce the number of surgical interventions, and result in minimally present postoperative complications, such as the patient's discomfort during the procedure. They also minimize the healing period in which it is expected to accomplish final prostetic reconstruction. Two clinical cases are shown in which densitometric measurements were compared by splitting technique and cllasic two-stage technique of dental implants placement. In the first case two implants in lower molar region using splitting technique and one implant in premolar lower region using two-stage technique were placed (Figure 8). Prosthetic suprastructure was completed within next 4 mounths. Values of bone density were measured in 10 points around each inserted implant after placement, after 4 and 12 mounths. After mutual comparison of average densities, the results showed almost nearly the same decrease of density for both surgical techniques in the followup period of 12 mounths. In the second case splitting technique is used in lower premolar and molar region, placing 3 dental implants (Figure 12). Values of bone density were measured in 10 points around each inserted implant after placement, after 4 and 12 mounths. The results showed again the same decrese of density compared with two-stage technique.

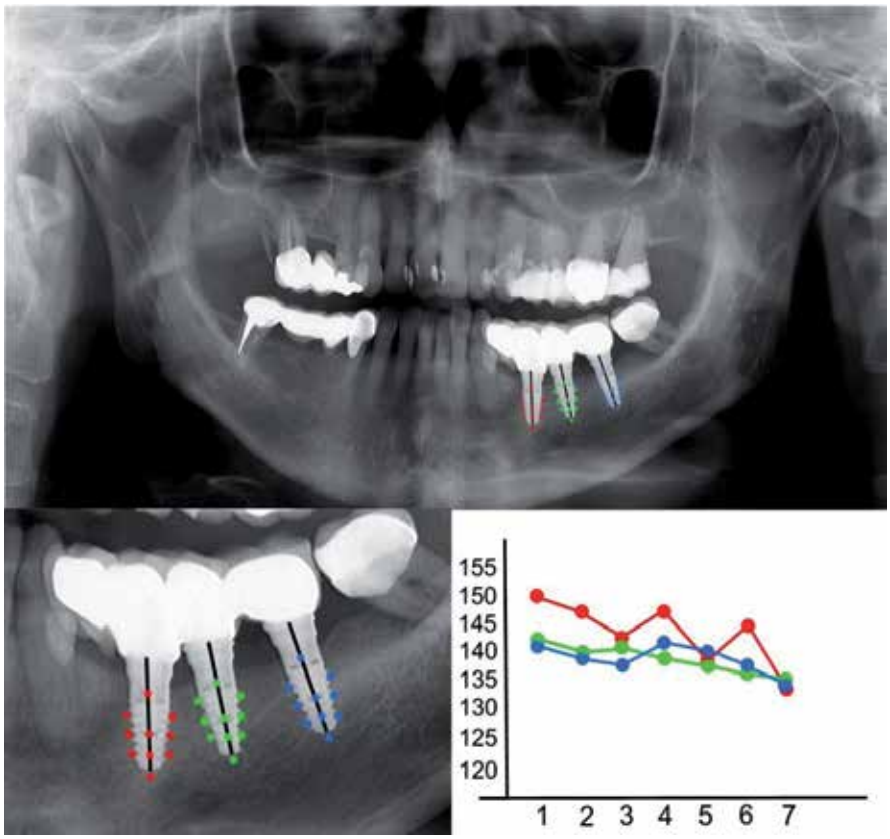

Fig. 12. Densitometric measurement of bone around 3 implants using splitting technique

3.4 Augmentation with autologous bone graft with simultaneous dental implant placement

Defect of the alveolar ridge of the left maxilla remained after extractions of the central and lateral incisors due to vertical root fractures (Figure 13) were augmented with the autologous bone block harvested from the retromolar area after two dental implants placement. Primary stability of the inserted implants was satisfactory. The gap between autologous graft and bone defect walls were filled with autologous bone chips harvested with bone scraper from the same harvesting site in the retromolar area. Augmented area was covered with the xenogenic bone substitude and resorbable collagen membrane. Densitometric measuremnt was performed six months after surgical procedure (Figure 14).

