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Bone Grafting

Recent Advances with Special References to Cranio-Maxillofacial Surgery

Edited by Raja Kummoona

BONE GRAFTING - RECENT ADVANCES WITH SPECIAL REFERENCES TO CRANIO-MAXILLOFACIAL SURGERY

Edited by **Raja Kummoona**

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Notice

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Meet the editor

Professor Raja Kummoona is a Fellow of the Royal College of Surgeons of England (FDSRCS), Emeritus professor of Maxillofacial Surgery of Iraqi Board for Medical Specializations, Fellow of the Royal Society of Medicine, Research Fellow of the Royal college of Surgeons of (1975-1977), President of Iraqi Dental Society (1977-1985), Registrar of Primary FDSRCS in Iraq (1985-1990), and is the most distinguished professor of the University of Baghdad (1991-1992). He is one of 40 top scientists in Iraq awarded a gold medal for 3 years (2000-2002) by presidential celebration. He has had many publications and contributions to science by advocating many surgical procedures and research in cancer surgery and flap reconstruction, TMJ surgery and maxillofacial injuries, orbit tumors and injuries, missile injuries of the face with advancing surgery of war injuries of the face worldwide. He contributed to research in cancer and was the finder of post graduate studies in maxillofacial surgery in Iraq. He has been the Editor of 'Neck Dissection, Clinical Application and Recent Advances', Feb 2012, IntechOpen. He has been Editor of 'Surgical Reconstruction of the Temporomandibular Joint', 2013. He has been Editor of the book 'Disease of the TMJ, Surgical Reconstruction, Clinical and Experimental Studies', April 2014, Science PG. He has been Editor of 'Missile War Injuries of the Face', 'Maxillofacial Injuries in Road Traffic' published by Science PG in 2014 and editor of the book 'Jaw Lymphoma and Orofacial Tumors', 2015, published by Science PG. He is the editor and member of Editorial Board of many international journals, President of the Society of Iraqi Maxillofacial Surgery, founder member of International Society of Head Neck Trauma, 2015, London and Chairman of the Department of Maxillofacial Surgery, College of Dentistry, University of Baghdad 1982-2000, member of the Council of College of Dentistry 1975-2000, Founder and chairman council of Maxillofacial Surgery, Iraqi Board for Medical Specializations, 1993-2010.

Contents

Preface

Bone grafting is an interesting topic that is required by craniofacial, orthopedic, plastic reconstructive, neurosurgeons and oral surgeons for reconstruction of bone defects either immediately or as elective surgical procedures for reconstruction after radical tumor or cancer surgery or in cases of post-traumatic missile injuries of the facial skeleton or limbs.

Bone grafting involves an important choice for both patients and surgeons The patient seeks the best possible reconstruction with the most natural looking results and to restore the functional activity of the bone defect. The surgeon tries to solve the patient problem and often must decide whether to perform a quick temporary reconstruction by metal prosthesis or carry out a carefully considered but more involved surgical procedure that combine an appreciation of form and function with understanding of tumor biology. Complete tumor resection of a bony type is always required for restoration of function, growth, and aesthetic feature. Also, bone grafting is required for restoration of function, growth, and aesthetic feature and in missing bone in RTA and in secondary phase of missile war injuries.

The ability to plan bone grafting comes only with experience, skill, and knowledge. In the early stage of practicing bone grafting, the results can be humbling. Competence, skill, and knowledge of the bone grafting technique can be achieved only through studying the patients' needs and requirements.

The book title (Bone Grafting - Recent Advances with Special References to Cranio-Maxillofacial Surgery) was chosen because there have been many different techniques of bone grafting of the facial skeleton been applied more than any other area in the body. Other specialties such as orthopedic surgery, reconstructive surgery, neurosurgery, plastic and oral surgery, and maxillofacial surgery use bone grafts from the iliac bone more than any other specialties.

Recently, the editor published a research study on experimental bone grafting studies on rabbits. The aim was to understand the cellular changes that occur after bone grafting between the stump of the mandible and bone graft and the role of mesenchymal stem cells and growth factor released from platelets to promote bone graft healing.

This book contains 8 chapters with the Introductory Chapter by the editor. The book is divided into three sections:

Section I, Craniofacial Surgery, contains three chapters

Section II, Orthopedic Surgery, contains one chapter

Section III, Research, Biological Aspect of Bone Graft and Bovine Type and its Application in Oral Surgery and Implantology, contains four chapters.

The book also describes the advances in the application of bovine bone graft from cadavers in both secondary hip replacement, in surgery of the ankle and foot and also for building alveolar bone.

This book required great support and effort from the book editor and from the publishing team. I would like to thank Author Service Managers, Edita Umihanic, Kristina Jurdana and Sara Petanjek for their great help and assistance. Also I would like to thank all the authors of the chapters for their great contribution in production of this book.

This is a very interesting book to the readers and top specialists in craniofacial surgery, orthopedic surgery, reconstructive surgery, neurosurgeons and oral surgeons who are interested in implantology.

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Section 1

Craniofacial Surgery

Chapter 1

Introductory Chapter: Bone Grafting and Its Application in Cranial-Maxillofacial Surgery. The Role of Mesenchymal Stem Cells

Raja Kummoona

Additional information is available at the end of the chapter

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1. Introduction

Bone grafting is not a new technique but has a longer history, and its important component in modern surgery begins nearly before three-and-a-half centuries.

The construction of first modern microscope by Galileo Galilei (1609) and the discovery of blood circulation by William Harvey in 1615, the first bone grafting operation was performed by a Dutch doctor in 1668, Jacob Van Meekeren. This doctor placed a piece of dog bone in a soldier's skull defect from war injury to heal in, but unfortunately, the soldier was often excommunicated by the church for being part dog, and he was pressing. The soldier did request his doctor to remove it because its presence upsets him, and the doctor discovered of how well bone grafting actually worked [1].

In the process of attempting to remove the bone graft, Van Meekeren discovered that the bone had healed too well, and he found it's actually irremovable. It was the first xenograft applied on humans. More than 150 years later, the first recorded allograft operation was performed by Dr. Walter in Germany. Later, Great British surgeon Sir William Macewen (1880) [2] (from Lexer) attempted the transplantation of bone by reconstruction of a diaphysis of a child's humerus arm from the leg of another patient and considered this as real success in bone allograft transplantation [1].

Bone grafting is an interesting topic practiced by cranial-maxillofacial surgeons and orthopedic surgeons for restoring continuity of bone after radical tumor surgery, road traffic accident with loss of bone segments, and in the cases of post-traumatic missile war injuries. Bone grafting has been used for reconstruction of congenital cranial and facial deformities and

for reconstruction of the temporomandibular joint (TMJ) by chondro-osseous graft in TMJ disease and hemifacial microsomia facial deformities.

Bone grafting is a surgical technique used to fix problems by using transplanted bone to repair, rebuild, and replace missing bones in order to repair bone fracture or to replace missing bone after tumor surgery and loss of bone in trauma injury in road traffic accident and in post-traumatic missile war injuries and has also been used for reconstruction of damaged joints [3]. It is extremely a complex technique which poses a significant health risk to the patient and its liability to fail.

Bone graft was used as filler and scaffold to facilitate bone formation and generally has the ability to regenerate completely but requires a very small fracture space or scaffold to do so [3].

Bone graft may be autologous bone as cancellous or cortical or cortical-cancellous types harvested from iliac crest or from rib. Allograft a cadaveric type of bone usually obtained from bone bank or as synthetic bone made of hydroxyapatite or other naturally occurring and should be biocompatible substances with similar mechanical properties to bone.

Bone grafting is possible because bone tissue has the ability to regenerate completely once the space is provided into which it has to grow as natural bone. Bone grafting or transplantation of bone tissue is beneficial in fixing bones that have been damaged or destroyed by war or required for building bone around transplanted tooth in dental surgery. Bone grafting is a technique which requires great experience, skill, and knowledge, and great advances of bone grafting occurred during the last 4–5 decades.

Many techniques were advocated and described for reconstruction of discontinuity defect of craniofacial regions after tumor surgery or congenital deformities or traumatic injuries. Bone graft is widely used and considered as the second tissue transplantation after blood transfusion.

We successfully applied bone grafting in cranial-maxillofacial surgery in the following clinical cases:

1. Reconstruction of the mandible after radical tumor surgery.
2. Reconstruction of the mandible and maxilla after missile war injuries of the face.
3. Reconstruction of the orbital floor with large traumatic bony defect.
4. Reconstruction of the frontal bone and anterior cranial fossa.
5. Reconstruction of receded chin by sandwich technique.
6. Reconstruction of the temporomandibular joint (TMJ) by Kummoona chondro-osseous graft.

2. Reconstruction of the mandible after radical tumor surgery and post-traumatic missile injuries as secondary phase

We had a long experience in reconstruction of half of the lower jaw by free bone graft from iliac crest as autograft, and the type of graft which has been used was cortical-cancellous bone graft as a block from iliac crest. We can reshape the graft according to the required defect. The graft was chosen because its rigidity gave the contour of the mandible, which is

highly vascular and applied with firm and rigid fixation. We used previously intermaxillary fixation (IMF) for the healing process for a period of 6 weeks, but nowadays, we change our technique by using rigid fixation without IMF, and we ask our patient to start functioning the jaw immediately with semifluid diet, based on Moss theory (the growth of bones is based on the functional demand of periosteal matrix of the facial skeleton) [4]. We noticed long fixation by IMF end with difficulty of mouth opening and spasm of muscles with damage to TMJ.

In children we do use rib graft for reconstruction of the lower jaw after tumor surgery. We face slight difficulties in manipulating the rib graft due to rigidity and mainly cortical type, and the amount of cancellous bone is very little, and also the rib is less vascular and less minable for cortical-cancellous bone graft from iliac crest with possibilities of pleural perforation.

Bone grafting been used for reconstruction of the mandible after radical excision of tumor surgery, and half of the mandible can be reconstructed by free bone graft from the iliac crest of cortical-cancellous type as one piece or two pieces with rigid fixation is required and IMF is not necessarily used, but mobilization of the jaw was required after few days for restoration of growth and function of the graft and the mandible. In some cases, the tumor involves half of the mandible, the body, and the ascending ramus, but without involving the condylar and subcondylar region, the tumor was resected at the level of subcondylar region, and the preserved condyle was fixed to the bone graft after reshaping the graft. The condyle with the graft was reimplanted in the glenoid fossa after firm rigid fixation through bone grafting. A series of cases were managed by the author by using this technique. We reported that tumor cases of cystic ameloblastoma do not involve the inner cortical plate, but the tumor involves the outer cortical plate and the cancellous bone. The outer cortical plate excised and the cancellous bone that involved by the tumor. The inferior dental nerve was preserved after complete excision of the tumor and decortication of the bed, and a piece of cortical-cancellous bone graft was used for reconstruction of the defect [5, 6].

We reported failure of the graft in two cases. In the first case, the area was subjected to deep X-ray therapy and the other to chemotherapy during the healing period.

3. Reconstruction of the mandible and maxilla as secondary phase of post-traumatic missile injuries

Bone grafting has been used for reconstruction of the mandible as secondary phase of missile war injuries. Sometimes, the situation is more complicated and requires flap surgery; our choice is the Kummoona lateral cervical flap [6] which has been used for the reconstruction of submental area previously subjected to high-velocity bullet injuries with a lot of scars in the submental area, and the lower lip was retracted down by scar with loss of mouth seal. The scar was excised, and the lateral cervical flap was used before 3 months of bone grafting followed by reconstruction of the bone defect by bone graft from iliac crest.

Bone grafting was done successfully for the reconstruction of defect and deformity of the maxilla by shell injuries. Previously, the area was explored and reconstructed by using bone graft from the iliac crest as cortical-cancellous bone after measuring and reshaping it. The margins of bony defect were decorticated, and the graft was successfully fixed with 0.25 mm of soft stainless steel wire; the aim was to restore the esthetic and function of the face.

4. Orbital floor reconstruction

Blowout injuries are quiet common with road traffic accident where the orbital floor content is displaced down to the sinus with herniation of orbital fat and incarceration of inferior oblique and inferior rectus muscles featuring enophthalmos and diplopia. The orbital floor defect measured if small can be successfully reconstructed by silicone rubber material sialastic (rubber silicone material) which is a biologically inert material, but once the defect is large, bone graft is harvested from the outer cortical plate of the iliac crest to simulate the floor, but our observation on bone graft of the floor might show some degree of resorptions in that case. An additional layer of silastic of 2 mm thickness is required to correct the case. It was noticed that membranous bone graft from skull vault is less liable for resorptions.

5. Reconstruction of anterior cranial fossa, orbital roof, and frontal bone

Severe craniofacial injuries may end with head injuries, with severe damage to the frontal bone, roof of the orbit, nose, and anterior cranial fossa. After the recovery of the patient from head injuries, the anterior cranial fossa is approached through bicoronal flap with craniotomy. The brain and dura are retracted backward, and the dura is repaired by the galea or temporalis muscle. The dura should be closed as watertight closure, the roof of the orbit and anterior cranial fossa was reconstructed by bone graft from the iliac crest with silastic, and the frontal bone was reconstructed by bone graft. The author successfully reported few cases with severe craniofacial trauma treated by this technique with collaboration with neurosurgeons.

6. Reconstruction of the chin

Hypoplasia of the chin or receded chin usually required bone grafting by sandwich technique by doing transverse osteotomy of the lower anterior border of the lower jaw. Bone graft was harvested from the iliac crest as horse shows cortical-cancellous bone graft inserted in between the two bones and fixed by rigid fixation with soft stainless steel wire of 0.25 mm or by plate, but some of these bone graft may show some degree of resorption. We prefer kidney-shaped silastic implant in three sizes, small, medium, and large, and the access for the silastic and bone graft is through submental incision [5].

7. Reconstruction of the temporomandibular joint (TMJ) in children

Reconstruction of the TMJ is of great challenge to maxillofacial surgeons because of difficulties of intubation in ankyloses and cases with hemifacial microsomia or first arch dysplasia syndrome and hypoplasia of the condyle.

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There are two successful techniques for reconstruction of the TMJ:

1. *Costochondral graft*
2. *Kummoona chondro-osseous graft*

Many other techniques have been used like sternoclavicular graft. This technique is only reported once or twice. This graft failed to restore growth of the condyle of TMJ but was used as gap arthroplasty, and this showed technical difficulties with a large head of sternoclavicular graft to fit small glenoid fossa [7].

The costochondral graft for reconstruction of the TMJ has been used since 1973 by Kennett [8] and experimental studies by Poswillo [9] on Macaca iris monkey to prove the viability of the graft and the cellular changes to simulate the condyle for restoration of growth and function of the TMJ. The objection about costochondral graft is that the junction between osteoid element and cartilaginous part is very fragile and easy to dislodge. Possibilities of pleural perforation and long duration of intermaxillary fixation (IMF) for 6 weeks end after the release of IMF. A spasm of muscles of mastication developed besides overgrowth of the graft was reported [10].

Kummoona chondro-osseous graft advocated in 1986 [11, 12] is the most popular graft nowadays because its junction between osseous element and cartilaginous cap is very stable but rigid fixation of the graft to ascending ramus with no IMF, and the child is advised to chew within the next few days to restore function and growth of the graft and the TMJ. This statement is based on Moss theory (1962) [4], the theory of functional demand of the periosteal matrix of facial skeleton.

Experimental research and study were done on a rabbit to demonstrate the viability of the chondro-osseous graft [12] and to demonstrate that the condyle is a growth center. At the end of the experiment, we did postmortem studies and observed an excellent union between the graft and ramus of the rabbit mandible.

Histological examination of the graft showed four zones. The first layer showed a thick articular layer of dense fibrocartilage due to the demand of hard masticatory process of rabbit food, the second layer showed several zones of active layers of round mesenchymal stem cells which represent the proliferative layer, and the third layer showed a series of hypertrophic chondrocyte passing through a series of changes. This layer represents the differentiation of mesenchymal stem cells to chondrocyte and osteocyte. These cellular changes represent the growth potential of the graft, and the fourth layer was an osteoid bone with bony trabecula and bone marrow spaces in between.

In the previous research on the bone and cartilage, they did find a G-protein-coupled receptor (CXCR4) predominately expressed in hypertrophic chondrocyte, while its ligand chemokine stromal cell-derived factor (SDF-1) is expressed in the bone marrow adjacent to hypertrophic chondrocyte. These findings explained the endogenous growth potential of the graft to continue to grow, repair, and remodel the condyle and restore growth of the mandible and midface in children for correction of facial deformity in the affected side.

8. Cytological changes that occurred through two mechanisms in the bones either distraction of bone or grafting bone defect by iliac crest bone graft and the role of mesenchymal stem cells

Our recent advances in bone research by experimental studies are by using rabbits as animal models for studding distraction technique for elongation of bones and studying the changes of bone grafting and the cellular changes associated and the role of mesenchymal stem cells.

Distraction is defined as the process of generating new bone by stretching distraction osteogenesis (DO). Traction on the living tissue can stimulate and maintain regeneration and growth by stimulating the proliferation of precursor cells.

The human body has an enormous regenerative ability to induce a regenerative ability and distraction osteogenesis (DO) which takes the advantages of this regenerative ability to induce the regeneration and remodeling of bone [13, 14].

We did experimental studies on rabbits by achieving distraction of the mandible [15] to demonstrate that the previous literature did not mention about the biological changes that occur in the gap created by osteotomy of the bone site desired during the latent period which is the key factor in distraction process and formation of new bone.

The mechanisms of surgical distraction technique are passed through three phases—phase one is the surgical phase, phase two is the latent period phase, and the third phase is the consolidation phase. Surgical phase is started by fitting distraction apparatus and gap creation by osteotomy. The second phase is the phase of the latent period where the biological and cellular changes occurred by formation of clot and granulation tissue with the release of growth factor (GF) from platelets with proliferation of mesenchymal stem cells derived from bone marrow, periosteum, and covering muscles with active fibroblast formation under the influence and action of growth factor (GF); this phase is the silent phase elapsed between 7 and 10 days.

Distraction of the lower jaw was achieved by using bilateral distractor designed for hand bone lengthening apparatus which was adjusted by transfixation by Kirschner wire of 1.5 mm which was passed through both mandibular bodies. Rhythmic distraction of both corticomized fragments was carried out at a rate of 1 mm/day at a rhythm of 0.5 mm twice daily for 10 days. The segments hold for 6 weeks till consolidation phase is completed, and bone formation and regeneration were evaluated radiologically.

Bone regeneration by distraction is a highly complicated and organized process. Through our research, we found in the histological studies revealed and demonstrated by our experiment that bone regeneration is based on membranous ossification preceded by formation of granulation tissue and mesenchymal stem cells derived from bone marrow of bone segments and periosteum lining and covering muscles with formation of active fibroblasts in the same direction of stretching forces by the influence of growth factor (GF) for formation of new bone, muscles, and even skin [13–15].

Bone grafting is also another interesting topic. We did research on rabbits [16] by resecting a piece of bone of 1.5 cm from the lower border of rabbit mandible and reconstructed by a piece of bone from the iliac crest of the rabbit of about 2 cm after decortication of both segments of the mandible and fixed by rigid fixation by stainless steel soft wire of 0.25 mm. The rabbits were divided for purpose of experiment into two groups: one bone graft was fixed without a cover by oxidized regenerating cellulose (Surgicel) in group A, and in the second group, group B, the graft was covered by oxidized regenerating cellulose mesh (Surgicel), to study the cytological changes associated with bone grafting and the differences between two groups of rabbits in healing process and cytological status.

The aim was to study the role of mesenchymal stem cells in bone grafting. In the experiment, 12 young rabbits of 3 months of age were divided into two groups, group A and group B, each group of five rabbits and two rabbits used as control. These rabbits were subjected to surgical osteotomy by excising 1.5 cm from the body of the mandible, and bone graft of 2 cm length was harvested from the iliac crest of the rabbit and fixed by rigid fixation by soft stainless steel wire of 0.25 mm. Oxidized regenerated cellulose (Surgicel) was used as mesh to cover the bone graft of group B; 3 months later, the experiment was terminated. The histological sections were obtained every 2 weeks, 4 weeks, and 8 weeks.

Histological and cytological changes of bone grafting was quiet interesting and showed formation of clot and platelet aggregation with releasing growth factor (PGF), and healthy granulation tissue was formed with mesenchymal stem cells derived from the bone marrow, periosteum, and covering muscles with formation of a large amount of fibroblasts and tiny small vessels. Osteoblasts were seen with the chondrocyte; these changes were noticed more with bone graft that was covered by Surgicel in group B. The role of Surgicel was to accelerate the healing process of bone grafting.

This research proved to be of great value to humans for better understanding of the cellular changes and the mechanisms associated with bone grafting and distraction for elongation of bones in children. The cellular changes of distraction technique and bone grafting by inducing mesenchymal stem cells are the same except distraction induced by stretching growth potential of bones based on Illizarov theory, and the bone grafting based on maximum contact between bone graft and bony segments after decortication of the segments with rigid fixation can be achieved by plating or by soft stainless steel wire of 0.25 mm.

The management of bony defect urgently required bone grafting after traumatic injuries, after missile war injuries, and after radical cancer surgery as an urgent technique.

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Chapter 2

Craniofacial Bone Grafting

Muzaffer Çelik

Additional information is available at the end of the chapter

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Abstract

Craniofacial bone grafting plays an important role in the reconstruction of the craniofacial skeleton. Most of the craniofacial surgeons accepted that bony defects of the face must be reconstructed with bone, and soft tissue defects must be reconstructed with soft tissues. Several studies have described the cranial bone grafting procedure that is preferred by most craniofacial surgeons worldwide. In the past and currently, alloplastic and other autogenous materials have been used to reconstruct the craniofacial skeleton. However, alloplastic materials have been abandoned as they are associated with a high risk of complications such as migration, infection, and underlying bone resorption.

Keywords: facial skeleton, skull graft, bony reconstruction

1. History

Craniofacial bone grafting plays an important role in the reconstruction of the craniofacial skeleton. Most of the craniofacial surgeons accepted that bony defects of the face must be reconstructed with bone and soft tissue defects must be reconstructed with soft tissues. Several studies have described the cranial bone grafting procedure that is preferred by most craniofacial surgeons worldwide [1–5]. In the past and currently, alloplastic and other autogenous materials have been used to reconstruct the craniofacial skeleton. However, alloplastic materials have been abandoned as they are associated with a high risk of complications such as migration, infection, and underlying bone resorption [6].

If vascularized free bone flaps and nonvascularized iliac bone grafts for mandibular reconstruction are eliminated, cranial bone grafts are the gold standard for use in the craniofacial skeleton. Because cranial bone grafts are composed of membranous bone, it is felt that they retain their bulk better than other types of bone grafts do, especially if they are rigidly fixed.

2. Necessity

Post-traumatic defects such as orbital floor fractures, postresection defects due to bone tumors, congenital bone defects, and esthetic reasons are some of the indications for the use of cranial bone grafts.

3. Mandibular reconstruction

For small mandibular defects and alveolar clefts, iliac bone chips and cranial bone chips are useful and mostly preferred by surgeons. In the alveolar cleft, our aim is to reconstruct the alveoli during primary cleft repair. In newborns, iliac bone chips are preferred for alveolar reconstruction. In adolescent or late repair, cranial bone chips are another useful graft material for alveolar reconstruction (**Photos 1** and **2**).

Mandibular contour defects should be reconstructed with cranial bone grafts because they have less tendency to resorb than other types of grafts do. Bone defects of the mentum should also be reconstructed with cranial bone grafts.

According to my personal experience, for full thickness defects of the mandible that are up to 10 cm in size, a nonvascularized, drilled iliac bone is the best choice.

One of the most important points when harvesting the iliac bone is to keep the muscle attachments intact and preserve all edges of the iliac bone. The iliac bone graft is harvested from the middle part of the iliac bone.

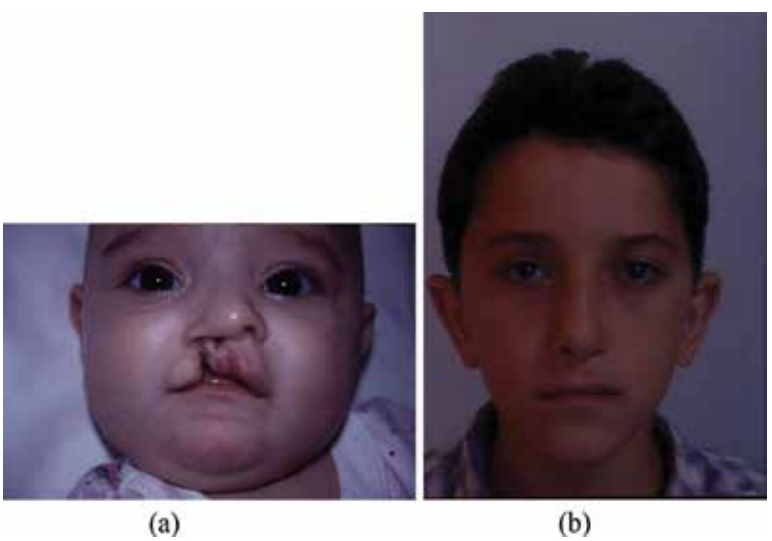

Photo 1. Alveolar bone grafting during the primary cleft lip repair.

Photo 2. Alveolar bone grafting during the lip-nose revision.

In patients with vertical mandibular asymmetry, the interpositional placement of the iliac bone graft or bone that is resected on the contralateral side is the most preferred in our practice (**Photo 3**).

Mandible is the most dynamic part of the of the oral and craniomaxillofacial region. It includes and neighbors the temporomandibular joint, glenoid fossa, teeth, muscles, ligaments, salivary glands, and the tongue. It creates the boundaries of fossas (submandibular, sublingual, submental, infratemporal, pterygomandibular, submasseteric, and so on) as well. Therefore, reconstruction of the mandible requires to restore all the functional, anatomic, and esthetic aspects.

The functional considerations would include the restorations of occlusion, fonation, mastication and swallowing. Anatomic reconstruction requires adequate three-dimensional maxillomandibular relation. The esthetic outcomes would have balanced facial harmony with symmetry and vertical dimension.

Today, mandible can be reconstructed via non-vascularized bones or different types of free flaps in association with stock or 3D custom-produced titanium screws and plates.

The use of non-vascularized bone grafts and modified approaches for reconstruction of atrophied mandible prior to dental implant and dentures is well defined in literature. However, for larger defects after trauma or neoplasm surgery for the vitality of the bone and soft tissue of the graft is still challenging. Recently, the use of vascularized osteocutaneous free flaps has decreased the morbidity and mortality percentages, especially since the oncological cases and

Photo 3. Interpositional bone grafting from left mandible to the right side.

osseointegrated dental implants that are installed on these flaps help to improve postoperative masticatory function.

Iliac crest and the fibula are the most favorable donor sides for oromandibular reconstruction with the advantages of minimal donor site morbidity, optimum pedicle length and diameter and two team approach. Various factors must be considered such as localization, residual bone dimensions with or without soft tissue defects, vessels, and bone volume for dental implant rehabilitation, when deciding which flap is the most suitable.

4. Iliac crest free flap

The iliac crest composite free flap has proven to be the one of the most effective and reliable choice for oromandibular reconstruction due to its appropriate and sufficient bone volume and corticocancellous structure and shape. It allows immediate reconstruction that avoids contour distortions of the mandible.

The iliac bone vascularization is maintained by circumflex arteries including the deep, lateral, superficial ones, epigastric superficial inferior and superior gluteal artery, and branches. The deep circumflex iliac artery (DCIA) is the principal blood supply for the flap. The incision should be designed according to need of the skin paddle. Following soft tissue dissection, the periosteum on the superior bone crest is elevated. Down to the level of the deep circumflex iliac artery, internal oblique and iliacus muscles are dissected and divided. According to shape of desired reconstruction of the mandible, the bone osteotomy and vascular pedicles are harvested, and rigid fixation is performed with by plates/screws. Finally, microvascular anastomoses of the vessels are finalized.

5. Fibula free flap

The free fibula flap has gained popularity due to its bone graft and vessel length for reconstruction of extended mandibular defects. Bone flap can be harvested with adjacent periosteum and soft tissue with or without a skin paddle. The incision starts 5 cm below the lateral epicondyle of the fibula and runs 10–15 cm in a distal direction. After elevating the skin flap anteriorly/posteriorly, the muscle dissection through the intermuscular septum to peroneal muscle and sural muscle is performed to identify fibula. A muscle cuff of 3 mm is left along the fibula. Anterior intermuscular septum is incised by protecting the anterior tibial vessels. Then hallucis longus and tibial muscle through interosseous membrane is dissected. Proximal and distal cuts are performed around minimum 7 cm blow the proximal neck and superior to lateral malleolus according to desired bone length of the reconstruction site. The soleus, hallucis longus, and tibial muscles are dissected to elevate the peroneal vessel pedicles. At the distal site, the outward traction of the bone would help to reach tibial posterior muscle with its raphae, which help to secure the vessels when the muscle dissection is carried out along this raphae. Facial and supra thyroid arteries and external jugular vein is generally preferred for anastomoses. By osteotomy cuts on the harvested fibula, the required shape for mandibular reconstruction is achieved.

6. Upper face and cranium reconstruction

For the entire upper face, including the nose and the cranium, bony reconstruction and cranial bone grafting are the best choices because they are membranous structures that have less tendency to resorb.

In terms of craniofacial surgeons' preferences for cranial bone grafts, this is the gold standard. Even for dorsal nasal reconstruction, the cranial bone is the most popular in craniofacial surgery departments [6, 7]. Rib cartilage has a high rate of resorption and undesired shrinkage. Diced or blocked rib cartilage is not incorporated into the underlying nasal bones. One of the fans of rib grafts was popular rhinoplasty surgeon Jack Sheen, who turned to use the cranial bone for nasal reconstruction.

7. Harvesting of cranial bone graft

Cranial bone grafts can be harvested as split-thickness or full-thickness bone grafts according to the condition of the recipient area. In the case of full-thickness cranial bone graft harvesting, a neurosurgeon should be involved in the surgery team. The desired shape and size of the bone graft are drawn on the skull after the scalp is subperiosteally dissected. Then the neurosurgeon opens burr holes at the planned areas. The burr holes are connected by cutting the cranium with an electrical craniotome. The dura is dissected carefully, releasing the cranial bone from the brain. In most conditions, harvested full-thickness cranial bone is split from diploe obtaining pieces of split-thickness cranial bone grafts.

Usually, the outer table of the cranium is used to reconstruct the donor site, which is fixated without any step to protect the cranium against undesired irregularities (**Illustration 1**). In children up to 10 years old, the diploe does not exist. The diploe may not exist in women and men who had a poor diet during their period of growth. In those with syndromic cranial anomalies, an undesired forehead shape may be corrected by transferring a nice part of the cranium to the forehead (**Photo 4**). In the case of split-thickness cranial bone graft harvesting, the desired shape is drawn on the temporoparietal skull with a pencil. Then, the graft area is cut down to the diploe with a special electrical osteotome. One of the edges of the bone graft is exposed, and a tiny wedge of bone is removed.

Then, a curved, tiny chisel or specially designed, L-shaped electrical osteotome is used to release the outer table of the cranium. Cranial bone graft-donor sites are reconstructed with tiny bone chip lamellae that are harvested from the area that is adjacent to the donor area (**Illustration 2**). This procedure is associated with a low incidence of patient complaints, thereby suggesting higher patient satisfaction [8]. This approach to cranial bone grafting appears to have a high patient acceptance [8].

Cranial bone grafts can also be harvested as bone chips, especially in patients who undergo alveolar cleft repair [9].

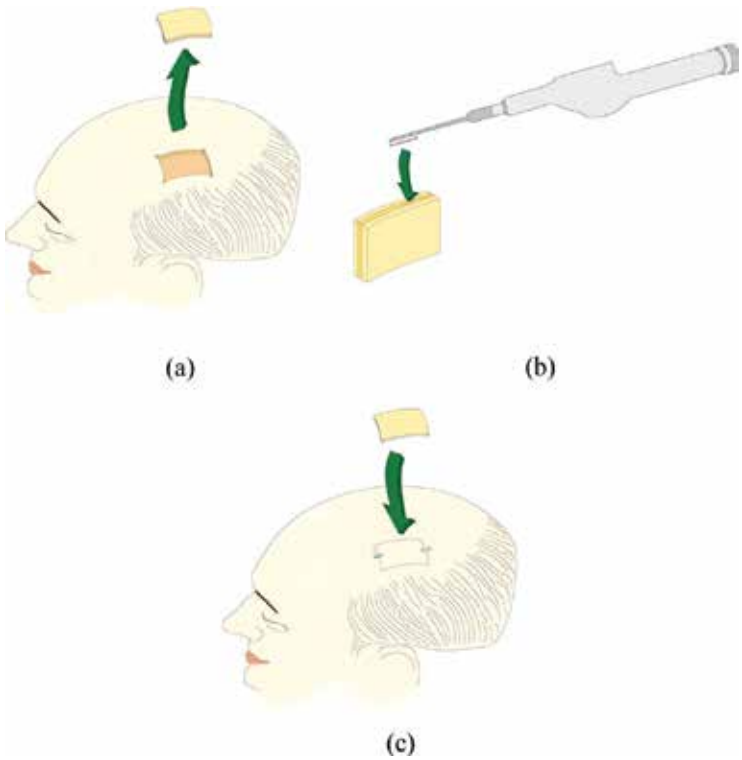

Illustration 1. Full-thickness cranial bone graft harvesting.

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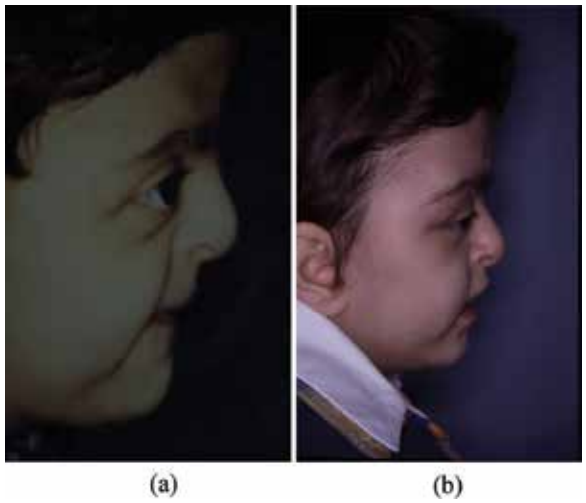

Photo 4. Temporoparietal bone block transferred to the forehead.

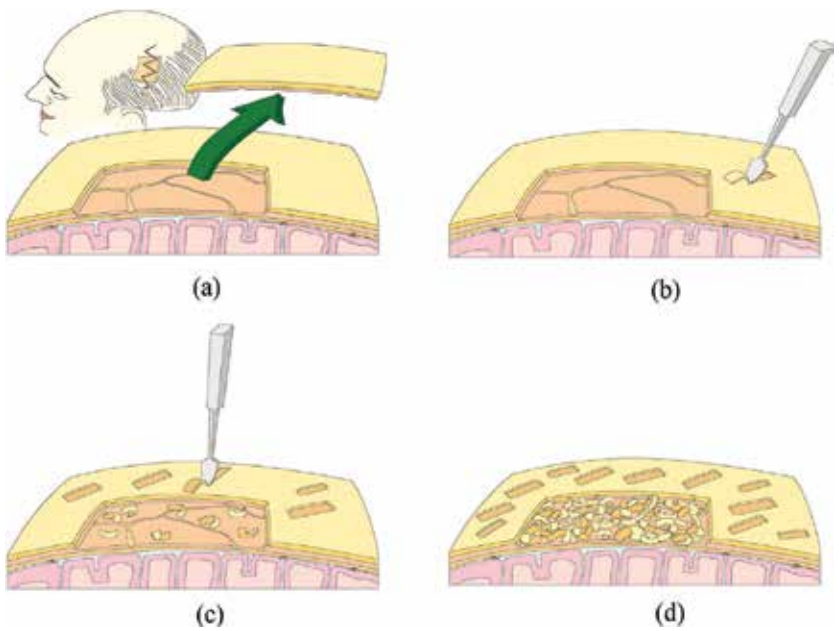

Illustration 2. Cranial bone graft donor site reconstruction.

7.1. Cranium reconstruction using cranial bone graft

Cranial defects must be reconstructed with cranial bone grafts. All alloplastics have different side effects and complications, while cranial bone grafts are durable and strong.

Split-thickness, outer table cranial defects may not be reconstructed, but we described a technique in which cranial bone chips were used to reconstruct the outer table of the cranium [8] (**Photo 5**).

Photo 5. Orbital floor and medial orbital wall reconstructed using split cranial bone grafts.

Full-thickness cranium defects must be reconstructed because of the possible risk of brain injury. For this purpose, the shape of the defect is drawn on the donor area, and a full-thickness graft is harvested and split into two pieces.

The outer table is used to reconstruct the donor site, avoiding any step deformity. The inner table is used to reconstruct the defect.

7.2. Orbital floor reconstruction with cranial bone

In the case of blow-out fracture of the orbit with a tiny bone defect on the floor, the size of the defect is drawn on the donor area, and the periosteum must be kept on the cranial bone. Then, the area around the graft is outlined with a tiny curved chisel. Finally, the graft that is 3 mm in thickness is harvested. This curved graft with a few fractures is inserted into the orbital floor through transconjunctival incision and is not fixed. The periosteum keeps the fractured, small cranial bones in one piece.

Large orbital defects are reconstructed with split-thickness cranial bone grafts. The shape of the defect is drawn on the temporoparietal cranium. After harvesting the desired graft, the surgeon fixes a miniplate to the harvested bone graft (**Illustration 3a** and **Photo 6**). Then, the graft is placed on the orbital floor and fixed to the anteroinferior part of the orbit (**Illustration 3b**).

7.3. Dorsal nasal reconstruction with cranial bone

According to my 22 years of experience, a cranial bone graft is the best choice for dorsal nasal reconstruction. Jackson et al. described the cranial bone grafting for nasal reconstruction in 1983 [2].

They published a long-term follow-up paper about the use of cranial bone grafts in dorsal nasal augmentation [6]. They mentioned that they formed various impressions about the use

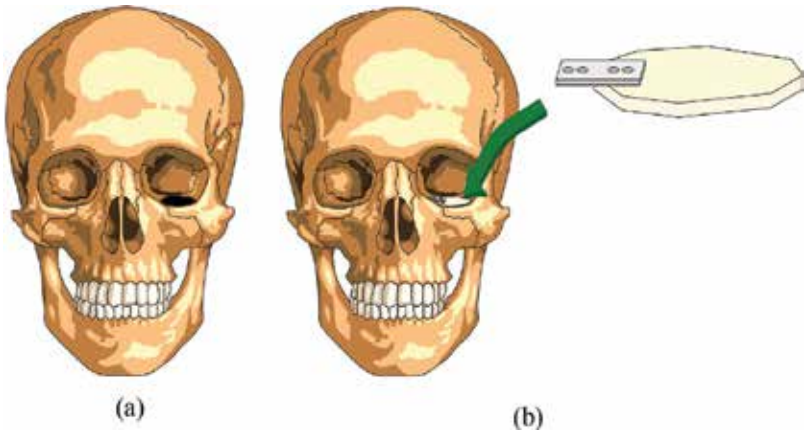

Illustration 3. Orbital floor reconstruction using split cranial bone graft.

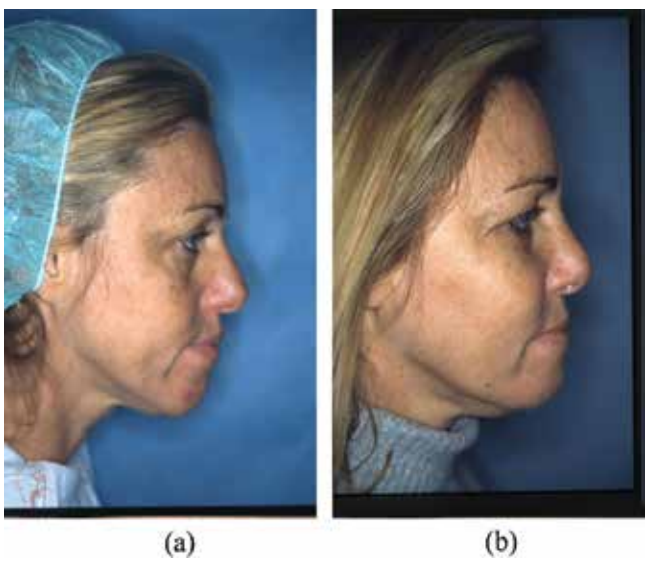

Photo 6. Malar augmentation with cranial bone graft.

of cranial bone grafts in other areas of the face. Cranial bone grafts are composed of membranous bone, and it is felt that they retain their bulk better than other types of bone grafts do.

In patients with cranial bone grafts on the nose, these grafts should be examined more effectively than grafts that are placed on other areas of the face because the grafts are in such an obvious and easily examined area and are covered with thin skin.

According to Jackson et al.'s technique, the cranial bone from the radix to the tip of the nose is used. However, this technique has two disadvantages: it causes the nasal tip to become rigid and the nose is rendered because of traumatic forces. Therefore, we described a modified technique for dorsal nasal reconstruction with the cranial bone [7]. We believe that the bony segment of the nose must be reconstructed with bone and cartilage segment and a cartilage

graft when performing anatomic nasal reconstruction. The idea for our technique arises from the principles of anatomic reconstruction, which means that the bony part of the nose is reconstructed with cranial bone and the cartilage part is reconstructed with cartilage (**Illustration 4**).