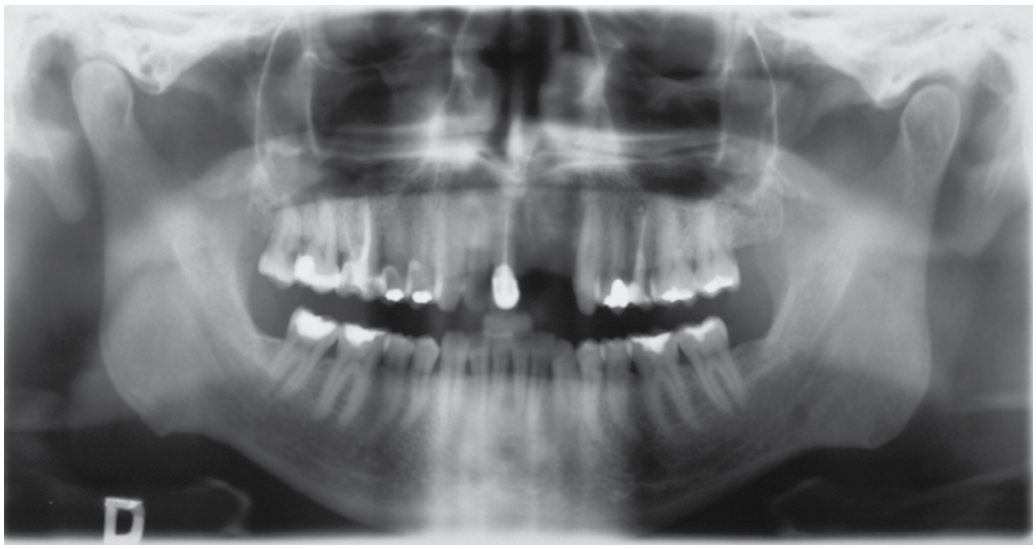

Fig. 13. Initial radiograph before surgical treatment

The results showed the higher decrese of density on bone grafts in comparation with bone alone.

3.5 Spreading technique in combination with autologous bone graft

Dental implant was placed after spreading the alveolar ridge bone due to long edentulous period (Figure 15). After implant placement infraction of the buccal cortical plate has remained. Defect was augmented with the autologous bone chips harvested with the bone scraper from the retromolar area, covered with β-tricalcium phosphate bone substitude and resorbable collagen membrane. Densitometric measurement was performed six months after surgical procedure, directly before final prosthetic restoration, and 12 months after surgery and 6 months after loading (Figures 16 and 17).

3.6 Alveolar ridge augmataion using rhBMP-2

In recent years, the delivery of osteoinductive factors such as bone morphogenic proteins (BMPs) have become an alternative approach to traditional bone grafting due to their capacity to enhance the natural ability of the surrounding tissues to produce bone healing and new bone and cartilage formation. In following case densitometric measurements were

compared between bone induced by rhBMP-2 and normal bone (Figure 19). Substantial loss of vertical ridge height was noted bilaterally in both the mandibular molar regions and were deemed insufficient without augmentation to enable placement of dental implants. Bone was augmented using human recombinant BMP-2 and 3 dental implant were placed 6 mounths after. Values of bone density were measured in 10 points around each inserted