According to our technique, the surgery is planned after the nose is examined. Nasal reconstruction is planned using cranial bone for the bony part of the nose and a double layer of ear cartilage for the distal part. The dimensions of the graft are measured on the nose and drawn on the skull with a marking pen. Then, the margins of the graft are cut down to the diploe with a special electrical osteotome. On one side of the margins, a wedge-shaped bone is removed to expose the diploe. Then, the bone graft is elevated using a tiny, curved chisel or an L-shaped electrical osteotome. After harvesting the bone and cartilage grafts, dorsally at the caudal end of the bone graft, the surgeon burrs away that is two millimeter in thickness. This burred area is deepened to create a space at the proximal end of the upper layer of the cartilage block. On the burred area, six small holes are opened using a tiny drill.

The cranial bone graft is shaped as desired with a contouring drill. The upper layer of the cartilage is fixed to the bone through these holes using 5-0 nylon sutures.

Then, another layer of cartilage graft is sutured posteriorly to the first layer, which is adapted to the bone graft that was created at the beginning of this step. The bone-cartilage block is

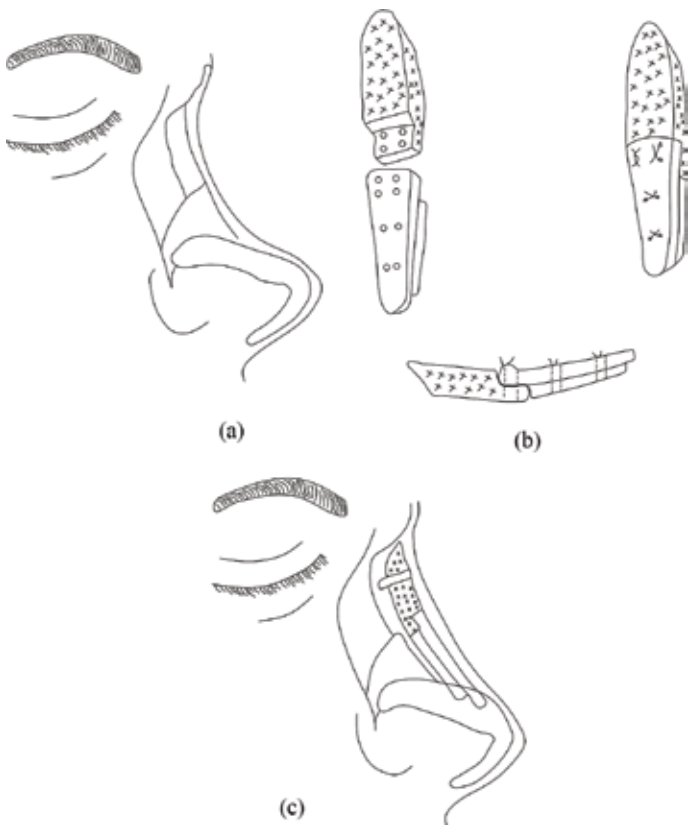

Illustration 4. Nasal reconstruction using both cranial bone and ear cartilage.

inserted in to the nose through an open approach incision, and the bony segment is fixated to the radix with a screw through a vertical glabellar incision.

7.4. Malar augmentation with cranial bone graft

Cranial bone graft is the first and best choice for malar augmentation in our practice. First, the malar area is exposed with subperiosteal dissection. Then, the augmentation thickness and size of the malar area are. The donor site is selected according to the size and shape of the required bone graft. Usually, a short, running Z incision is used for harvesting (**Illustration 2**). The desired graft is harvested and shaped using a contouring drill. When the graft is ready to be inserted, the best location is selected according to its esthetic appearance during a visual examination. Two screws are used for fixing the cranial bone graft to the underlying malar bones.

7.5. Cranial bone grafting for fixation during the maxillary osteotomy

If maxillary osteotomies are performed to elongate or advance the maxillae, there is a significant risk of the relapse, even fixation with a plate and screw. When we examine the maxillae of patients with a cleft palate, there is retrusion and hypoplasia. In patients with these conditions, the maxillae should be advanced and elongated.

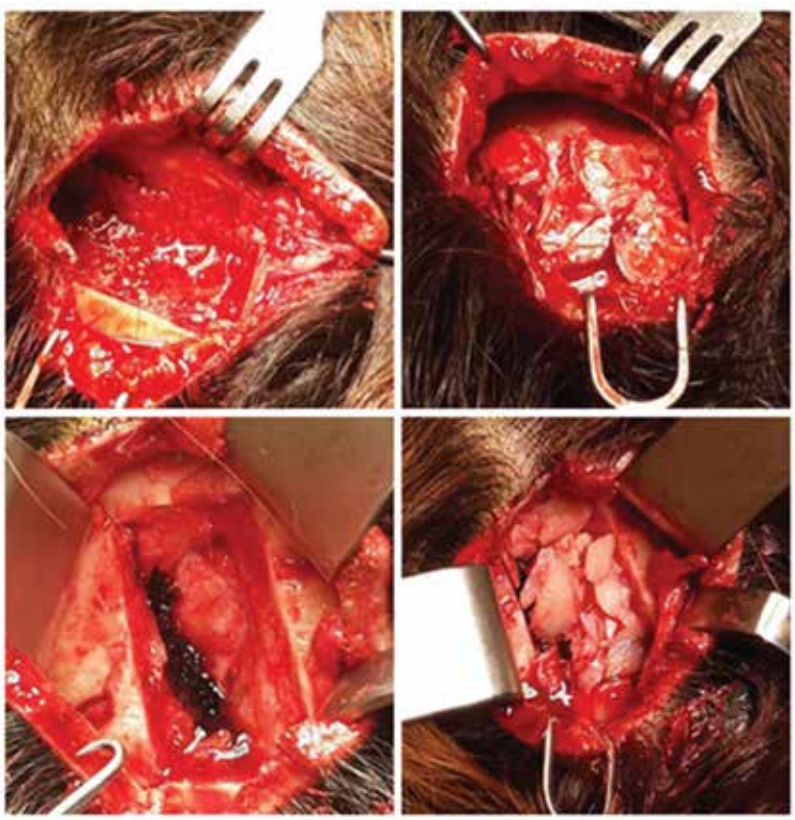

Photo 7. Reconstruction of the donor site of cranial bone graft.

Elongation and advancement are performed using Le Fort I osteotomy, and there is no bony contact between the advanced part and the maxillae.

However, this condition is subject to the relapse because of advancement and elongation. In most patients with midface retrusion, when the maxillae are advanced, malar retrusion becomes more prominent. Therefore, a cranial bone graft is used to both provide strong fixation of the Le Fort I osteotomy and augment malar retrusion. Usually, a preplanned, large split-thickness cranial bone graft can solve both problems on one side of the face.

Cranial bone grafts are used for all types of upper face bone defects and chin defects, mandibular contour augmentation, periapertural augmentation, and augmentation of asymmetric deformities (**Photo 7**).

8. Cranial bone graft donor site reconstruction

The most important concern, in my entire experience with cranial bone grafting procedures, is managing the donor site of the bone graft, such as the donor site cavity through harvesting and weakening of the cranium. The most frequent patient complaint, following cranial bone grafting for esthetic indications, is the presence of a cavity at the donor site. I previously described a technique for cranial bone graft donor site reconstruction. The cranial bone graft donor site is reconstructed with tiny bone chip lamellae that are harvested from the area that is adjacent to the donor site. This approach to cranial bone grafting appears to have high patient acceptance. Our 15-year experience with donor site reconstruction following cranial bone grafting has demonstrated that the procedure is simple, safe, and satisfying. Herein, we provide a detailed description of our technique for donor site reconstruction.

9. Surgical technique

As mentioned before, donor site reconstruction following cranial bone grafting involves the use of thin bone chip lamellae that are harvested from the cranial bone that is adjacent to the donor site.

We have successfully performed this procedure in more than 200 patients in a 15-year period. This reconstruction technique is applicable to mild-to-moderate donor site defects, and is useful for treating in both, split- and full-thickness donor site defects.

An initial Z-type scalp incision is made, followed by subperiosteal dissection to create a periosteal cover for the grafted bone chips. The galea over the periosteum is preserved to ensure that the periosteal blood supply is adequate. The use of subperiosteal dissection is essential in order to close the reconstructed donor site. Following graft harvesting of the cranial bone, thin cranial bone lamellae are harvested from the adjacent cranial bone by using a curved chisel (**Illustration 2** and **Photo 5**). The bone dust and small bone chips that are obtained during harvesting are collected and also used for reconstruction. The thin harvested bone chips are placed in the donor site cavity to over-correct the defect. Then, a block of gelatin sponge

is placed over the bone grafts to avoid displacement during the operation. The procedure is completed by closing the periosteum and skin in separate layers.

Among the patients we have treated, the reconstructed defects initially appeared to be undercorrected, which prompted us to modify our procedure by overfilling the cavity with cranial bone chips.

As a craniofacial surgeon, I observed my mentors Henry Kawamoto and Ian Jackson perform cranial bone graft harvesting. My main concern was that patients might not accept the appearance of the donor site defect, especially when the procedure was performed for esthetic reasons. I described a technique of nasal bony reconstruction and performed it in a large number of patients. My initial experience with this procedure demonstrated that there was poor patient acceptance of the cranial bone grafting procedure owing to the presence of a defect at the donor site. Therefore, I reconstructed the donor site defect using tiny bone chips that are harvested from the cranial bone that is adjacent to the donor site. After the procedure was introduced, a higher proportion of my patients accepted the use of cranial bone grafts. The bone dust and small bone chips that are obtained during harvesting are also collected and placed in the donor site cavity, along with the tiny harvested bone chips. Overcorrection is advised to account for the potential dead spaces between the bone chips. Several reports have described reconstructing the donor site following full-thickness cranial bone grafting by splitting another full-thickness bone graft or using a split graft.

In our experience, reconstructing split-thickness and full-thickness donor sites with cranial bone chips is a simple, safe, and satisfying procedure. This technique is useful to fill the donor site during cranial bone grafting, which is a concern for esthetic surgeons.

10. Discussion

Overall, scientific studies have shown that bony defects of the craniofacial structures must be reconstructed with autogenous bone grafts. Although some studies have found that irradiated bone or autoclaved homografts are useful for bony reconstruction, our clinical experience contrasts with these studies.

Alloplastic materials such as medpor, silicon, and hydroxyapatite are not useful for bony reconstruction of the craniofacial skeleton. For defects of the mandible that are up to 10 cm in size, drilled, nonvascularized iliac bone grafting is an easy and suitable reconstruction method. The donor site of nonvascularized iliac grafts must be harvested at the midportion of the iliac bone instead of at the edge of the anterior part. For large or total reconstruction of the mandible, free iliac or fibula bone grafts are preferred. Free iliac grafts are superior to free fibula grafts for dental restoration because of their spongy structures.

As I mentioned before, cranial bone grafts are the best choice to reconstruct the upper face and cranium. Some research studies investigated resorption of cranial bone grafts on the craniofacial skeleton, and they showed minimal rates of resorption [5, 6]. According to my 23-year experience with cranial bone grafts, these grafts have minimal or no resorption. In the past, surgeons used materials from other sites for dorsal nasal augmentation: the rib, iliac crest, olecranon,

mandible, or cartilage. It was reported that a cranial bone graft can be harvested by a surgeon who has had proper training, with an extremely low incidence of serious complications [10–13].

Cranial bone resorbs less than other bone does, does not warp, has a hidden donor site, and has an excellent shape [5]. Solid silicone, silicone sponges, medpor, and proplast are easy to use but lead to a high rate of complications in Caucasian patients. This does not occur in Asian people.

Nasal reconstruction with a calvarial bone graft from the radix to the tip of the nose may cause problems such as pain in the nose, graft fracture, graft displacement, and an immobile nasal tip [6].

We believe that the bony segment of the nose must be reconstructed with bone and the cartilage segment should be reconstructed with a cartilage graft when anatomic nasal reconstruction is performed.

In conclusion, nasal reconstruction with a cranial bone-ear cartilage complex facilitates anatomic reconstruction, creating a flexible nasal tip that benefits from the use of autogenous materials.

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Chapter 3

Bone Graft Types

Yaşar Mahsut Dinçel

Additional information is available at the end of the chapter

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Abstract

Bone grafts have been used by surgeons for a variety of purposes including filling bone cysts, reconstruction of bone loss after trauma and tumor resections and osteogenesis in fractures with union problems. In recent years, a significant increase in the use of bone grafts for reconstructive purposes has necessitated bone grafts of much greater shape and size. Although the use of avascular bone transfers is becoming more preferred due to benefits such as good osteogenic properties, resistance to infection and hypertrophy over time, nonvascular bone grafts have a wide range of use in fracture repair and reconstruction, with new developments in bone morphogenetic protein and stem cell support areas resulting in the proliferation of bone banks. Bone grafts are evaluated in three main groups as follows: autografts, allografts and xenografts. We have compiled the types of bone grafts.

Keywords: autografts, allografts, xenografts, review, literature

1. Introduction

Bone grafting is a surgical procedure that has been used for many years, especially in the fields of orthopedics, neurosurgery, and plastic surgery. Bone grafts are used for filling cystic defects, for bone fractures and arthrodesis treatment, and also for traumatic bone defects or loss of bone lesions that occur after removal of the tumor. It has been reported that allograft use increases in revisions of arthroplasty and in vertebral fusions in the last 10 years [1].

Bone grafting is needed and used in orthopedic surgery and plastic surgery for the fracture repair and skeletal reconstruction of the craniofacial region during the first century. Although the first recipe, up to the seventeenth century, continued to improve day by day in terms of better understanding of the pathophysiology of bone grafts and developing new techniques,

especially for the removal of vascular bone grafts. Nonvascular bone grafts are still widely used in fracture repair and reconstruction, along with new developments in bone morphogenetic protein and stem cell-supporting areas, leading to more favorable use of vascular bone transfers due to benefits such as good osteogenic properties, resistance to infection and over time hypertrophy.

The graft is a dead structure, providing new bone formation and replacing the new bone implant. While grafts are rapidly incorporated into the body, others integrate differently. Some of the grafts cause inflammation and are rejected. Some grafts are completely inert. However, successful results are usually obtained with bone grafts.

Bone and periosteum were expressed at the beginning of the nineteenth century with biological potential. Reliable and pioneering clinical applications began with the reconstruction of a diaphysis of a child's arm-bone by Macewen in 1881 [2].

Autogenous cancellous bone graft is now considered the "gold standard" for fracture healing, to fill spine fusions and bone defects. The main reason for this is the essential components [osteoprogenitor cells, osteoconductive hydroxyapatite collagen matrix and bone matrix protein (BMP)] that help bone healing [3].

Grafts are used as a skeleton to provide bone formation and support wound healing. The grafts also act as a mineral reservoir to aid in the formation of new bone. Bone grafting is a surgical procedure. It aims to replace missing bones with the artificial or natural substitute material of the patient's own body. As the natural bone grows, it usually replaces the graft material completely and results in a completely new bone area [4].

Different sources and origins and various bone graft categories and graft replacements are available. Despite the availability of a wide variety of options, the availability of autologous bone grafts may be limited. In addition, the procedure for collecting material is associated with many complications [5].

Bone grafting has been used to stimulate the healing process over a period of 300 years [6], but the mechanisms by which research has emerged over the last 30 years have been uncovered.

Fresh autogenous bone graft stimulates osteogenesis by three main mechanisms [7]. The first is the direct addition of osteogenic progenitor cells to the local population. These cells are necessary for the differentiation of new bone forming cells. Secondly, autogenous bone grafting is a structural cage for the attachment of osteoblasts, and osteoconductivity for matrix support for supporting cells. The third mechanism is osteoinduction. It refers to the ability of the bone graft to pick up the surrounding mesenchymal cells and direct their differentiation to bone and cartilage forming cells.

Autografts contain a large number of bone growth factors that stimulate the growth of new blood vessels into the graft and encourage migration of bone-forming cells to the injury site. Bone grafts also function as structural support.

The healing of the bone graft and healing occurs with the same healing phases such as induction, inflammation, soft callus, hard callus and remodeling. The mechanical conditions around the

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fractured have a great effect on the morphology. According to Wolff's law, compression-electro-negative areas during bone remodeling increase bone formation. Tension-electropositive areas increase bone resorption [8]. Mechanical stabilization is very important in the healing of the defect [9].

Autogenous bone grafting can cause significant donor site morbidity if large structural parts are required [10]. Permission to receive grafts in limited quantities of the donor site, variable graft quality, increased the duration of anesthesia, blood loss and cost are important postoperative complications. For these reasons, surgeons have searched for other options for grafting in the management of bowel and skeletal defects.

Bone grafts that are used effectively in the treatment of bone defects are named differently according to the source: autograft, allograft and xenograft.

2. Autografts

Autograft is the transfer of a piece of tissue in the same individual without the veins that will continue to bleed from one place to another. Autogenous bone grafts; osteogenic, osteoinductive and osteoconductive capacities. It also forms a living cell source that is not immunologically rejected. Autografts are better than allografts and xenografts [11].

Autografts are preferred, especially where osteogenesis is the primary goal, because in autografts, "creeping substitution" develops much faster. In addition, autografts contain osteoblasts, bone marrow and blood cells, osteogenetic induction capacities and osteogenesis contributing bunions. Other types of grafts cannot contribute to osteogenesis because they stimulate the immune response [12].

There is an important distinction in autografts in terms of osteogenetic activity. In spongious built and compact autografts, "creeping substitution" develops in completely different form and speed. The open and hollow structure of the spongious bone allows for easier diffusion of newly formed vessels during the revascularization phase. Microanastomoses are easier to establish and blood supply to the graft is provided early. However, a more compact bone graft creates a barrier to vascular invasion. Vascular penetration occurs only through Haversian channels. In addition, osteogenesis and callus formation is easier because the large area surface area of the spongious bone contains many more osteoprogenitor cells [13].

The region where autografts are taken also has an importance in terms of osteogenesis. For example, osteoprogenitor cells, such as grafts, iliac crest bone grafts, primitive reticulocytes, immature hematopoietic cells, integrate rapidly into the bone to which they are implanted. In order for all the above rules to be valid, the autograft should first be well established [12].

During the well-established grafting, it is first observed that the new vessels of the grafted microcavities have formed a mesenchymal stem cell pool in the graft. These cells have the capacity to differentiate into osteogenic, chondrogenic or even fibrogenic cell lines.

The direction of this differentiation determines local, nutritional and electromechanical forces. For example, high oxygenation and compression allow mesenchymal stem cells to develop in the direction of osteoblast. Low oxygenation and compression lead to chondroblast, high oxygenation and tensile forces lead to fibroblast growth [14].

Despite these features of autografts, they can exhibit up to 50% resorption, sequestration and inadequate integration [15]. Development of alternatives to bone autografts due to limited donor sites and potential donor-acquired morbidity has been a constant focus [16]. The success of bone grafts is also due to the presence of osteocompetent cells in the graft, the availability of the recipient site and the lack of immunological response [17].

After transplantation of bone autograft, a number of basic histologic events take place in the recipient area [18]. After transplantation, the graft is covered with hematoma, inflammatory events occur in which the inflammatory cells reach the site, and then new blood vessel formation takes place. Nonvascularized autografts turn into necrosis over time. Most of the osteocytes in the graft die, but those with superficial settling can survive [19]. The blood vessels originating from the recipient site proliferate into the remaining graft tissue and the recipient osteocytes mesenchymal stem cells multiply in the graft. Vascular growth occurs from the Haversian channels present in the graft. Initially, osteoclastic resorption activity is increased, resulting in reduced graft porosity and durability. Cancellous bone is revascularized in a short period of 2–3 days due to open structure. Conversely, revascularization of the cortical bone may last up to 2 months. The fact that vascular touch is invasive into the graft, bringing osteoblasts to the site and osteoblasts' new bone production is a "creeping substitution" phenomenon that occurs in normal fracture physiology [20]. Resorption of necrotic bones in the cortical bone graft is incomplete and therefore the final live and dead bone-mixed touch cannot reach the cancellous bone [21].

Autografts; cancellous, nonvascularized cortical, vascularized cortical and bone marrow. Different grades have osteogenic, osteoconductive and osteoinductive properties.

2.1. Cortical and cancellous autografts

Cortical bone grafts are less successful as biocompatibility than autogenous cancellous bone grafts. Due to the low porosity of the cortical bone, it is difficult and slow to move the vascular structures into the graft. The cortical bone contains fewer osteoblastic progenitor cells than the trabecular bone. The cells in the cortical bone are less resistant to transplantation because of the diffuse oxygen and less nutrient transfer [6].