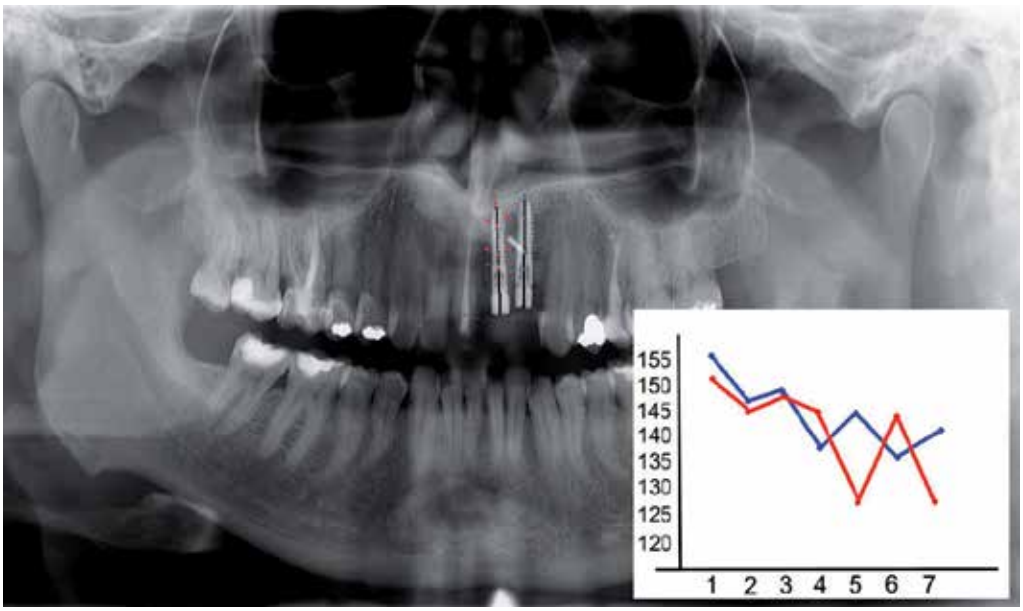

Fig. 14. Densitometric measurement of augmented bone around 2 implants placed simultaneouslly with the autologous bone graft (the fixation screw is positioned between the osseointegrated implants)

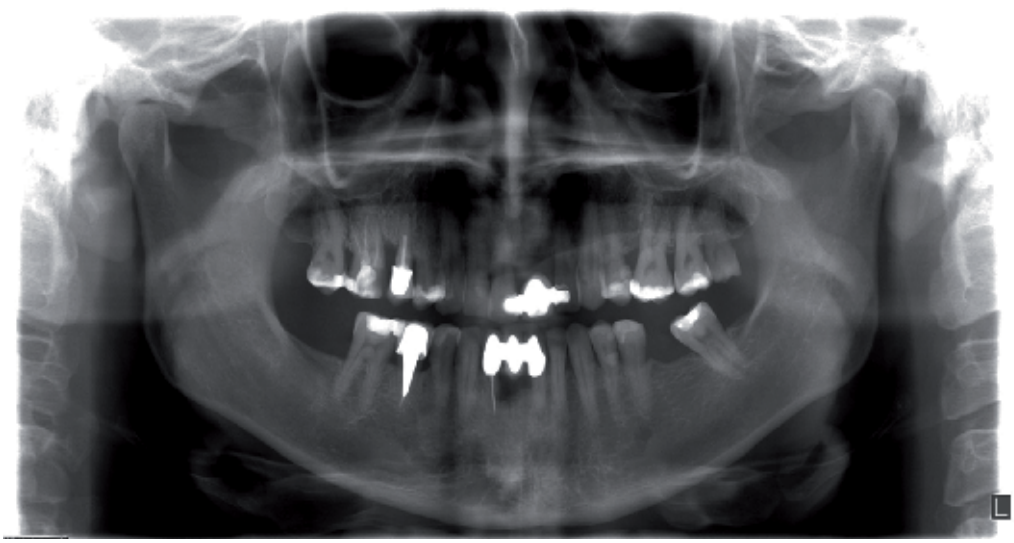

Fig. 15. Initial radiograph before surgical treatment

implant after placement, after 4 and 12 mounths. In this case, the results shows slighty decrease of density in bone induced with rhBMP-2 in comparison with classic two-stage technique of dental impalnts placement in the follow-up period of 12 mounths.