Cortical bone grafts are a good choice for repair of segmental bone defects smaller than 5–6 cm. Fibula, costa and iliac crest can be used as cortical bone autograft. Osteoprogenitor is poor in cells, osteoconductive and osteoinductive properties are low. They provide strong structural support. The recipient is joined with a creeping substitution process on the tissue. The recipient is fed with plasmatic imbibition from the capillary structures in the wound bed. Complete revascularization does not occur before 1–2 months. This time it is two times that of the cancellous bone. Cortical autografts are less revascularized and less remodeled than spongious autografts. Autologous cortical bone grafts are good choice for bone loss above 5–6 cm. However, vascular grafts are preferred because of the 25–50% failure rate of nonvascular grafts over 12 cm in bone loss [22]. Cortical porosity for revascularization and repair is one of the most important

reasons for the occurrence of graft fracture, delayed union or nonunion, especially in large cortical grafts. Cortical grafts initially have structural durability. But between 6 and 18 months of age, about one-third of your power is lost in the stages of re-vascularization and restructuring. Over time, it approaches normal structure and reaches to the power of normal cortical bone in about 2 years. Nonetheless, nonviable bone islands in the graft continue to survive [23].

Autologous cancellous bone grafts are currently known as the most effective graft material for spinal fusion, filling bone defects and bone healing in fracture treatment. Osteogenic bone and bone marrow cells, osteoconductive collagen and mineral matrix, matrix proteins and osteoinductive matrix proteins are transplanted into autogenous cancellous bone. It has been shown that primitive osteogenic cells survive posttransplantation and transform into osteoblasts in the new bone tissue developed after autograft application [24]. Although the cancellous bone is known to be osteoinductive, there is no evidence that inductive proteins and cytokines are active in autologous cancellous grafts [22].

Cancellous bones are fast revascularized grafts. Surface osteoblasts and endosteal cells can transplicate. Creeping acts as an osteoconductive substrate that effectively supports substitution. The cancellous graft cannot provide the acute structural support provided by the cortical graft. Although cancellous autografts do not initially have carrier-carrying properties, bone grafting builds upon the graft and structural integrity of the bone with the recipient bones begins to form. As bone mass increases, endurance increases and the resulting new texture is restructured in the direction of Wolf's rules. However, it can be strengthened as fast as cortical graft for 6–12 months. Among the sources of cancerous bone, the posterior iliac crest comes first. The most frequent site of grafting is iliac crest. Major complications were reported as 8.6% and minor complications as 20.6% [25]. Other sources of grafts are Gerdy's tubercle, distal radius and distal tibia [22].

2.2. Endochondral and membranous autografts

While endochondral bone is originated from cartilage, membranous bone is originated from mesenchymal tissue. While craniofacial skeleton is formed by membranous ossification, most of the axial skeleton is formed by endochondral ossification. In graft viability, the interaction between the local mechanical environment and the cortical or cancellous nature of the bone, rather than the embryonic origin of the bone, has been shown to be important [26]. Membranous bone is generally preferred when grafting to the endochondral bone.

2.3. Vascularızed autograft

With the progress of microsurgical techniques, autografts are frequently used in vasculature. When both artery and vein are anastomosed during transplantation, approximately 90% of the osteocytes survive and there is no osteoclastic resorption of the bone for incorporation and boiling. Areas where grafts can be removed: fibula, costa, tibia, olecranon and iliac wing. The most preferred graft is fibula graft [27]. McKee [28] reported the first microvascular anastomotic bone graft transfer at a meeting. Then the first free bone grafting articles were published [29]. In 1977, the first bone skin free flap was published [30]. Although bone grafting with vascular anastomosis using microsurgical techniques is described in the literature, "free bone graft" is used, although "free vascular bone graft" has been used in the terminology [31].

Resorption and subsequent osteoconduction and remodeling are not observed as in nonvascular autografts and therefore are more resistant to the first 6-week period than nonvascular autografts. Osteogenic cells in vascularized bone grafts undergo less resorption. More cells live in grafts than nonvascularized grafts [32]. They do not need a good vascular space where they are transported. Vascularized bone grafts have been shown to be biomechanically superior [33].

Vascularized bone graft acts as if there is a simple fracture on the contact surfaces of the field. Bone healing in the fracture line "Creeping substitution" occurs here. These grafts can maintain 60 min of life at room temperature. There are publications that show that reperfusion grafts will partially return in up to 6 h of ischemia [34].

2.3.1. Vascularized fibula autograft

It is the gold standard in bone free skins because it can provide bone mass, pedicle length and skin pedicle. Both endosteal and periosteal circulation are provided. Bone healing is very good. It is the type of flap that should be considered when the receiving bed is not very good due to radiotherapy, infection and scar. Disadvantages include the possibility of damage to the peroneal nerve and tibial arteries.

2.3.2. Vascularized iliac crest autograft

The ileum may be transferred as a deaf circumflex iliac artery based bone autograft or as a superficial gluteal artery based bone autograft. With this autograft, up to 4–10 cm of bone can be carried. They are used in mandibula and sub-articular reconstructions. Continuation of endosteal and periosteal circulation, donor site morbidity is low and long pedicle is a very useful flap. Hypoesthesia due to the injury of the lateral femoral cutaneous nerve and the risk of herniation are disadvantages. Posterior iliac crest is a potential source of bone used when there is segmental bone loss, such as radiotherapy, trauma and tumor resection. It is also used in diaphyseal pseudoarthrosis treatment of long bones. The bone segment that can be transferred is limited to 4–6 cm.

2.3.3. Vascularized rib autograft

Flaps can be prepared via endosteal or periosteal pedicles. It limits the use of the vascular pedicle short.

2.3.4. Vascularized scapula autograft

The lateral part of the scapula can be transferred so that the circumflex scapular artery is fed from musculoperiostal branches. Scapula autograft can be preferred for mandibular, orbital and maxilla reconstructions.

2.3.5. Vascularized calvarial autograft

Vascularized calvarial autograft can be transferred with the scalp. Vascularized transfer of calvarial bone is especially preferred for craniofacial reconstructions. Bone quality is very good. The risk of intracranial injury is the disadvantage.

2.3.6. Vascularized metatarsal autograft

This graft is commonly used for the first finger of the foot. It is also applied in the form of composite tissue transfer of the first and second fingers of the foot.

2.3.7. Vascularized radius autograft

The radial artery gives periosteal and skin perforator branches. These vessels are fed with radial side forearm bone skin blade. A 6–12 cm of the radial bone may be included [35].

2.3.8. Vascularized wrist dorsal and volar autograft

Vascularized bone grafts are widely used in the repair of many carpal pathologies. Many pedicle grafts such as radial volar, radial dorsal and second metacarpal have been described [36].

2.3.9. Vascularized medial femoral condyle autograft

The flap was first populated by Sakai [37]. Eighty per cent of the cases of the major pedicle of the flap are descending genic artery with medial branch of superficial femoral artery. The corticoperiosteal bone graft can be removed from the medial femoral condyle up to 8×13 cm^2. It is a source of grafts for the repair of relatively small bone defects.

2.4. Nonvascularized bone autografts

2.4.1. Nonvascularized iliac crest autograft

It is a very good resource for cortical and cancellous bones. Easy to reach, can be taken in any amount. The disadvantage is that the donor area is also postoperative pain. Anterior grafts can be taken from superior iliac spine and crest tubercle. No damage to the tendinous ligaments is important to prevent the possibility of gait disturbance. The lateral femoral cutaneous nerve may be damaged during graft retrieval. Paresthesia on the outside of the leg results in hypoesthesia. If large segments are taken from ileum, herniation due to inguinal ligament integrity may occur.

2.4.2. Nonvascularized calvarial autograft

Calvarial bone split grafts are used. In an adult patient, the average thickness of the calvarium is assumed to be 7 mm. The thickest part of the bone is the parietal area behind the coronal suture. Calcium is not preferred in children and adolescents because of the risk of dura and brain damage during graft ingestion.

2.4.3. Nonvascularized costa autograft

Costa grafts are used for mandibular or craniomaxillary zone reconstructions. Unlike whole-layer costa grafts, partial thickness jeans grafts provide cortical bone with a larger surface area. The regeneration ability of the casts allows the partial autogenous cortical graft to be taken several times over the same donor site.

2.4.4. Nonvascularized autografts of radius, trochanter major and olecranon

Cortical and cancellous bone needs are not among the preferred donor sites. They are preferred in situations where the iliac crest is unavailable.

2.4.5. Bone marrow

Bone marrow can be used alone as an osteogenic graft. Bone marrow obtained after aspiration; cytokines, osteoblastic progenitors such as other bone marrow-derived cells, and a rapidly revascularized absorbable biological fibrin matrix. An average of 1400 connective tissue progenitors was found in the iliac crest aspirated bone marrow [38]. Bone marrow should be used immediately after aspiration.

3. Allografts

Bone transfers are genetic characteristics made between different individuals. The first literature report was made by MacKewen [39]. Allografts; porous structures contain many chemical domains that are retained by progenitor cells and endothelial cells. They also contain growth factors in the bone matrix that are released when resorbed by osteoclasts. The allograft bone also contains a small amount of bone morphogenic protein with osteoinductive properties.

Demineralization increases the bioavailability of growth factors in the allograft bone matrix. In addition, demineralization prevents HIV infection [40].

In the early allograft use, the graft cells were completely destroyed and the skeleton of the bone roof served as a scaffold. Fresh bone allografts result in both humoral and cellular immunological responses, which allow the graft to be recognized by the recipient. Antibody production results in cell lysis and vascular destruction resulting in graft rejection. The frequency of allograft rejection depends on the degree of antigen mismatch between the graft and the recipient. Vascularized bone allograft rejection is seen on postoperative third day. The first affected members have been shown to be osteocytes and vascular endothelium [41]. Rejection can be suppressed by the use of cyclosporine. Every allograft causes an immunological reaction in the recipient.

The alignment of allografts is different than that of autografts. Both vascular invasion and perivascular new bone formation are slower. This adaptation also affects the size of the graft, the level of immunological response to the graft and the conditions under which allograft is stored [12].

The immunological response to allografts results in the sensitization of the recipient to histocompatibility antigens in osteogenic and hematopoietic cells, leukocytes, blood vessels, nerves and connective tissue matrices within the graft. Therefore, this is a secondary immunological response. This is a cellular immune response [42]. Herndon et al. allografts have found widespread use, demonstrating that the immune response with frozen allografts decreases. Attention has shifted to allograft preservation techniques [43].

If allografts are separated from their cells, they are prevented by immunological reactions. The bones obtained from cadavers are used as osteoconductive skeleton by decellularization [19]. Thus, the disease is prevented from passing between people. Among the processes used are irradiation, debridements, ultrasonic washing, liquid nitrogen, ethylene oxide and deep freezing. Allografts are prepared and maintained in tissue banks.

Frozen-dried bones are poorly immunogenic to both the humoral and cellular immune system. However, passing the bone through these processes destroys osteoinductivity when changing mechanical properties [44]. Bone banks were needed for the use and development of these methods and bone banks were established in many parts of the world.

3.1. Bone banks

The preferred age range for choosing donors for bone banks is from 16 to 65 [45]. Donors; acute or fatal chronic infection, malignancy, exposure to radiation in the area to be caught, venereal disease, hepatitis, slow virus diseases, AIDS or HIV infection, drug use, steroid use for more than 1 week, diffuse osteoporosis, immune complex disease, connective tissue disorders and long-term insulin-dependent diabetes should not have an anamnesis such as grafting with live virus vaccine in the near future [46, 47]. Donors can live in cadaver. Live donors require adequate physical examination and a good anamnesis, and a detailed autopsy is required for cadavers.

3.2. Demineralize bone matrix

Demineralized bone matrix (DBM) is used to fill bone defects and voids as an osteoconductive and osteoinductive material. DBM is rapidly re-vascularized and is also a good carrier for autologous bone marrow. There are differences between tissue banks and firms according to DBM acquisition phases [22]. Studies have shown that DBM results in long bone pseudoarthroses and bone loss similar to autologous bone grafts [48]. DBM can be used by mixing with cancellous graft to increase and intensify autologous bone graft when bone loss is large. It can also be considered as an alternative in patients who cannot use autologous bone graft [22].

3.3. Morselized and cancellous allografts

They are osteoconductive. They provide mechanical support against compression. They are prepared by freeze-drying (lyophilization) and vacuum packaging. It can be used to fill cavities formed after curettage in bone cysts and to remove bone surfaces in periarticular metaphyseal fractures.

3.4. Osteochondral and cortical allografts

They are obtained from the pelvis, costume, femur, tibia and fibula and used in major bone and joint loss. They also provide both structural and mechanical support for the treatment of periprosthetic fractures. They carry osteoconductive properties.

4. Xenograft

Another species is the use of the bones of living things. Bones from various animal species have been tested since allograft is an expensive method of providing. However, they were abandoned because of their high immunity, insufficient biomechanical qualities and foreign body reaction [49].

Dog tibia, calvarial bone transplants have been reported for the defect in human bones [49]. But it has been understood that it is not useful in the human body [50]. Cell-free and demineralized xenografts have been used but have been shown to destroy bone morphogenic proteins and other growth factors [51].

5. Conclusion

Bone grafting is the most commonly used method for increasing bone regeneration in surgical procedures [52]. More than 2 million bone grafting procedures have been performed worldwide every year. Immediately after blood transfusions, it is the second most common tissue transplant [52]. Important when deciding on the use of bone grafts; patient factors, environmental factors, the experience of the surgeon and the economic dimension of graft use.

Conditions limiting the use of autografts and allografts have accelerated the development of bioceramic technology. As the future of bone grafting procedures, new technologies will emerge in the isolation and production of recombinant human bone morphogenic proteins and growth factors and in the application of autogenous stem cells.

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Section 2

Orthopedic Surgery

Chapter 4

Allogenic Decal-Bone Grafts: A Viable Option in Clinical Orthopedics

Surendar Tuli

Additional information is available at the end of the chapter

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Abstract

One has to resort to allogenic source of bone grafts especially in filling up of large or multiple containable cavitary lesions, structural reconstruction of large circumferential osteoperiosteal defects, extensive spinal fusions for gross deformities, or extensive operative reconstruction after total joint replacements. These procedures demand an abundant quantity of bone material in which a patient's (recipient's) body cannot supply without significant morbidity and risks. At present most of the allogenic bone banks use deep-freezing or freeze-drying or radiation for long-term preservation. The techniques maintain sterility, reduce immunogenicity, and provide adequate structural integrity; however, such procedures reduce the bone-forming biological activity and are expensive. We have worked for clinical translation of the basic research performed by Marshal Urist (1965–1994). After extensive experimental observations, we have been using partially decalcified allogenic bone as grafts in clinical cases since 1978. Favorable outcome has been observed in benign cystic lesions, wide-gap grafting, and spinal fusions. Minimum follow-up for declaring "success" or "failure" of the procedure was 2 years after implantation.

Keywords: bone grafts, allogenic bone grafts, allogenic decal-bone grafts

1. Introduction

Bone grafting is a standard orthopedic procedure performed in clinical practice. Autogenous graft is the gold standard and the preferred graft used. However, allograft bone continues to play an important role in many orthopedic reconstructive procedures. One has to resort to the allogenic sources especially in filling up of large or multiple containable cavitary lesions, structural intercalary reconstruction of large circumferential osteoperiosteal defects, extensive

spinal fusion for gross deformities or severe instabilities especially in children, and repeat surgeries after total joint replacements. These procedures demand an abundant quantity of bone which the recipient's body cannot supply without significant morbidity and risks. At present popularly allogenic bone is preserved by deep-freezing or freeze-drying or by radiation for long-term preservation. These techniques have been shown to maintain sterility, reduce immunogenicity, and provide adequate structural integrity; however, such procedures also reduce the bone-forming biological activity and are expensive (**Table 1**). Autoclaving and radiation completely destroy bone inductive principles.

One of the most exciting works during the latter half of the twentieth century (1965–1994) has been the clinical translation of the basic research performed by marshal Urist [1, 2].

After extensive experimental work [3, 4], we have been using partially demineralized allogenic bone (decal-bone) as grafts in clinical cases. For preparation and preservation of allogenic bone graft, we used the techniques described by Urist (1965–1987). We aimed at removal of approximately 50% of mineral from the graft, thus retaining adequate structural integrity (**Table 2**). We used this material since 1978, and we closely observed the clinical results on long-term bases in 67 benign cystic lesions, 32 wide-gap graftings, and 11 posterior

	Fresh autogenous	**Fresh/unprocessed allogenic**	**Frozen freeze-dried allogenic**	**Partially decalcified**	**Deproteinated allogenic**
Osteoinduction	++++	+++	+++	++++	0
Osteoconduction	+++	++	++	+++	++
Osteogenic	++	0	0	0	0
Immunogenicity	0	+++	++	?+	0
Mechanical strength	+++	+++	++	++	++
Cost	?+	+	+++	+	+++

Frozen grafts require thawing, freeze-dried grafts require hydration before implantation, and the unused graft cannot be re-preserved.

Table 1. Commonly used bone grafts in clinical orthopedics.

A. Treatment with 0.6 MHcl:

- Removes mineral and exposes BMP and other growth factors on matrix
- Opens and cleanses vascular, Haversian, Volkmann channels, and lacunar spaces of cells and debris
- Reduces antigenicity, chemosterilizes, and is virucidal

B. Treatment with ethanol:

- Preserves without denaturing the bone morphogenetic protein (BMP) and other osteoinductive principles (OIP), chemosterilizes, and is virucidal
- Leaches out fat ex vivo
- These processes as a rule are done by the host scavenger cells in vivo in un-demineralized bone grafts

Table 2. Processing of allogenic decal-bone.

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or posterolateral spinal fusion. Minimum follow-up for declaring "success" or "failure" of the grafting procedures was 2 years after implantation. We prefer to use the expression of "decal-bone" because the whole process of decalcification is performed in vitro.

2. Preparation of allogenic "decal-bone" graft

Human bones were obtained from freshly (posttraumatic) amputated extremities, under strict aseptic operation theater conditions. Soft tissues and periosteum were removed from the bones using sharp instruments. After a minimum of three washings with normal saline, the bones were placed and immersed in 0.6 M HCI solution for 3–5 days in a domestic refrigerator. The solution was changed every 24 hours. The partially decalcified bone was washed with normal saline to remove any traces of acid, sealed in 80–90% ethanol, and kept in a domestic refrigerator at about 4–6°C (**Figures 1** and **2**). The stored bone was used between 1 and 12 months of preservation. Osteoporotic bone from old persons would be ready by the third day; however, fully mineralized bone from athletic or healthy persons may take 5 days for achieving 50–40% decalcification. Bone obtained from total knee replacements in the operating rooms was another material processed for use as decal-bone.

When required for implantation, the preserved bone was washed thoroughly with normal saline. The superficial surface of graft was pared using a sharp scalpel, and it was cut to the required size to give a snug fit in the host bed for structural grafting, generally fixing to the host bone using an intramedullary nail. For filling large cystic cavities of bone, the decal-bone was cut like matchstick silvers with thickness and width of 4–6 mm and washed with normal saline. The cavities after thorough curettage were compactly packed with the matchstick graft.

For spinal fusions the recipient bed was decorticated, and abundant graft was placed oriented along the long axis of the spine. In cases with gross mechanical instability, a suitable implant with multi-segmental fixation was employed as an adjunct. Standard operative principles for such extensive procedures were followed with modifications to suit individual requirements.

For giant cell tumors of bone (GCT) prior to 1986, en bloc resection and structural reconstruction was performed as a standard procedure. We had an opportunity to observe the behavior of large allogenic segmental graft used for such patients. Global observation however advised less aggressive joint-sparing intralesional procedures since approximately 1987.

Currently for all containable cystic lesions including GTC, we use and recommend the following steps for grafting:

I. Perform thorough intralesional curettage through an adequate window.

II. Aspirate the debris completely.

III. Fill up the cavity with hydrogen peroxide for 3 minutes, and clean the cavity with normal saline.

IV. Fill the cavity with absolute ethanol (80–90%) for 3 minutes.

V. Remove ethanol and wash with normal saline.

VI. Do compact filling of the cavity with decal-bone grafts.

VII. After wound closure protect the limb with suitable cast, and follow the standard post-operative care.

This routine has appreciably reduced the incidence of infection and recurrence, and the success rate has markedly improved.