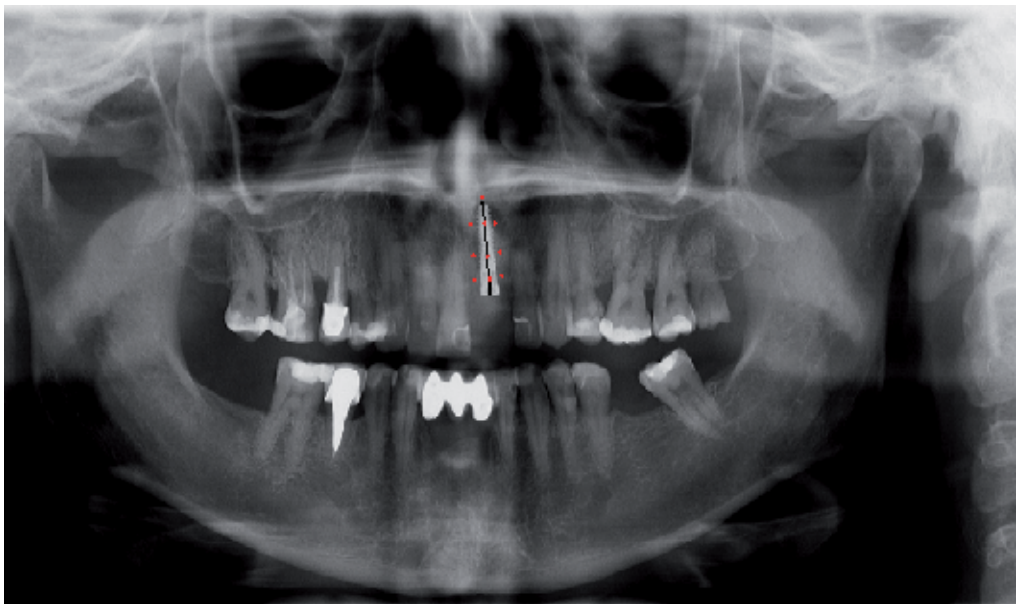

Fig. 16. Densitometric measurement of augmented bone around dental implant after spreading technique, 6 months after surgical procedure

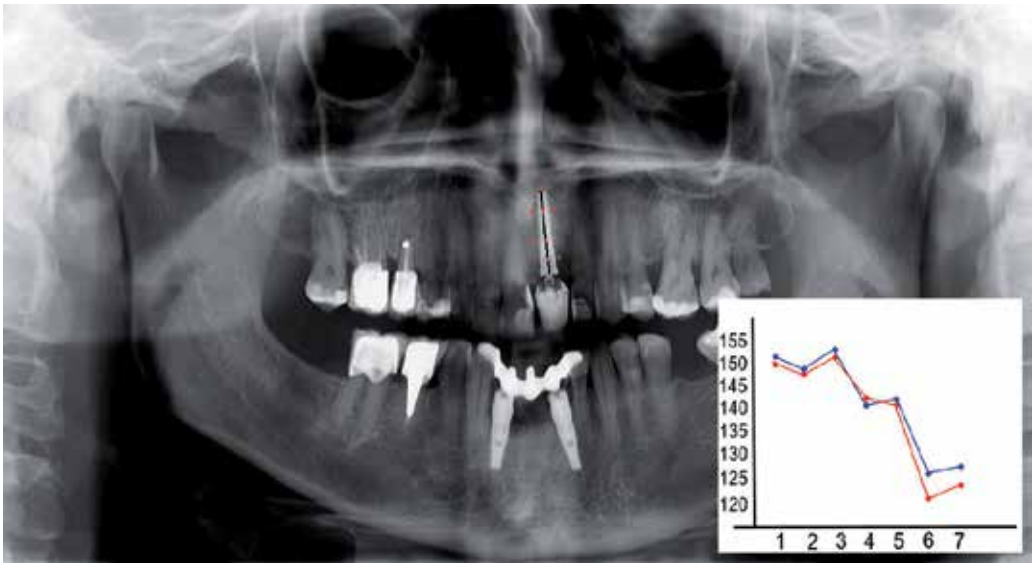

Fig. 17. Densitometric measurement of augmented bone around dental implant after spreading technique, 12 months after surgical procedure

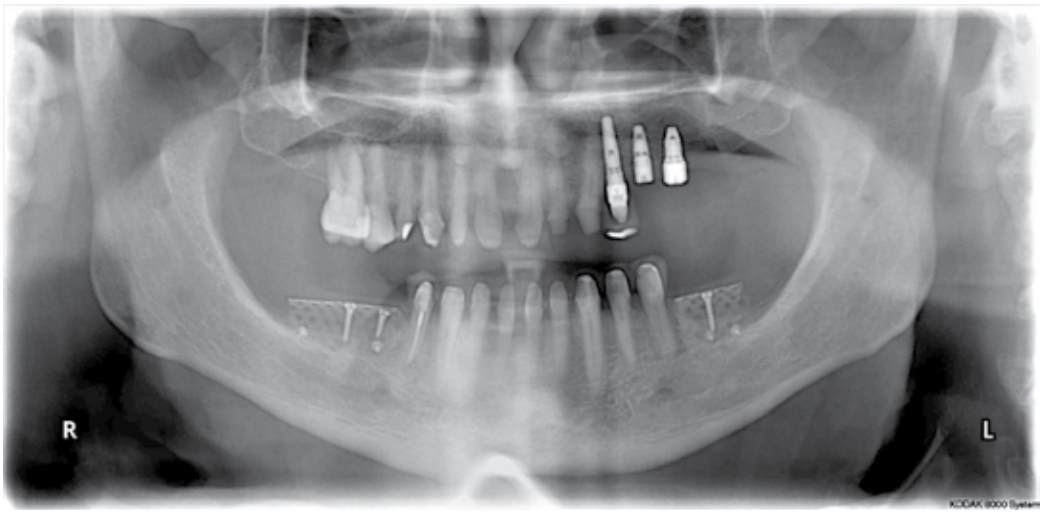

Fig. 18. Radiograph taken after placement od rhBMP-2

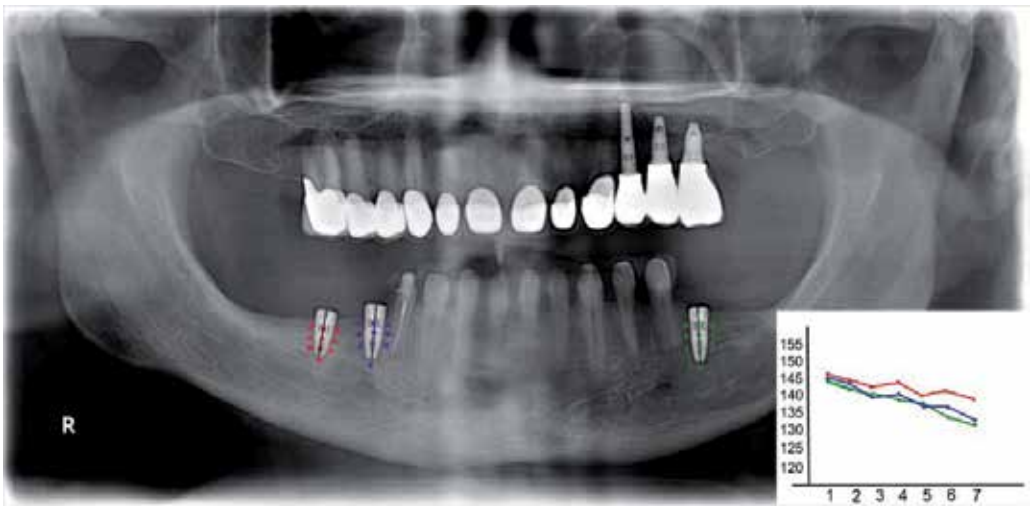

Fig. 19. Densitometric measurement of augmented bone around 3 implants using rhBMP-2

3.7 Importance of bone density in implant dentistry

Currently the use of osseointegrated implants to treat partially or completely the endentulous arch is considered realible and predictable, with a success rate of 98% or higher. The success of dental implant treatment is associated with good primary implant stability. Primary stability corresponds with bone density and it has been determining factor in treatment planing, implant design, surgical approach, healing time and initial progressive bone loading during prostetic reconstruction. Secondary implant stability results after formation of secondary bone contact of woven and lamellar bone. Bone density is related directly to the strenght of the bone and it seems to be a vital factor in the achievement of osseointegration. For assessing bone quality several classification systems and method were introduced. The most popular method was introduced by Lekholm and Zarb

Quality 1	Homogenous compact bone
Quality 2	Thick layer of cortical bone surrounding dense trabecular bone
Quality 3	Thin layer of cortical bone surrounded by dense trabecular bone of favorable strenght
Quality 4	Thin layer of cortical bone surrounding a core of low-density trabecular bone

Table 3. Classification of bone density by Lekholm and Zarb

who listed four bone qualities found in the anterior regions of the jawbone (Table 3). Their scale of bone quality ranges from 1 where is composed of homogeneuous compact bone to 4 where is a thin layer of cortical bone surrounding a core of low density trabecular bone. Their classification has recently been questioned due to poor objectivity and reproductibility because it provides only a rought mean value of the entire jaw. Misch proposed five bone density groups independet of the regions of the jaws based on macroscopic cortical and trabecular bone characteristic, their tactile sence during implant placement, location and CT values. (Table 4). The percentage of bone contact is significantly greater in cortical bone than in trabecular bone. An antherior mandible (D1, D2) provides the highest percentage of bone in contact with implant compared with posterior maxilla (D4) which offer less areas of bone contact with implant. Its reasonable to say that the period of osseointegration is longer in maxilla (4-6 months) than in mandible (3-4 months) and it coresponds with implant success. The male patients had higher average bone density value than that in female patients. That constatation could be explained with the hormonal peculliarities in females and generally higher bone mass in males.