2.1. Cytological and histological observations

In addition to clinical and radiological assessment, postimplantation observations were made by (i) periodic fine-needle aspiration cytology (FNAC) from the graft and perigraft area,

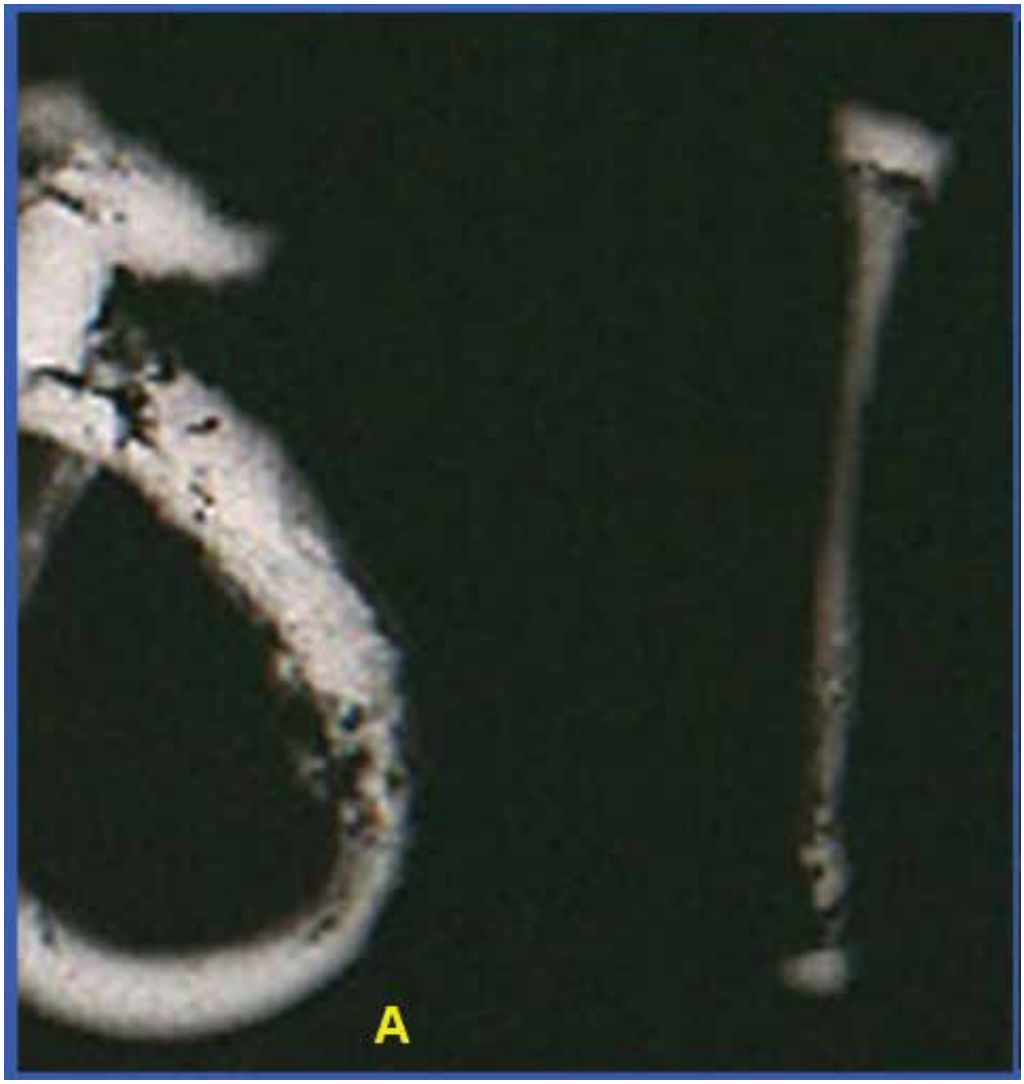

Figure 1. After complete decalcification, the bone (radius in picture) becomes soft like leather.

(ii) by periodic core biopsy of the graft after tetracycline administration, and (iii) by histological studies of the retrieved graft in cases of reoperation.

2.1.1. Observations

Cavitary cystic lesions: the observations regarding cystic lesions are listed in **Table 3**. The success after curettage and bone grafting was uneventful in 60% of cases of giant cell tumor (GCT), in 75% of cases of aneurysmal bone cyst (ABC), and in 85% of cases of unicameral bone cyst (UBC) **Figure 3**. Further success by supplementary curettage and bone grafting was obtained in 10% of cases of GCT, 25% of ABC, and 15% of UBC. Supplementary bone grafting was required because of unexplained resorption of the graft or low-grade infection leading to sequestration and resorption of the graft. Six cases of GCT failed because of uncontrolled

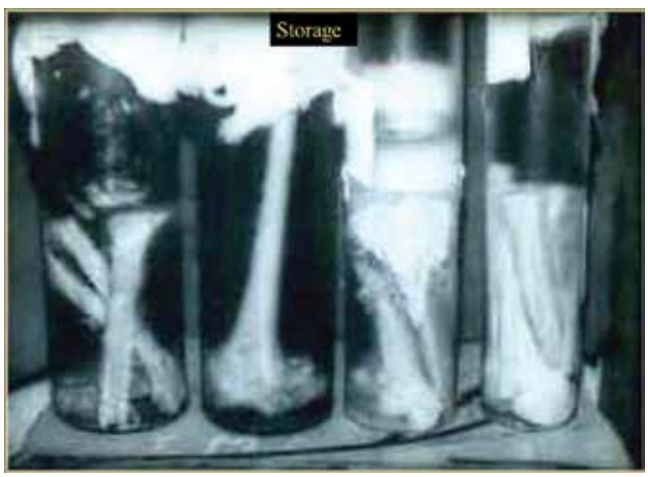

Figure 2. Partially decalcified bone stored in ethanol.

Pathology	Success		Sup. success	Failure
Typ. giant cell tumor	18	60%	10%	30%
Aneurysmal bone cyst	21	75%	25%	
Unicameral bone cyst	11	85%	15%	
• Fibrous defect	6	6		
• Solitary chondroma	3	3		
• Chod. myxoid fibroma	2	2		
• Poly-OFD	4	3	1	
• Multiple enchondromas	2	1	1	

• Number is too small to express as valid percentage.
Poly-OFD = polyostotic fibrous dysplasia.

Table 3. "Cystic lesions" of bone (67) treated by curettage and allogenic decal-bone grafting.

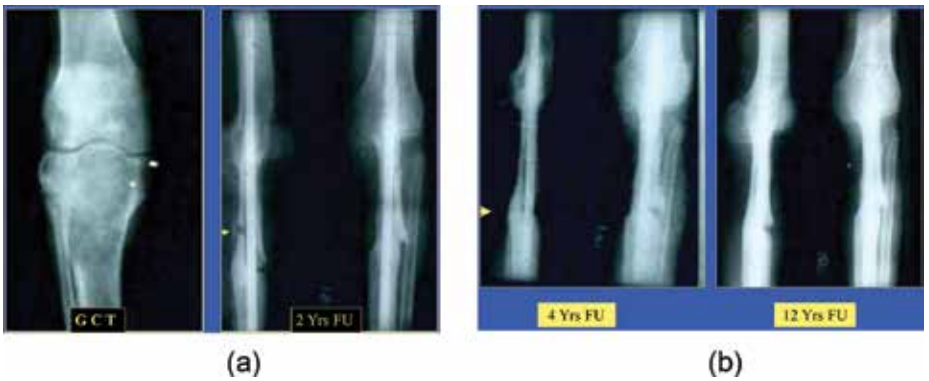

Figure 3. A recurred giant cell tumor of distal femur with pathological fracture. The healed status achieved by intralesional curettage and decal-bone grafting following 12 years.

Figure 4. A case of polyostotic fibrous dysplasia. The cyst in the trochanteric area remained healed after curettage and decal-bone grafting. After about 14 years of gap, another cyst in the supra-acetabular region needed treatment.

infection (three cases) and massive recurrence of tumor (three cases). All patients of solitary osseous lesions healed successfully. Polyostotic fibrous dysplasia (4), multiple fibrous defects (6), enchondromas (3), chondromyxoid fibroma (2), and enchondromatosis (2) by their biological nature may need reoperation for an increase in the size of the lesions which were insignificant at the time of the first surgery. One patient of fibrous dysplasia had to undergo a second operation for a new lesion, and one patient of enchondromatosis is awaiting surgery for the additional area (**Figure 4**).

3. Intercalary structural bone grafts

Observations regarding 32 cases of structural bone grafts used for large osteoperiosteal gaps are summarized in **Table 4**. The largest group was 28 cases of giant cell tumors, 40% of these obtained uneventful success (**Figure 5**), 38% needed supplementary operation in the form of

Pathology		Success	Supp. success	Failure
Giant cell tumor	28	40%	38%	28%
• Aneurysmal bone graft	1	1	–	–
• Unicameral bone graft	1	1	–	–
• Central fibrosarcoma	1		–	1
• Traumatic extrusion	1	1	–	–

• Number is too small to express valid percentage.

Table 4. Large osteoperiosteal gaps (32) and structural grafts (1979–1999).

further autologous bone grafts for areas of non-union at host graft junctions or for pseudarthrosis in the intermediate part of the graft 20%, for control of infection, or for a combination of these factors (18%).

Six patients were considered a failure because the reconstruction failed. Two had recurrence of tumor, one had uncontrolled infection, and these ended up in amputations. In three patients despite two attempts at supplementary grafting, the areas of pseudarthrosis did not heal; these patients accepted an orthosis till further decision. One case of malignant fibrous histiocytoma failed because of recurrence of tumor within 4 months of limb salvage attempt.

Of the 11 patients of posterior or posterolateral spinal fusion, 10 were considered to have obtained satisfactory osseous fusion based upon clinical assessment and stress X-rays done 12–24 months after the operation (**Figure 6**). One young nurse who had posterior fusion along with Steffi's fixation at L3–L4 for spondylolisthesis was considered a failure because of the implant breakage observed 2 years after operations.

3.1. Cytological and histological observation

Fine-needle aspiration cytology (FNAC) was done from the perigraft region (in 20 patients) between the 10th and 40th day after grafting. The FNAC showed high cellularity composed of

Figure 5. Intercalary reconstruction after en bloc resection of giant cell tumor of distal femur. Note gradual incorporation and remodeling as observed in the 12-year follow-up.

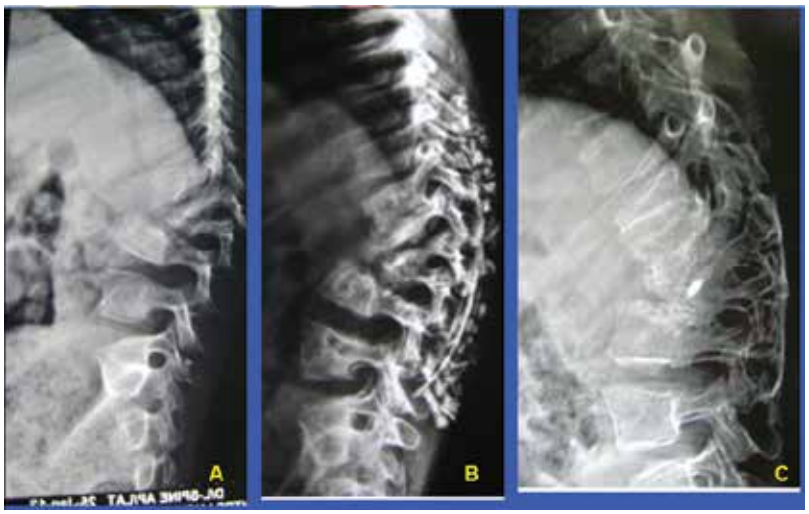

Figure 6. A child having tuberculosis of the spine involving multiple vertebrae: posterior spinal fusion was performed using allogenic decal-bone. Note the incorporation of the graft with remodeling during a 3-year follow-up.

polymorphs, lymphocytes, and macrophages between 10 and 20 days. The cellularity gradually reduced with relative increase in the number of lymphocytes. By the 40th postimplantation day, the macrophages were practically absent, and one could see appreciable osteogenic activity by the presence of osteoblasts and osteoclasts. No cellular immune reaction was discernable.

Periodic core biopsy in early stages and biopsy of the graft in patients who required a second operation showed histological and tetracycline fluorescence evidence of neo-osteogenesis between 6 and 12 weeks. The fluorescence in the implanted allogenic bone was quantitatively the same as the bone of a patient's iliac crest in specimens available 12 months after the grafting.

3.2. Discussion

Contents of bone grafts and their roles: In general calcium hydroxyapatite, the predominant mineral in bones provides an inert framework providing mechanical stability and offering a lattice work for penetration of neocapillaries, reparative tissues, and osteoconduction (**Table 5**). Only the most superficial bone-forming cells in fresh autografts which survive getting nutrition by tissue perfusion provide direct osteogenic activity. In allografts no viable cells are expected; however, the debris of dead cells act as the most potent immunogenic agent. Organic matrix provides the most potent bone morphogenetic (bone induction principles)

Ca hydroxyapatite	Mechanical stability
Autogenous surviving cells	Osteogenesis
Allogenic non-surviving cells	Immunogenesis
Matrix	Bone morphogenetic agents(weak mechanical strength)

Table 5. Contents of bone graft and their role.

agent. Its viability however depends upon the influence of processes of allograft preparation and preservation [5–10].

Bone graft incorporation: The biological process of incorporation of bone grafts is practically similar to that of a fracture healing. Under favorable environment, the following major steps occur in a cascadal fashion from the time of placement of the bone graft in the recipient bed to its incorporation and remodeling according to Wolff's law: (i) hematoma formation and its organization by invasion of neocapillaries surrounded by perivascular pluripotent mesenchymal cells; (ii) osteoclastic and phagocytic resorption of nonviable mineral (calcium hydroxyapatite), cellular debris and marrow fat, and tunneling of the graft-making channels for ingrowth and propagation of neocapillaries and osteoprogenitor cells; (iii) conversion (tissue engineering) of osteoprogenitor cells to osteoblastic cells under the influence of local osteoinductive agents (bone morphogenetic protein, other inductive agents, and growth factors present in the organic matrix of the bone graft) and the platelets. Laying down of the new bone (neo-osteogenesis) on the surface of matrix framework and along the vascular spaces/channels; and (iv) remodeling of the newly formed bone to conform to the trabecular pattern along the lines of functional loading and stress (according to Wolff's law). These events are a slow process; the grafted area needs protection with repetitive physiological axial or functional loading. The most challenging clinical condition of structural (intercalary) bone grafting for large osteoperiosteal gaps in the lower limb may take 2–4 years for adequate incorporation permitting unprotected loading [11, 12]. The mechanical strength of the reconstruct is weak for 1 and 1/2 to 2 years, after which the strength increases by more neo-osteogenesis. The least time is taken in a cavitary pathology which offers a very large osteogenic bed and copious surface for intimate contact with the graft [13–15]. As incorporation takes place from periphery to the center, the time taken for large cavities and large grafts is correspondingly higher. In un-demineralized cortical (e.g., fibula) graft, 20–30% (deepest sector) of the grafted bone may never get incorporated; it may stay incarcerated surrounded by newly formed bone.

By HCL decalcification we aimed at the removal of nearly 50% of mineral, thus providing adequate structural integrity. The said treatment removed all cell debris and fat providing opened-up channels for ready penetration of neocapillaries and perivascular mesenchymal cells. Acid demineralization also removed the mineral from the surface of the bone, along the vascular channels, and on the lacunar spaces, thus exposing the matrix for intimate contact with the invading perivascular tissues, and facilitated the interaction between the graft matrix (most active osteoinductive principle) and the pluripotent mesenchymal cells from the host. The technique used by us does not destroy the biological osteoinductive property of the bone matrix.

Immunogenicity of allogenic bone is now better understood. Fresh unmatched and untreated allogenic bone inevitably evokes an immune response in the host. The immune response in general is delayed and mild and develops slowly; however, it results in "unexplained" graft resorption and delay or failure in its incorporation. In clinical practice deep-freezing, freezing, freeze-drying, and irradiation are currently employed to reduce immunogenicity. Pure BMP from an allogenic source or even a xenogenic source is considered to have negligible immunogenicity. We feel that a simple treatment of allogenic bone by HCL decalcification and ethanol preservation practically eliminates the antigenic material (cells and debris) to permit unhindered incorporation in clinical practice as observed in our cases. Overall analysis

in our clinical material has been approximately 80–90% successful for benign cavitary lesions; for impaction grafts in revision joint surgeries, 50–70% success for structural reconstruction in circumferential osteoperiosteal gaps; and 70–90% clinical success in extensive spinal fusion. Supplementary procedures for obtaining success in difficult cases, especially for osteoperiosteal gaps, are an accepted norm in 20–30% of cases. The success rate in our clinical cases is compatible with the observations of the outcome where allogenic bone was used from more sophisticated bone banks. Allogenic bone graft is a rational option when the recipient's patient owns bones that are inherently defective (e.g., fibrous dysplasia, enchondromatosis).

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Conflict of interest

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Section 3

Research, Biological Aspect of Bone Graft and Bovine Type and its Application in Oral Surgery and Implantology

Chapter 5

Biology of Bone Graft and the Use of Bovine Bone for Revision of Total Hip Arthroplasty with Acetabular Reconstruction

Carlos Roberto Galia, Fernando Pagnussato,
Tiango Aguiar Ribeiro and Luis Fernando Moreira

Additional information is available at the end of the chapter

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Abstract

The use of bone graft in orthopaedic surgeries has become essential in many situations in which there is a bone defect. This includes bone tumour procedures, fracture operations where there was a loss of bone tissue and revision arthroplasty surgeries. In this chapter, we will introduce aspects related to history of bone transplantation, the biological and mechanical aspects, as well sterilization, transmission of infectious diseases and biological safety, and concluding, the functioning of tissue banks and screening of musculoskeletal tissue donors.

Keywords: bone graft, revision of total hip arthroplasty, acetabular reconstruction, biological and mechanical aspects of bone tissue, tissue banks

1. Introduction

The use of transplants in orthopaedic surgeries has become essential in many situations in which there is a bone defect. This includes bone tumour procedures, fracture operations where there was a loss of bone tissue and revision arthroplasty surgeries [11, 44, 53]. The latter, revision arthroplasty, has considerably increased since the rate of primary arthroplasties has grown substantially in recent years. Some kind of musculoskeletal tissue is transplanted into 10 to 15% of orthopaedic surgeries performed in the United States. Annually, about 650,000 bone-based grafts are distributed by the American Tissue Banks, clearly highlighting

the importance of processing, controlling and storing of this type of material becoming one of the major transplanted tissues [44, 59].

Bone tissue can be processed in many ways. It can be stored longer and has been implanted till recently without prior testing compatibility as opposed to transplant of other organs and the vast majority of soft tissues. Nevertheless, the bone tissue can be obtained from patient's own body called autograft, from another donor of the same species (living or cadaver donors) called homograft, from donors of another species xenograft and from non-organic biomaterials [2]. Despite the success of these implants, patients who require transplant may wait quite a few years on transplant lists. These lists have grown considerably in recent years, either from public hospitals or from private ones [3].

Deep-frozen homologous graft is the most common used bone graft, but its use is limited due to shortage and although small, the risks of transmitting contagious diseases and even cancers should not be overlooked [11, 44]. On the other hand, though homologous lyophilised (freeze-dried) grafts have virtually eliminated the risk of transmissible diseases is still lacking availability and therefore, alternative biomaterials from synthetic or natural source have been considered and carefully studied. Among these materials, the use of bovine lyophilised grafts is a suitable alternative with many advantages over autologous or homologous lyophilised graft ones due to the great physicochemical and structural resemblance to human bone and their practically endless availability [13, 15, 16, 45]. As a xenograft however, it may possibly present adverse immunological and inflammatory reactions [16]. Nevertheless, our physicochemical processing protocols have been developed in order to significantly reduce these problems, decreasing antigenicity and thus, turning the bovine freeze-dried bone an important biomaterial for large scale use in reconstructive surgery [14, 63].

The bone tissue transplanting is far from being a novel issue, but it is necessary an increased knowledge about some kinds of grafts, their mechanical and biological aspects, sources and a careful analysis of outcomes. Bone transplant is responsible for an improvement in patient's quality of life; in some cases, return to daily life activities and promotion of patient social reinsertion. This chapter is intended to present a brief review of the history of bone grafts, indications for use, storage details and differences of processing techniques as well as to present the experience of our hip orthopaedic surgery group with the use of lyophilised bovine xenograft in almost two thousand orthopaedic procedures.

2. History of bone transplantation

In the first centuries of the Christian era, the idea of tissue transplantation between individuals of the same species and even of different species arose. Cosmas and Damian, considered the pioneers of bone transplantation, in the second century of Christian era removed a tumour-affected leg and soon implanted a leg taken from a recently killed Moro. But success in bone transplantation only came in 1880 with Sir William MacEwan who reconstructs a patient humerus with bone graft from another patient's leg. But Job van Meekren in 1668 was the first who realise a xenograft bone transplant. Meekren transplanted part of the skull of a

dog in a traumatic defect of the skull of a soldier, who was excommunicated by the church. The soldier asked the surgeon to remove the graft, but due to time elapsed, this could not be completely removed since it was already fully integrated. Ollier and Barth in nineteenth century concluded, though not fully correct, that bone and periosteum remained viable when transplanted, contributing to new bone formation; arising the primary concept that cells survive in the graft even when removed from donor [3, 52]. In an important and considered a classical work, Albee concluded that the most suitable tissues for transplant are those originated from connective tissue such as bone, fat and fascia [12, 19, 30].

3. Biological aspects

The bone tissue is composed of 10% water, an inorganic part (mineral, mainly hydroxyapatite) corresponding to 65%, and an organic part corresponding to 25%, being this latter part consisting of a collagen type I matrix with low molecular weight proteoglycans and non-collagen proteins [21].

When affected by severe bone loss or osteolysis, a condition founded in several cases of total hip arthroplasty revision, the bone tissue cannot be repaired, even though it is a tissue with high recovery power. In these cases, it is necessary to use bone grafts or bone substitutes (biomaterials) that fill this gap and restore patient's bone stock [21].