Bone density	Description	Tactile analog	Typical Anatomical Location	CT values
D1	Dens cortical	Oak or maple wood	Anterior mandible	>1250 HU
D2	Porous cortical and coarse trabecular	White pine or spruce wood	Anterior mandible posterior mandible Anterior maxilla	850-1250 HU
D3	Porous cortical and fine trabecular	Balsa wood	Anterior maxilla Posterior maxilla Posterior mandible	350-850 HU
D4	Fine trabecular	Styrofoam	Posterior maxilla	150-350 HU
D5	Soft bone with incomplete mineralisation	Styrofoam		<150 HU

Table 4. Classification of bone density by Misch

Another bone classification by Tomaso and Vercellotti has universal application and can be used in all fields of bone surgery expecially in implantology. The classification outlines the quantitative characteristics of the cortical crest and separately the density of spongy bone mineralization (Table 5).

Quantitative cortical thickness classification	
0 mm	Thickness of cortical crest at the site of recent tooth extraction after few months
1 mm	Thickness of cortical crest at the site of tooth extraction after several months
2 mm	Thickness of cortical crest at the site of tooth extraction after a few years
3 mm or more	Thickness of cortical crest at the site of tooth extraction after several years and characterized by a reduction in spongy bone resulting in partial merging oft he buccal cortical and lingual cortical bone
Qualitative spongy bone density classification	
High density	The tomographic image is prevalently radiopaque and grayish-whitish in color
Medium density	The tomographic image is rather radiopaque and grayish in color
Low density	The tomographic image is radiolucent and grayish-blackish in color

Table 5. Classification of bone density by Tomaso and Vercellotti

Implant stability can be measured by non-invasive clinical test methods (i.e. insertion torque, the periotest, resonance frequency anaysis). Insertion torque is a method that records the torque required to place the implant. The Periotest M (Figure 20) is a measuring device for use in dental practises and is designed for the following range of applications:

1. Assessment oft he osseointegration of dental implants
2. Diagnosis and assessment of periodontopathies (the Periotest Mmeasuers the damping characteristics oft he periodontium and, indirectly, tooth mobility, which it outputs In the form of a Periotest value)
3. Assessment oft he occlusal load
4. Control of the treatment's progress

The unit scale ranges from -08 to +50. The measuring procedure is electromechanical. An electrically driven and electrically monitored tapping head percusses the test object (tooth or implant) 16 times. The entire measuring procedure requires approximatelly 4 seconds. The tapping head is pressure sensitive and records the duration of contact with the test object. Loose teeth or implants display a longer contact time and the Periotest values are correspondingly higher, while sturdy teeth and implants have a short contact time and result in low Periotest values. The Periotest M should not be applied in the following cases:

all types of acute apical periodontitis and acute trauma (dislocation, root fracture, alveolar process fracture).
Another method, resonance frequency anaysis (RFA) and their instrument called Osstell mentor are commonly used in clinical studies (Figure 20). The technique is contactless, non-invasive, patients experience no pain sensation from the measurement and the measurement takes 1-2 seconds.The unit of Osstell measurement is the implant stability quotient (ISQ) that is calculated from the resonance frequency and ranges from 0 to 100 units.