Bone grafts can be of three types, depending on the location of its origin: cortical, cancellous or cortical-cancellous. These grafts can be used in three modes: blocks, segments or morselised. These different types of bone grafts will provide distinct mechanical and biological responses. The cortical bone is less osteogenic than the spongy one, showing however, a higher structural quality, for long periods and even in the absence of adequate integration. In revision arthroplasty surgeries cancellous bone graft remains the choice due to its greater osteogenic features [11, 18, 27, 34, 49, 50, 61].

A sequence of events begins after transplantation of a spongy bone, starting by an inflammatory response. This is followed by macrophage invasion, neovascularization and differentiation of mesenchymal cells into osteoblasts that place an osteoid layer on a remaining necrotic trabecular bone. Viable nuclei are reabsorbed by osteoblasts and the matrix is eventually replaced by necrotic trabeculae in the newly formed bone [57]. Urist and Hernandez in their classical study demonstrated ectopic bone formation after implantation of demineralised bovine bone matrix in rabbit's muscular tissue. This discovery showed that certain substances present in bone matrix induce cell differentiation. All these events are probably mediated by inducing protein factors, called bone morphogenetic proteins (BMPs) that have great osteogenic activity [39, 43]. These BMPs belong to a superfamily of proteins called transforming growth factors beta (TGF-ß) responsible for inducing growth. The TGF-ß is responsible for cell growth, differentiation and embryo formation. BMPs have been shown to be important regulators in the development and regeneration of skeletal tissue [43]. Buma and colaborators detailed Galia [14] in their PhD thesis, in 2000 and 2004 (**Figures 1** and **2**), respectively, demonstrated the relationship between time elapsed from grafting till to biopsies for histological analysis. The wider the interval, the larger

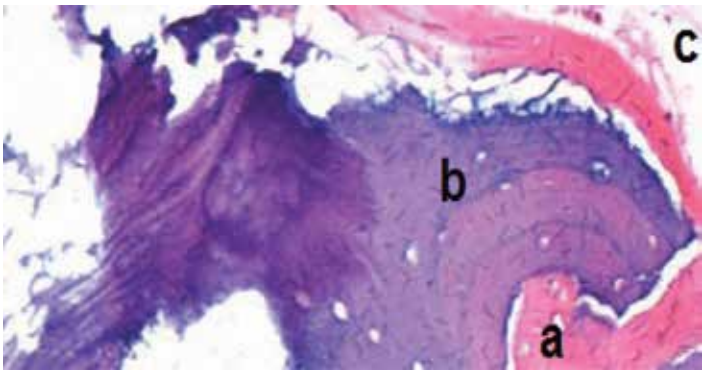

Figure 1. A representative case with a severe bone loss before a primary total hip arthroplasty was submitted to a revision total hip arthroplasty with acetabular bone reconstruction with bovine lyophilized xenograft. This patient was affected by recurrent dislocation of the revised arthroplasty. An inadequate positioning of the acetabular component was the aetiology. A biopsy of the acetabular bone graft reconstruction was done in the second revision surgery. In this case, the time elapsed from the first revision with the bone grafting and the second revision made to reposition the acetabulum was nine months. a: new bone formed; b: lyophilised bovine xenograft; c: fibrous tissue.

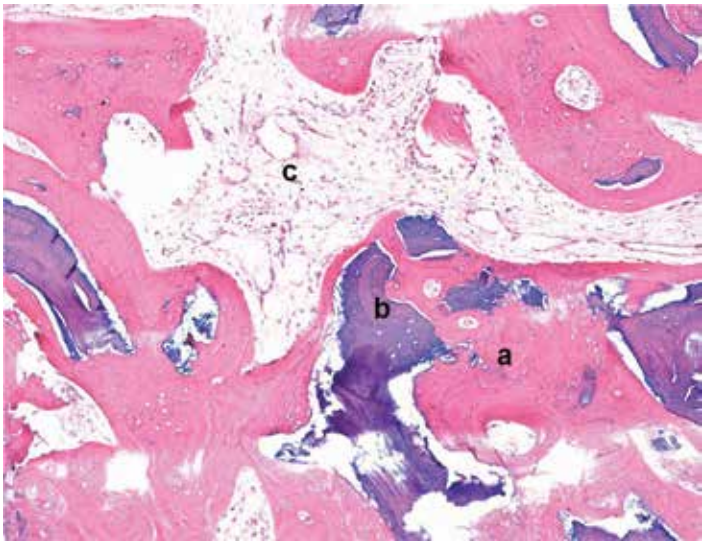

Figure 2. Another patient with a severe bone loss before a primary total hip arthroplasty was submitted to a revision total hip arthroplasty with acetabular bone reconstruction with bovine lyophilized xenograft. This patient was affected by recurrent dislocation of the revised arthroplasty. An inadequate positioning of the acetabular component was the aetiology. A biopsy of the acetabular bone graft reconstruction was done in the second revision surgery. In this case, the time elapsed from the first revision with the bone grafting and the second revision made to reposition the acetabulum was 43 months. a: new bone formed; b: lyophilised bovine xenograft; c: fibrous tissue.

the amount of new bone formation and the smaller the residual bone graft, though Tiango et al. in 2015 [44] in a case series resulted from his PhD thesis was notable to reconfirm these findings, probably due to the small number of cases with biopsies in the study [5, 17, 18, 58].

Out come in bone transplantation also depends on antigenic reaction factors. Some grafts have proved to be extremely antigenic, depending on the antigens present in these grafts. The antigenic reactions are mediated by bone marrow cells and not by T or B lymphocytes. Indeed, *in vitro* studies demonstrated that lineages of bone marrow granulocytes appear to be responsible for this immune response [7]. Moreover, *in vivo* studies in rabbits also showed that fresh grafts, frozen grafts and lyophilised bovine xenograft express distinct immune responses. Fresh and frozen grafts led to systemic response in rabbits as opposed to lyophilised bovine xenograft where rabbits were not able to develop such systemic immune response, demonstrating that the lyophilisation process clears up immunologically the graft by removing all the fat and bone marrow cells [13].

4. Mechanical aspects

The different mechanical aspects of the various types of graft play a key role when a graft is to be chosen. These differences can lead to distinct end-results and therefore acquaintance with these mechanical aspects is of a paramount prominence.

Several physical properties of human and bovine trabecular bone have been reported [26, 54]. The compressive strength to mechanical forces varied between 2.44 and 6.24 MPa in both, human and bovine bones. In Young's module analyses the results of both bones varied between 70 and 673 MPa [42]. Galia et al [15]. in 2011 obtained a similar result between lyophilised bovine and lyophilised human bones for the scanning electron microscope analyses on the pore sizes of the trabecular bone. The mean pore size was 316 μm, ranging from 91.2 to 497.8 μm to bovine lyophilised bone and 333.5 μm, ranging from 87.2 to 963.9 μm to human lyophilised bone. Macedo et al [36]. in 1999 in an *in vitro* study demonstrated that frozen lyophilised bovine bone defrosted or rehydrated for an hour have similar compressive load and deformation rates. Cornu et al [6] in 2001 showed that lyophilized morcelised and impacted human bones were mechanically superior to morcelised and impacted deep-frozen ones. However, the resistance of both bones was similar after impaction [36].

5. Sterilisation, transmission of infectious diseases and biological safety

The sterilisation is a crucial issue involving grafts and it can change their mechanical properties, as well as may affect transmission of diseases or even tumours [60, 64]. Radiation as Cobalt 60 can significantly reduce bone mechanical properties and even in low doses can destroy morphogenetic properties, in a progressive dose-dependent manner over the bone biomechanics [10]. Several authors studied gamma radiation and other sterilisation methods (ethanol and ethylene oxide at 55°C) and their effect on graft osteoinduction. Gamma radiation with 25 kGy inactivate HIV virus but reduces osteoinduction capacity of grafts in nearly 40%; ethylene oxide at 55°C provides an almost complete loss of this important potential. On the other hand, ethanol has not affected osteoinduction [10, 64]. However, toxic factors should be observed. Ethylene

oxide may be maintained in the graft and may interact or be released when in contact with any liquid, including blood. Gamma radiation may be toxic when in direct contact with fat that is present in the grafts [27, 37]. Autoclaving is another way to sterilise grafts. However, xenografts must be sterilised at a temperature of 132°C to inactivate infectious proteins, i.e., prions, though this method reduces the mechanical resistance of xenografts in approximately 70% [62].

Frozen grafts from tissue banks undergo several protocols for donor selection before being used for transplantation. Graft rejects are reported to reach 20 to 30% [4, 51]. But even with these severe protocols for selection, transmission of an infectious disease may occur [25, 53, 55, 59. Sugihara et al [53] in 1999 reported the presence of tumour cells in frozen femoral heads and suggested inclusion of histopathological examinations as part of the screening protocol for donor tissues [41, 53].

The lyophilisation process until now has not been linked with infection as confirmed by Shibuya et al. [48], Ledford et al. [29] and Ribeiro et al. [44] who also reported no clinical signs of infections in patients submitted to revision of total hip arthroplasty with acetabular reconstruction, as well as no case of bovine spongiform encephalopathy disease (BSE) and its new variant the Creutzfeldt-Jakob disease (CJD). It is believed that lyophilisation process chemically inactivates most of the bacterial agents, viral and prion agents to a safe and acceptable level to be used in humans as reported by Wenz et al. [63] and among us, by Galia et al. [16] and Rosito et al. [45].

6. Processing

Several bone grafts storage ways are used all over the world. The two most widely accepted and used are the deep-frozen (−80°C) and lyophilisation ones.

Extremely low temperatures (<−80°C) are achieved by using temperature-controlled freezers equipped with power generators, just in case of lack of electric power cut, and alarms triggered by the system when the temperature increases. In these special freezers, a bone tissue can be maintained for up to 5 years. It should be noted that very low temperatures do not have a role in the sterilization of bone tissue.

Proposed and diffused by the Tissue Bank of USA Navy in 1951, the lyophilisation process became the technique in which bone is washed, centrifuged, decellularised, chemically degreased and subjected to physical processes of cleaning and sterilisation, and this way, only the protein-mineral matrix still remains in the graft, which is later frozen and then dehydrated. All these processes decrease antigenicity, tumour cell transmissions and inactivate prions [28, 35]. The lyophilisation is an important method of processing and storage for musculoskeletal tissues that allows not only the use of human bones, tendons and fascia (allogeneic) but also and mainly the bovine (xenogenic) [27, 31, 33, 39]. Currently, in major health centres, different types of bone grafts are available for reconstructive orthopaedic surgery: frozen and lyophilised autologous, (allogeneic) and lyophilised bovine grafts (xenogeneic) [2].

The frozen human graft (allogeneic) was the most used and widely accepted but the number of Tissue Banks in our country and in other developing countries is not enough to overcome the huge demand as well as the number of donors [47]. In the same way, as mentioned, there is a

risk of transmission of diseases and tumour cells [32, 34, 41, 53]. The vast majority of USA Tissue Banks produce lyophilised human grafts (allogeneic); however, there are few studies on its use or production. Therefore, lyophilised human grafts are still not widespread used among us [8, 56].

The bovine bone has a chemical composition, porosity, size, shape and biological behaviour similar to its human counterpart, although controversial bovine grafts is commonly used in dentistry surgeries and reconstructions [14]. It provides structural support, osteoconduction and a high content of calcium and phosphorus; essential factors for the newly formed bone tissue [40]. For these reasons, its use is growing in orthopaedic surgeries as reported by Prof. Galia [14] and by his co-workers in his research group, Rosito et al. [45, 46], Henning et al. [23] and Diesel et al. [9].

The most important issue is whether the graft has been processed and stored according to the standards of Associations of Tissue Banks and by national and international health authorities.

7. Tissue banks

Musculoskeletal tissue banks such as in the USA, there is an institution – the American Association of Tissue Banks (AATB) – to regulate and supervise the functioning of all in-country tissue banks since 1976 [1]. In Brazil, however, is the Ministry of Health, under the regulation order No. 55 (Dec. 2015) [24] that provides the technical regulations for the functioning of musculoskeletal and skin tissue banks of human origin, determining guidance from the facility characteristics, screening of living donors or cadavers up to equipment and human resources [24]. These regulations, though quite strict, have greatly improved the quality of tissue processing and safe use. For accrual, the decision to accept or reject a donor is carried out by the chairman of the Bank of Musculoskeletal Tissues (BMST) after rigorous tests and following an established protocol [38]. As the protocols used by the Tissue Banks, one must consider the history, physical examination and laboratory tests of the donor (**Table 1**).

Summary protocol for screening of musculoskeletal tissue donors (bone, tendon and osteochondral)			
NAME:	REGISTRY:		
DATE OF BIRTH:	AGE:	SEX:	COLOUR:
TYPE OF DONOR: ( ) DECEASED - BRAIN DEATH (BD) ( ) DEAD - CARDIORESPIRATORY ARREST (CRA) ( ) LIVE	TIME OF BD OR CRA:		
DATE AND TIME OF COLLECTION: ___/___/___ ___:___	COLLECTION LOCATION:		
DATE AND TIME OF CLINICAL/SOCIAL SCREENING: ___/___/___ ___:___	NAME AND RELATIONSHIP OF FAMILIAR SCREENED:		

ANAMNESIS **(The Tissue Bank should carry out a risk assessment for the selection of tissue donors in case one or more of the situations below are observed)**		
BODY INSPECTION	**YES**	**NO**
presence of physical signs that have been at risk of malignant or sexually transmitted diseases, such as:		
skin or mucosal lesions, including genital and perigenital or anal and perianal lesions		
scars or surgical incisions prior to removal of tissues		
jaundice		
hepatomegaly		
diffuselymphadenopathy		
uncontrolled local infection at the time of donation, including bacterial, viral, fungal or parasitic infections		
presence of piercing, tattooing or permanent makeup without evaluation conditions regarding the safety of the procedure performed, done in the last 12 (twelve) months; if the procedures have been performed in establishments regularized by health surveillance, the period to be considered is 6 (six) months, except for the case of piercing in the oral and genital region, in which the period to be considered is 12 twelve months		
use of injectable drugs or needle punctures suggestive of drug use in the donor's body;		
CLINICAL / SOCIAL HISTORY	**YES**	**NO**
history of chronic, systemic and autoimmune disease capable of impairing the quality of tissues to be donated		
history of travel and exposure to infectious agents, as well as the prevalence of local infectious diseases		
use or exposure to toxic substances in the last 12 (twelve) months, such as cyanide, lead, mercury and gold, which may be transmitted to the receptors at doses likely to endanger their health		
vaccine history, as provided in Ordinance No. 2,712, of November 12, 2013 or to replace it		
laboratory tests anti-cytomegalovirus (CMV) (IgG) and anti-Toxoplasma (IgG) reagents		
history, clinical data or presence of risk factors for transmission of Chagas' disease, syphilis, Zika or Yellow Fever		
history, clinical data or presence of risk factors for transmission of human immunodeficiency virus (HIV-1 and -2), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-lymphotropic virus (HTLV I and II)		
EXCLUSION CRITERIA FOR TISSUE DONATION:	YES	NO
unknown or undetermined cause of death		
disease or history of disease of unknown aetiology		
existence or history of malignant disease except for primary basal cell carcinoma, cervical carcinoma in situ, and some primary tumours of the central nervous system		
transplanted organs		
therapeutic use of tissues within a period of less than 12 (twelve) months, except in the predicted situation in "diseases caused by prions", whose condition is definite exclusion for the donation		
uncontrolled systemic infection at the time of donation, including bacterial, viral, fungal or parasitic infections, or significant local infection in donor tissues		
(HIV antibody + p24 antigen from HIV, including screening for antibodies against subtype 1, group O, and subtype 2), HIV NAT, HBsAg, anti-HBcIgG or IgG + IgM, antibody + HCV antigen, HCV NAT, antibody against HTLV I and II, anti-Trypanosoma cruzi antibody, anti-treponemic or non-treponemic antibody.		
laboratory results of anti-CMV (IgG) and anti-Toxoplasma (IgG) reagents. (the donation may be accepted but the transplant surgeon who will decide on the use of the donated tissue must be informed)		
people who have undergone xenotransplantation		
risk of transmission of diseases caused by prions; this risk applies, for example, to:		
donors diagnosed with Creutzfeldt-Jakob disease, or with the variant of this disease or with a family history of non-iatrogenic Creutzfeldt-Jakob disease		
history of rapidly progressive dementia or neurodegenerative diseases, including those of unknown origin		
donors treated with human growth hormone or other hormones of non-recombinant pituitary origin and recipients of corneal, sclera and Dura mater transplantation, as well as persons who have undergone undocumented neurosurgical intervention (in which it may have been used Dura mater)		

indications that the results of analyses of the donor blood samples will not be valid due to:		
the occurrence of haemodilution greater than 50% where a pre-transfusion and/or infusion sample is not available or where there are no validated laboratory tests to be used in this type of sample		
treatment with immunosuppressive agents		
risk sexual practice in the past twelve (12) months, including:		
individuals who have had sex in exchange for money or drugs or their respective sexual partners		
individuals who have been victims of sexual violence or their respective sexual partners		
male individuals who have had sex with other same-sex individuals or their sexual partners		
individuals who have had sexual intercourse with a person with HIV infection, HBV, HCV or other sexually transmitted infection and blood or sex partners		
individuals who are sexual partners of patients in a renal replacement therapy program and of patients with a history of transfusion of blood components or blood products		
individuals who have a history of incarceration or compulsory non-domicile confinement of more than seventy-two (72) hours, or their sexual partners		
WEATHER TIME AND TEMPERATURE CONDITIONS	**YES**	**NO**
Time between cardiorespiratory arrest (CRA) and tissue withdrawal of up to 15 h after PCR, if the donor body is not kept under refrigeration; Or up to 24 hours after PCR if the donor's body is refrigerated within 12 hours after PCR.		

LABORATORY EXAMS			
Exam	Result	Exam	Result
haematocrit/haemoglobin		Leukocytes	
erythrocyte sedimentation rate (ESR)		Blood glucose	
Bilirubins		Alkaline phosphatase	
Aspartate transaminase		Alanine transaminase	
Clog tests		Platelets	
Urine analysis		Urine culture test	
Detection of HBV surface antigen (HBsAg)		Antibody detection against HBV capsid (anti-HBcIgG or IgG + IgM)	
Detection of antibody against HCV or combined detection of antibody + HCV antigen		Detection of antibody against HIV or combined detection of antibody against HIV + HIV p24 antigen. This test should also include screening for antibodies against subtype 1, including group O, and subtype 2;	
Detection of the antibody against HTLV I and II		Detection of anti-Toxoplasma antibody (IgG and IgM)	
Detection of the anti-Trypanosoma cruzi antibody		Epstein-Barr	
Detection of anti-CMV antibody (IgG and IgM)		Antibody detection against HBV capsid (anti-HBcIgG or IgG + IgM)	
NAT of HCV		Detection of anti-treponemic or non-treponemic antibody to syphilis	
HIV nucleic acid (NAT) detection test			
RADIOGRAPHIC EXAMINATION			
(Osteoarticular segments and long bones, kept as such for future transplantation, should undergo radiological examinations for measurement and diagnosis of structural abnormalities) Result:_____			

Source: Musculoskeletal Tissue Bank (BTME) of São Vicente de Paulo Hospital - Passo Fundo. Tissue Bank Unit of the Hospital de Clínicas of Porto Alegre (UBMT-HCPA).

Table 1. Screening protocol for musculoskeletal tissue donors.

8. Conclusion

There are many differences when comparing distinct types of graft. Thus, it is essential the implementation of protocols for processing and quality control of all types of bone grafts. This measure will facilitate the monitoring and analysis of the results and shall provide grafting material of better quality, thoroughly tested and readily available.

There is no doubt on the importance of bone transplants in orthopaedic surgery, especially in hip procedures. Their results are well known and, to some extent, predictable when used by experienced surgeons. It is undeniable, however, that we still know little about several issues regarding host-graft interaction. Therefore, further studies have yet to be carried out to attempt to address concerns on this matter that sometimes are overlooked or underestimated in order to achieve best clinical responses, increased biosafety and lower complication rates, i.e., better surgical outcomes [20, 22].

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Chapter 6

Update on Bone Grafting Materials Used in Dentistry in the Bone Healing Process: Our Experience from Translational Studies to Their Clinical Use

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Additional information is available at the end of the chapter

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Abstract

The use of bone grafts is important to preserve the alveolar bone ridge height and volume indispensable for dental implant placement. Despite the highly successful outcomes for the implant-supported overdentures, it seems that a majority of edentulous individuals have not pursued implant-based rehabilitation. Among the reasons cited for this, discrepancy between highly successful therapy and its acceptance is the cost of the treatment. Therefore, the development of biomaterials for bone grafting with comparable characteristics and biological effects than those renowned internationally is necessary. In addition, domestic manufacture would reduce the high costs in public health arising from the application of these biomaterials in the dental field. The aim of the following chapter is to offer an update on one bone grafting material frequently used in dentistry through an assessment of anorganic bovine bone graft in small and medium experimental models as well as its clinical use.