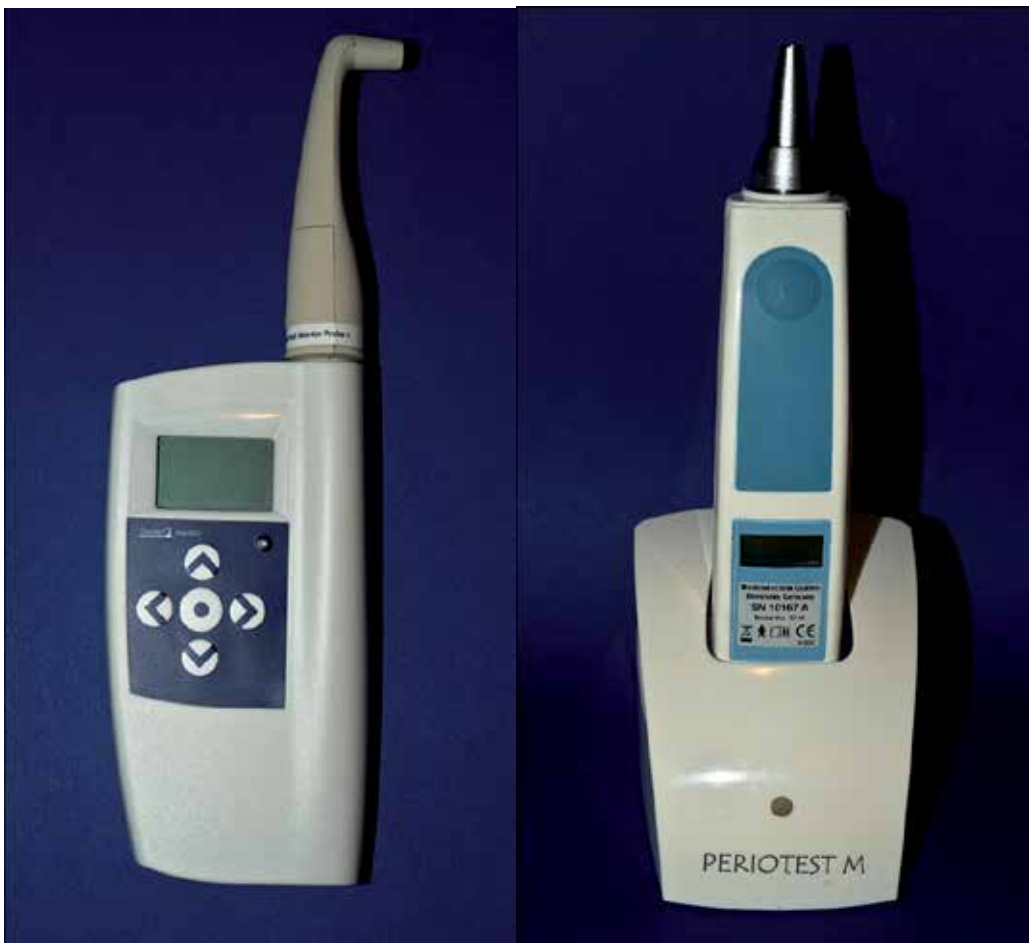

Fig. 20. Osstell mentor (left) and Periotest M (right)

Turkyilmaz et al found strong correlations between mean bone density scaned by CT, insertion torque and resonance frequency analsysis for the early loading protocols of dental implants. Autors suggested that primary stability is achived for early loading of dental implants when CT value is over 528 HU, insertion torque value is 32 Ncm or 45 Ncm and RF values higher than 65 ISQ.

In the end of this chapter there is a need for discusion of implant success criteria which were proposed by Albrektsson et al. Implant treatment, to be regarded as successful, need to meet the following criteria:

1. No radiolucent zone around the implant
2. The implant is acting as an anchor for the functional prosthesis
3. Confirmed individual implant stability
4. No suppuration, pain or ongoing pathologic processes

4. Conclusion

In this chapter CADIA measurement were described and its values were in strong correlation with CT values. Described CADIA modification is designed to monitor changes in bone density around implants and to compare it with other images. If there is a need to precisely determine a densitometric value, original stepwedge is inevitable, CADIA measurement were follow-up with Osstell device witch was helpful tool for determination of primary stability. Primary implant stability is in strong correlation with implant success.

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Since Dr. Branemark presented the osseointegration concept with dental implants, implant dentistry has changed and improved dramatically. The use of dental implants has skyrocketed in the past thirty years. As the benefits of therapy became apparent, implant treatment earned a widespread acceptance. The need for dental implants has resulted in a rapid expansion of the market worldwide. To date, general dentists and a variety of specialists offer implants as a solution to partial and complete edentulism. Implant dentistry continues to advance with the development of new surgical and prosthodontic techniques. The purpose of Implant Dentistry - The Most Promising Discipline of Dentistry is to present a comtemporary resource for dentists who want to replace missing teeth with dental implants. It is a text that integrates common threads among basic science, clinical experience and future concepts. This book consists of twenty-one chapters divided into four sections.

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