Keywords: bovine bone graft, new bone formation, critical size bone defect, sinus augmentation, osteoconduction

1. Introduction

Bone graft is an implanted material that promotes bone healing alone or in combination with other material(s) [1]. The use and success of bone grafts in the medical field date from the

beginning of the nineteenth century. In this regard, Albee in 1915 had depicted that there were successfully bone transplants in animal models, as well as in humans, since 1809 [2]. Nowadays, bone graft implantation is the main treatment modality for bone defect repair and reconstruction [3]. In oral and maxillofacial areas, bone grafting aims to replace the volumetric bone loss that frequently occurs by systemic pathologies, periodontal defects, and tooth loss [4].

The mechanisms underlying the bone healing promoted by a bone graft are osteogenesis (osteodifferentiation and subsequent new bone formation by donor cells derived from the host or graft), osteoinduction (induction of undifferentiated and pluripotent cells to develop osteogenesis into the bone-forming cell lineage), and osteoconduction (the ability to support the attachment of osteoblast and osteoprogenitor cells and the migration and ingrowth of these cells within the three dimensional architecture of the graft) [5, 6], in combination or alone [7].

Bone grafting materials are classified as autografts (derived from the same individual), allografts (derived from a different individual from the same species), xenografts (derived from a different species), and alloplasts (derived from synthetic sources) [8]. Autografts are the "gold standard" in the reconstruction of bone defects due to their osteoconductive as well as osteoinductive properties [9]. Although they present excellent biological outcomes, they also have a number of drawbacks. In this regard, the use of autografts increases the operative time due to graft harvest, increases the donor site morbidity, and increases the graft resorption. In addition, they represent a big challenge for the operator, since they need to be mold and have limited availability, especially in the pediatric population [10]. Allografts are typically obtained from human cadavers and require to be processed before being used [11, 12]. Allograft bone is available as cortical, cancellous, corticocancellous forms, or as demineralized bone matrix. They can be processed as mineralized or demineralized, fresh, fresh-frozen, or freeze-dried forms [13, 14]. Among the benefits of allografts are their availability in different shapes and sizes. This is particularly advantageous, since it avoids donor site morbidity [15]. The major disadvantages of allografts are related to the transmission of diseases and the graft rejection. In order to decrease the risk of transmitting infectious diseases, allografts need to be treated. The techniques employed include treatment with hypotonic solutions, acetone, ethylene oxide, or gamma irradiation that may eliminate cellular and viral particles [16]. However, these processes eliminate the bone cells and denature proteins present in the graft altering the osteoconductive and osteoinductive properties and eliminating the osteogenic properties [17]. In addition, allografts are capable to induce immunological reactions that interfere with the bone healing process leading to rejection of the graft [15, 18–20]. Xenografts are frequently derived from bovine, porcine, and coral sources [7]. The effectiveness of different bone processing techniques has made possible the use of these materials for medical applications [21, 22]. Bovine bone is one of the most popularly used xenografts. This source material is desirable because it is readily available an inexpensive. However, bovine bone grafts require proper preparation to avoid risks such as transmission of zoonoses [23]. Several studies have shown that organic or inorganic matrix derived from bovine bone is biocompatible and osteoconductive [23, 24]. These important biological properties allow the apposition of newly formed bone by osteoprogenitor cells and the partial remodeling by osteoclasts and osteoblasts of the host [25]. Moreover, the large interconnecting pore volume and its composition encourage the formation and ingrowth of new bone at the implantation sites. Ideally, a synthetic bone graft should be biocompatible and causes minimal fibrotic changes [26]. Synthetic bone grafts

are osteoconductive and have been shown to integrate to bone [27]. There are many available kinds of synthetic graft materials, including bioactive glasses, a- and b-tricalcium phosphate (TCP), and synthetic hydroxyapatite [27].

Bioactive glass or "bioglasses" has been widely used as bone substitutes because of their ability to join and integrate with the bone tissue by forming a layer of active apatite on its surface, with characteristics similar to bone [28]. These biomaterials are resorbable, and dissolution of their products (soluble silicon and calcium) upregulates seven families of genes present in osteoblasts (bone forming cells), thus promoting osteogenesis [28, 29]. Among synthetic materials, synthetic hydroxyapatite, a crystalline phase of calcium phosphate found naturally in bone minerals, exhibits initial mechanical rigidity and structure and demonstrates osteoconductive, as well as angiogenic properties in vivo [30]. Because of its physicochemical characteristics, synthetic hydroxyapatite is a biocompatible and osteoconductive material [31]. This material allows to keep the space filled extremely well providing a physical matrix for the deposition of new bone. For these reasons, synthetic hydroxyapatite has high success in the fields of biology, medicine, and dentistry.

Due to the high popularity of dental implant surgery, the demand for alveolar ridge reconstruction, including sinus augmentation and immediate implant procedures, increased. This new trend in dentistry for implants boosted the development of new grafting materials in dentistry. Ideally, a bone graft should be biocompatible, biodegradable, osteoconductive, and osteoinductive, structurally similar to bone, easy to use, and cost-effective [7]. Within these parameters, a growing number of bone graft alternatives are commercially available and frequently used in dentistry.

Different types of bone grafts are available in the international market. However, it is essential to have a wide variety of them to improve the competitivity of each product in terms of quality, commercial value, and clinical use. Therefore, the development of biomaterials for bone grafting, produced by domestic manufactures, with comparable characteristics and biological effects than those renowned internationally, is necessary in order to reduce the high costs in public health arising from the application of these biomaterials in the dental field. The aim of the following chapter is to offer an update on one bone grafting material frequently used in dentistry through an assessment of an organic bovine bone graft in small and medium experimental models, as well as its clinical use.

2. Translational studies

We evaluated and compared the effects of a bovine bone graft made in Argentina with a commercial bovine bone graft recognized for its osteoconductive effects, on bone healing process in experimental models in rats (experiment 1) and rabbits (experiment 2).

2.1. Materials and methods

The research and all procedures involving live animals were processed after approval by the Clinical Hospital, School of Medicine, University of Buenos Aires, Argentina. Animals were

maintained in keeping with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.1.1. Experiment 1 in rats

A total of 12 young male adult Wistar rats (175 ± 10 g) were housed at room temperature (21 ± 1°C), 55 ± 10% humidity, under 12-hour light/dark cycles. They were fed a standard rodent diet (Ganave SA, Argentina) and deionized water "ad libitum." Body weight was recorded 3 times per week.

2.1.1.1. Surgical procedure

Rats were anesthetized by intraperitoneal injection with ketamine hydrochloride [0.1 mg/100 g body weight (BW)] and acepromazine maleate (0.1 mg/100 g BW), (Holliday-Scott S A, Buenos Aires, Argentina). Hind legs were shaved, and the medial aspect of tibiae was exposed. A critical size bone defect (CSBD) (1.6 mm × 2 mm) was made with a fissure bur under copious irrigation with saline [32]. Bone defects were filled as follows: Group 1 (n = 6): The right tibia of each rat was filled with the bovine bone graft Synergy Bone Matrix (SBM), (Synergy Bone Matrix, Odontit Implant Systems, Argentina, Lot No: E11121216), while the left tibia was unfilled and used as control. Group 2 (n = 6): The right tibia of each rat was filled with the bovine bone substitute SBM (Lot No: E11121216) and the left tibia with the bovine bone substitute Bio-Oss (BO), (Bio-Oss, Geistlich, Switzerland, Lot No 100238).

Taking into account that the remodeling phase in the rat takes approximately 21 days, a healing period of 4 weeks was used to assess the late healing response. Animals were sacrificed after 24 days by carbon dioxide inhalation.

2.1.2. Experiment 2 in rabbits

A total of 15 adult male New Zealand rabbits (weight 3.0 ± 0.5 kg) were housed in individual cages with grid floating floor of stainless steel in a 12 m^2 insulated and equipped room with a continuous extraction system air renewal. They were maintained at room temperature (21 ± 1°C), 55 ± 10% relative humidity, and under 12-hours light/dark cycles. Cleaning and disinfection of excreta trays are made daily. Prior to surgery, animals had an adaptation period of 10 days. Pelleted commercial diet and deionized water were supplied *ad libitum* throughout the experiment with the exception of the first 3 days after surgery, in which they were fed with fresh leafy vegetables. Body weight was assessed 3 times per week during the morning.

2.1.2.1. Surgical procedure

All surgical procedures were carried out under aseptic conditions and in accordance with ISO 10993-6: 2007. The experimental surgery was performed under general anesthesia by an intramuscular injection of 1 mg/kg of acepromazine maleate and an intramuscular injection of ketamine hydrochloride and xylazine (Holliday-Scott S A, Buenos Aires, Argentina), at a dose of 35 and 5 mg/kg, respectively. The surgical site was shaved and scrubbed with iodine. A parallel skin incision was made along the inferior border of the mandible on both sides.

After exposing the masseter muscle, subperiosteal elevation of the muscles detachment was performed exposing the body of the mandible. A standardized CSBD of 5 mm diameter on each side of the mandible was created with a slowly rotating trephine bur, under constant and copious irrigation with saline [33, 34]. Once the bone was excised, each biomaterial (SBM, Lot No: H13010002 or BO, Lot No 100148) was implanted in the corresponding side of the mandible according to the following groups: Control group: CSBD without bone graft; SBM group: CSBD in the right mandible filled with SBM; BO group: CSBD in the right mandible filed with BO. Animals were sacrificed at 4, 8, and 12 weeks postsurgery by a lethal intravenous administration of sodium pentobarbital (Euthanyle® Brouwer, S.A., Argentina).

Postoperatively, rabbits were given a 0.5 ml subcutaneous injection of penicillin and streptomycin antibiotic (Dipenisol Retard, Bayer Argentina) every 48 hours for 7 days. In order to supplement the analgesia, intramuscular injection of 1 mg/kg of Nalbufina (Nalbufine 10 Richmond S.A, Argentina) was administered as premedication and every 12 hours during 3 days postsurgery.

The 5-mm diameter CSBD created in the rabbit jaw for implantation with bone grafts is generally considered to be the appropriate critical size to evaluate bone graft materials [35–37]. Bone remodeling in the rabbit is approximately three times faster than in humans. Therefore, a healing period of 4 weeks was considered appropriate to evaluate the early response to bone healing. Likewise, for the evaluation of delayed bone repair, in relation to the amount of neoformed bone and the reabsorption of the biomaterials used, the period of 8 to 12 weeks was considered [38, 39].

In both experiments, the wound was closed with absorbable sutures, after filling each bone defect. Each animal surgical area was disinfected with iodine, and animal behavior was observed and recorded.

2.1.3. Bone mineral density evaluation in rats

Total skeleton bone mineral density and bone mineral content (BMD and BMC, respectively) were measured "in vivo" under light anesthesia at the end of the experiment (day 24) using a total body scanner with software designed specifically for small animals (DPX Alpha 8034, Small Animal Softer, Lunar Radiation Corp, Madison, WI) following a previously described technique [40]. Rats were scanned under light anesthesia. The precision of the software in determining total body BMD was assessed by measuring one rat five times after repositioning between scans, both on the same and on different days [40]. The coefficient of variation (CV) was 0.9% for total skeleton BMD. A specific region of interest (ROI) was manually traced at the site of the critical size bone defect for the first animal evaluated. Once established the ROI for the first animal, we used the same ROI to evaluate the BMD at the site of the bone defect in all the animals. The BMD CV of the studied area was 3.5% for the proximal tibia. All the analyses were carried out by the same technician in order to minimize inter-observer variation.

2.1.4. Histological and histomorphometrical analysis

At the end of the experimental period, tibiae (experiment 1) and mandibles (experiment 2) were removed and cleaned of soft tissue. All specimens were prepared for histological evaluation according to a standard protocol for undecalcified sections, as previously described [41], and stained with hematoxylin-eosin. The analysis was "blinded" performed with respect to

the rendered treatment. Images of the histological sections were captured by a digital camera (Olympus DP 10; Olympus Optical, Tokyo, Japan) connected to a light microscope (Olympus CX 31; Olympus Optical). Digital images were saved for static histomorphometrical analysis (experiment 2) using Image-Pro Plus 4.5 software. The following criteria were used to standardize the analysis: the total area (TA) to be analyzed was delineated on the composite image. The TA (mm^2) corresponded to the area of the mandible where the surgical defect was previously created, and it was considered 100% of the area to be analyzed. The newly formed bone area (NFBA) and the remaining graft particles area (RGPA) were then delineated. Both, NFBA and RGPA, were located entirely within the confines of the TA. The NFBA and RPGA were also calculated in mm^2 and the percentage calculated according to the following formula: 100-NFBA/TA. Values obtained from each animal were used to calculate the means and SD of each control and experimental group. We evaluated bone volume (% BV/TV): the percentage of cancellous bone within the total measured area and the percentage of remaining particles (% RP/TV) of either SBM or BO.

2.1.5. Biomechanical tests in rabbits

Biomechanical measurements were performed using a three-point bending test (Instron 4411, Universal Testing Materials). The equipment consists of a load frame in which is placed the material to be test (test tube) and a control console that provides calibration controls, programming, and test operation. The installed load cell allows the measurement of compressive forces exerted on the crosshead specimen. IX Series software was used to pick up data analysis of testing bone material. Control, SBM, and BO specimens were cleaned of soft tissues and cut in squares of 20 × 20 mm in the area where the bone defect was done. Bones were weighed and measured. Then they were placed one by one on the rollers, and the bone fracture was performed to evaluate elastic modulus (Mpa) and shear modulus (Mpa). Compression test was performed to evaluate compressive strength (KgF/mm^2).

2.1.6. Statistical analysis

Results were expressed as mean ± standard deviation (SE). Data were analyzed using parametric tests according to data distribution and "a posteriori" tests. Data were analyzed using one-way analysis of variance (ANOVA). The Bonferroni multiple comparisons test was performed when significant differences were found. Student's t-test for independent samples was used to compare both bone grafts at each end point. Statistical analyses were performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered significant.

3. Results

3.1. Experiment 1 in rats

3.1.1. Bone mineral density evaluation

Non statistically significant differences were found in BMC and BMD of tibiae filled with SBM and BO (**Figure 1**), whereas SBM and BO exhibited higher BMC and BMD than the control group (**Figure 1**).

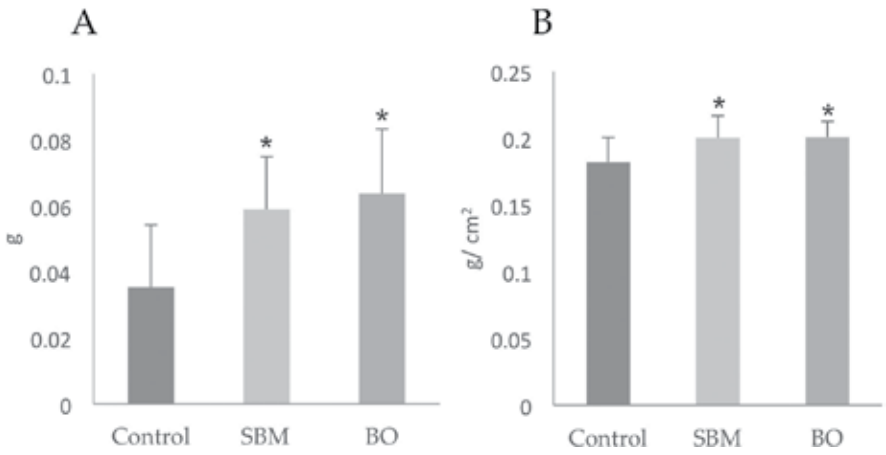

Figure 1. Total skeleton bone mineral content (A) and bone mineral density (B) in control, Bio-Oss (BO) and synergy bone matrix groups (SBM) (*$p < 0.05$ vs. control).

3.1.2. Histological analysis

Cross-sections of tibiae showed remaining particles of each bovine bone graft in the area of the CSBD. Multiple particles of either SBM or BO, of different shapes and sizes, surrounded by laminar bone tissue were observed in the bone medullary space (**Figure 2A**, **B** and **C**). This finding indicates that both bone substitutes were osteoconductive. Proper bone healing was observed in tibiae from both groups. No signs of inflammation were observed; this result suggests biocompatibility (**Figure 2A**, **B** and **C**). After 4 weeks, blood vessels with small angiogenesis and revascularization foci formed in the CSBD implanted with either SBM or BO. The samples also showed mature Haversian systems forming a thin interface within the NBF represented by bone growth and surfaces covered by osteoblasts and fibroblast-like cells surrounding the bone grafts, which implied active osteogenesis. The present study suggests that the bovine bone graft SBM presented similar properties of biocompatibility without inflammatory signs to that of BO. Moreover, SBM also exhibited similar osteoconductive properties to BO, allowing a normal bone formation surrounding the particles.

3.2. Experiment 2 in rabbits

3.2.1. Histological analysis

As expected, control rabbits did not exhibited NBF at any of the studied times. Instead histological samples exhibited normal development of fatty bone marrow with occasional remnants of hematopoiesis foci in the site of the CSBD (**Figure 3A**, **D** and **G**). CZBD filled with each of the bovine bone grafts did not evidenced "foreign body" reaction at any of the studied times. Bone healing over time was accompanied by a progressive inflammatory response consistent with the expected histological stages of repairing. In addition, SBM and BO groups presented NBF characterized by trabecular bone growth and the presence of osteoblasts and fibroblast-like cells (**Figure 3**). Both samples exhibited blood vessel formation with small angiogenesis and revascularization foci and Haversian mature systems the implanted grafts, forming a

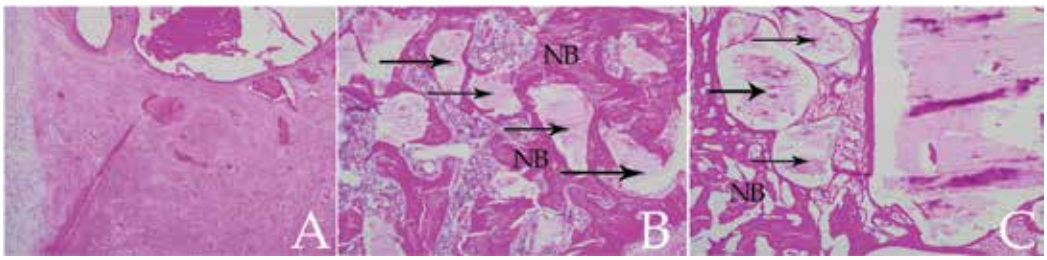

Figure 2. Histological evaluation in rats. Photomicrography of the critical-sized bone defects at 10× or 40× magnification and stained with Hematoxylin-Eosin. A: Control group exhibited the presence of fibrous tissue. B: Critical-sized bone defect filled with synergy bone matrix (SBM). C: Critical-sized bone defect filled with Bio-Oss (BO). Black arrows indicate SBM or BO particles; NB: new bone formation surrounding the particles.

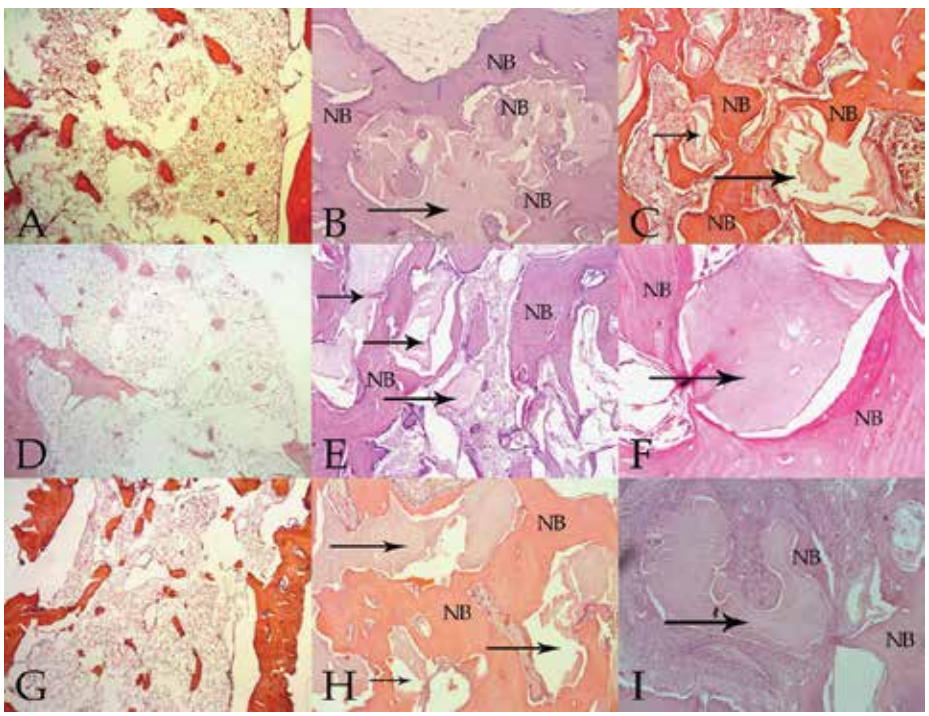

Figure 3. Histological evaluation in rabbits. Photomicrography of the critical-sized bone defects (CSBD) at 4×, 10× or 40× magnification and stained with Hematoxylin-Eosin. Control group exhibited the presence of fibrous tissue at 4 (A), 8 (D) and 12 (G) weeks. CSBD filled with synergy bone matrix (SBM) and Bio-Oss (BO) showed new bone formation surrounding each particle at 4, 8 and 12 weeks. B, E and H: SBM at 4, 8 and 12 weeks. C, F and I: BO at 4, 8 and 12 weeks. Black arrows indicate SBM or BO particles; NB: new bone formation surrounding the particles.

thin interface with the bone forming tissue. The presence of traces of bone substitutes and the formation of blood vessels with small foci of angiogenesis and revascularization was also evident. Moreover, we observed the presence of mature Haversian systems around the bone grafts (**Figure 3**). These findings indicate that SBM and BO are osteoconductive and histologically substantially equivalent.

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3.2.2. Histomorphometrical analysis

Histomorphometrical analysis showed an increase in new bone formation (% BV/TV) that was time dependent for both bone grafts compared to the control group ($p < 0.05$) (**Figure 4A**). A time dependent reduction in the percentage of remnant particles of each device (% RP/TV) was also observed. As control group was not filled with any of the grafts, RP/TV remained in 0. Since the size of the granules of SBM was bigger than that of BO, it is speculated that this factor contributed to the low percentage of RP/TV observed for BO (**Figure 4B**).

3.2.3. Biomechanical tests

Both grafting materials exhibited a significant increase in elastic modulus, shear modulus, and compressive strength at 4, 8, and 12 weeks (**Figure 5A**, **B**, and **C**). This finding suggests that the biomechanical properties of the newly formed bone were equivalent for the two grafts evaluated. Moreover, the quality of the newly formed bone was superior to that presented by the control group.

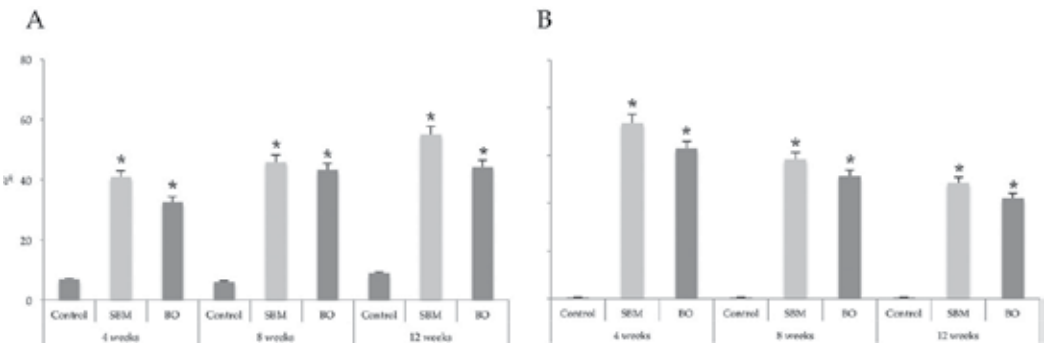

Figure 4. New bone formation (A). Histomorphometrical analysis of rabbit mandible showed an increase in bone formation in the critical sized bone defects filled with synergy bone matrix (SBM) or Bio-Oss (BO), *$p < 0.05$ by one-way ANOVA, followed by Bonferroni multiple comparisons test. Remnant particles of each device (B). Histomorphometrical analysis of rabbit mandible showed a time-dependent decrease in the remaining particles in the critical sized bone defects filled with SBM and BO, p = N.S between SBM and BO by one-way ANOVA.

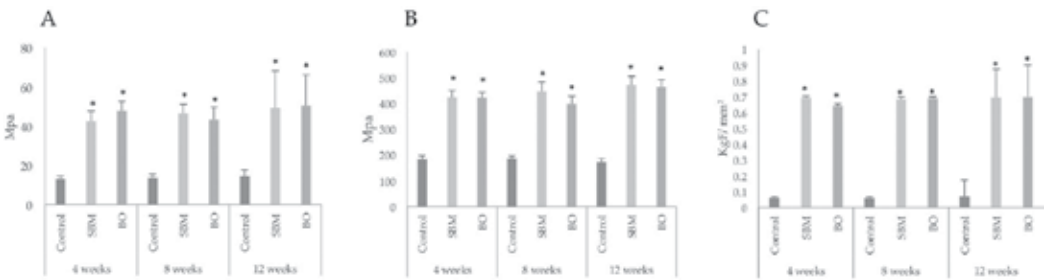

Figure 5. Biomechanical tests. The new bone formed in the critical sized bone defects filled with synergy bone matrix (SBM) and Bio-Oss (BO) presented better quality than the control group exhibited by higher elastic modulus (A), shear modulus (B) and compressive strength (C). *$p < 0.05$ by one-way ANOVA, followed by Bonferroni multiple comparisons test.

4. Case report

A 54-year-old female patient was referred to the Department of Clinical Operative and Prosthesis II, Dental School, University of Buenos Aires, Buenos Aires, Argentina, for the rehabilitation of the edentulous maxilla. Radiographic and cone beam computed tomography (CBCT) exhibited severe atrophy in the posterior region of the maxilla (**Figure 6**). The medical history did not reveal any systemic disease, and the patient did not reported to be under any medication. The patient aimed to rehabilitate the upper jaw with a fixed implant-supported

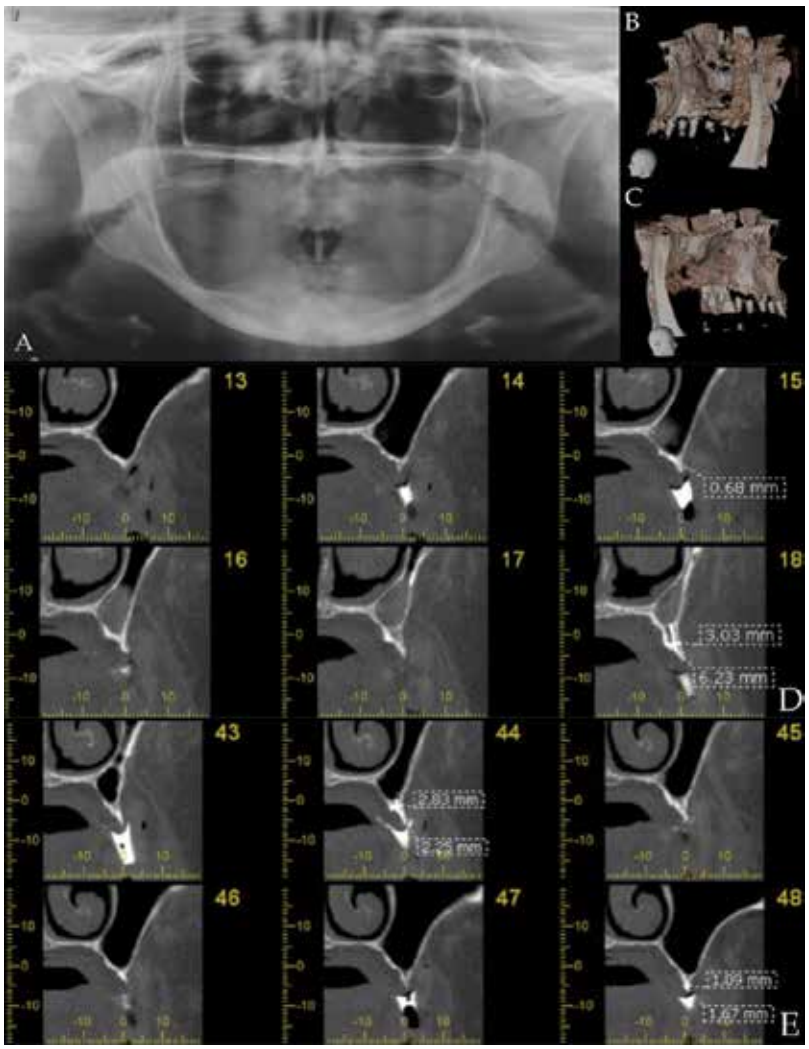

Figure 6. Preoperative diagnostic images. All images exhibited a dramatic loss of bone in the upper left and right maxilla. A: Panoramic X-ray showing edentulous maxilla and mandible. B, C. 3-D reconstruction of the left (B) and right (C) maxilla with the surgical stent. D, E: coronal cut from a cone beam computed tomography scan from the left (D) and right (E) maxilla.

prosthesis. The proposed treatment plan was divided in two stages. The first stage included the confection of a complete upper denture, as well as a surgical and radiological stent, and the reconstruction of the posterior maxillary alveolar ridge. The second stage, after 6 months, consisted in the placement of four dental implants in the posterior maxilla. All the clinical procedures were conducted under the patient's written informed consent. The purpose of this clinical case report was to provide clinical evidence of the efficacy of a new bovine bone graft in the bone healing process.

4.1. Sinus elevation surgery and guided tissue regeneration

The bilateral sinus elevation procedure was performed using the technique previously described by Tatum [42]. Briefly, after anesthetized with infiltrative local carticaine hydrochloride 4% with adrenaline 1:100.000 (Totalcaína Forte, Microsules Bernabó, Argentina), a mucoperiosteal flap was elevated with releasing vertical incisions. Once exposed the buccal wall of the remaining alveolar process and the anterolateral wall of the Highmore antrum, a surgical stent was used to locate the lateral window. An oval osteotomy was performed with high speed handpiece and a round diamond bur under copious irrigation with saline, leaving a "bone island," in the lateral wall of the sinus, attached to the Schneider membrane (**Figure 7**). This fragment of bone was then turned medially and positioned toward the sinus floor. The sinus membrane was then elevated across the floor and up the medial wall. A bilateral guided bone regeneration procedure was performed using a mixture of SBM. The graft was covered with a rebsorbable collagen membrane (BioCollagen, Bioteck, Italy). Finally, the flap was repositioned and sutured without tension. The patient was instructed to perform oral hygiene and to rinse 2 times a day during 7 days with chlorhexidine digluconate 0.12% for disinfection of the surgical wound. Amoxicillin-clavulanate 875 mg was prescribed twice a day for 7 days, and 500 mg of naproxen was administered every 8-12 hours for 5 days to control postoperative pain. Soft diet was also recommended. The sutures were removed after 7 days. CBCT scans and panoramic x-rays were obtained preoperative, 6 months after stage 1, and 4 months after stage 2. A biopsy of each treated area was taken with a trephine bur during the implant placement surgery.

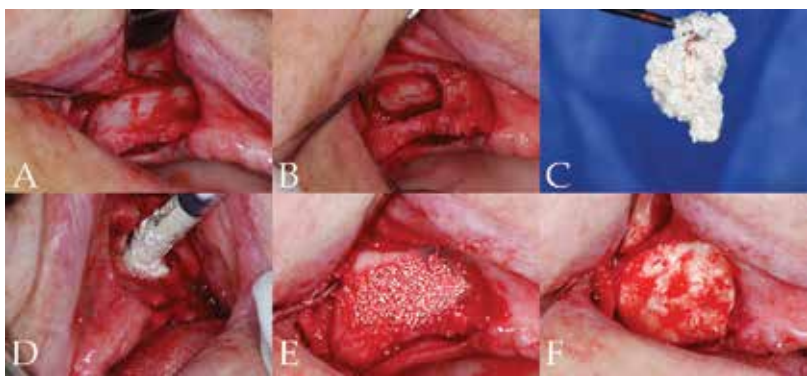

Figure 7. Sinus elevation surgery and guided tissue regeneration. A: Elevation of a mucoperiosteal flap. B: Oval osteotomy and "bone island" in the lateral wall of the sinus attached to the Schneider membrane. C: Synergy bone matrix (SBM). D, E: Placement of SBM for guided bone regeneration. F. The graft was covered with a rebsorbable collagen membrane.

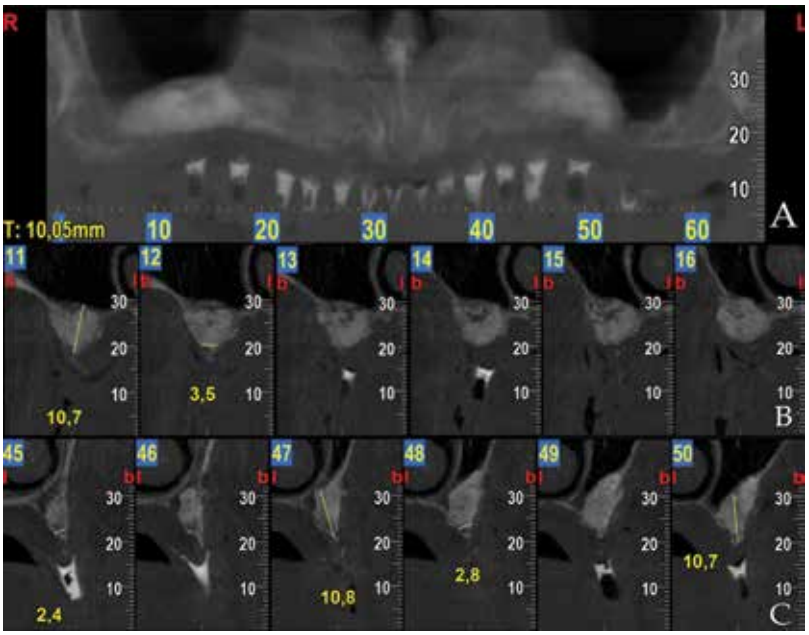

Figure 8. Postoperative CBCT (6 months after the sinus elevation surgery). A, B, C: There was an increase of 10.7 mm and 10.8 mm in the height of the alveolar crest, and an increase in the alveolar crest width of 3.5 mm and 2.8 mm in the right and left side, respectively.

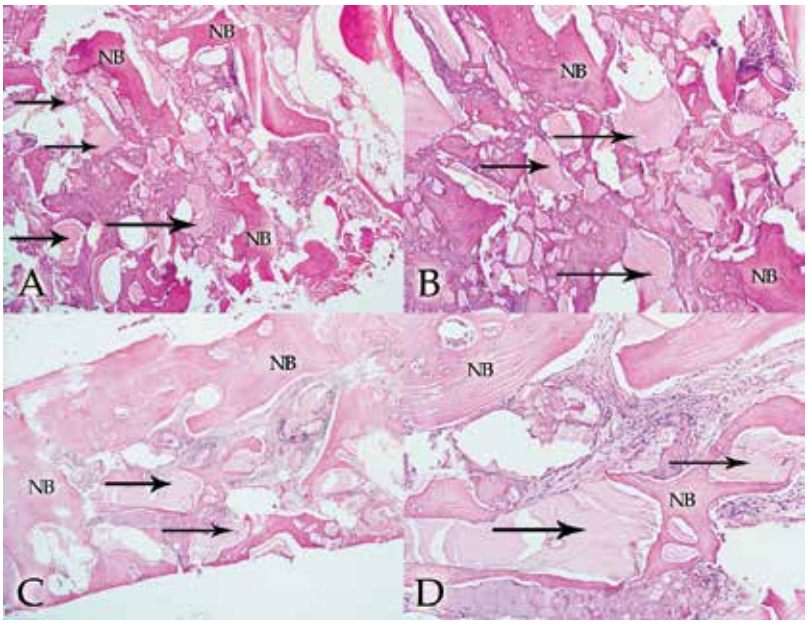

Figure 9. Histological evaluation of the areas grafted with synergy bone matrix (SBM) at 4×, 10× or 20× magnification and stained with Hematoxylin-Eosin. New bone formation surrounding each particle was observed in right (A, B) and left (C, D) grafted sinus. Black arrows indicate SBM particles. NB: new bone formation.

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During the first surgical stage, a postoperative follow-up 7 days after the procedure revealed that the edges of the flap wounds faced each other, and there were no signs of dehiscence or inflammation. The patient did not report any discomfort, pain, or inflammation of the treated areas. The postoperative CBCT, taken 6 months after this surgery, exhibited an increase of 10.7 mm and 10.8 mm in the height of the alveolar crest and an increase in the alveolar crest width of 3.5 mm and 2.8 mm in the right and left side, respectively (**Figure 8**). Six months after the sinus lift surgery, dental implants were placed in the areas that received the bone graft (stage 2). Dental implants in the areas grafted achieved primary stability, indicating that there was an accurate bone quality after the placement of the bone graft. Consistent with the digital imaging findings, histological evaluation of the bone samples retrieved during the implant surgery revealed that SBM particles were osteoconductive. All particles were surrounded by new bone formation (**Figure 9**). There were fibro-angiogenic and fibrous areas associated to

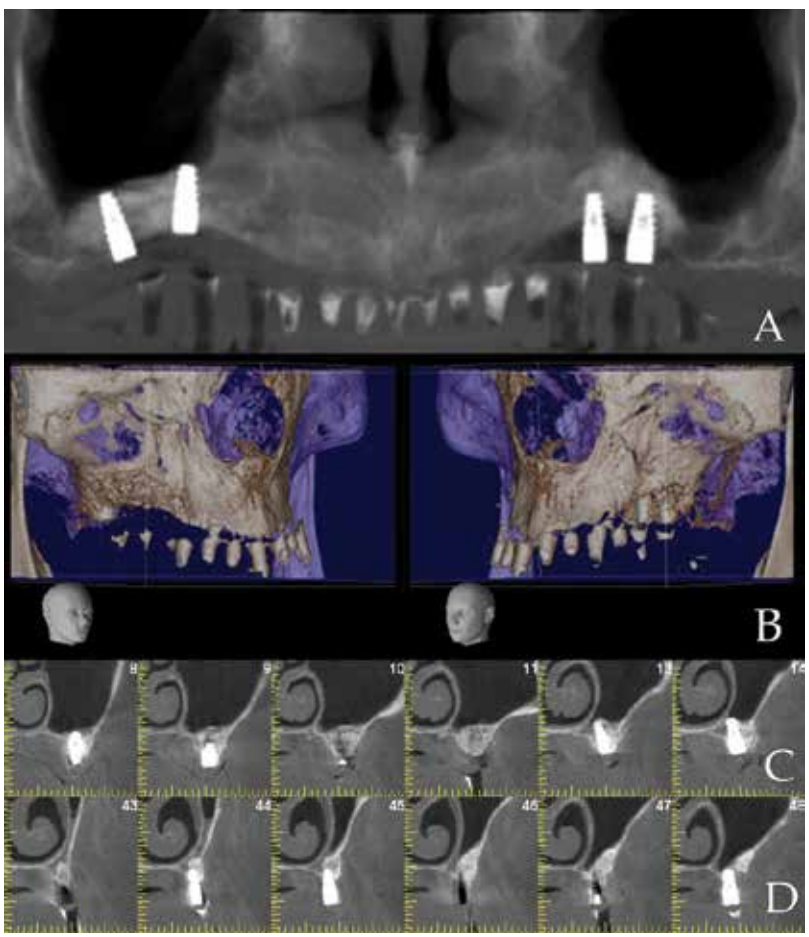

Figure 10. Post-dental implant placement diagnostic images. All images showed bone gain in both sides of the maxilla that persisted after the placement of dental implants. A: Panoramic X-ray from a CBCT showing the increase in alveolar bone height and dental implants on the right and left side. B: Reconstruction of the left and right maxilla with the surgical stent. C, D: Coronal cut from a CBCT scan from the left (C) and right (D) maxilla.

SBM, as well as gradual regression of associated fibrosis. The bone formation pattern was lamellar and trabecular, and the presence of osteoblast at the surface of the trabeculae, as well as osteocytes, was also observed. There were no signs of inflammation or bone sequestrae.

Postoperative 4-month control digital images exhibited osseointegration of the implants (**Figure 10**). No peri-implant radiolucencies were observed. The regenerated bone gain by the graft placement in both sides was preserved (**Figure 10**). Clinical assessment of the dental implants did not exhibited mobility of the implants, and a solid-deaf sound when performing percussion tests showed proper bone healing. The patient did not report pain, and there was no leakage of purulent material or signs of inflammation. In addition, the grafted bone presented the similar density than the perisinusal bone at both sides.

5. Discussion

The results of the present report provide evidence for the biocompatibility and osteoconductive properties of Synergy Bone Matrix. Bone graft implantation is the main treatment modality for bone defect repair and reconstruction [3]. In this sense, demineralized bovine bone offers excellent biocompatibility and physicochemical properties due to its mineral similarity with the host tissues [43].

SBM and BO are inorganic bovine bone xenografts indicated for bone defects filling due to their osteoconductive properties. In experimental models, the bone defect above a critical size requires a scaffold to guide bone repair. Deproteinized bovine bone mineral is osteoconductive and provides excellent biocompatibility because it has similar physicochemical characteristics to that of the mineral component of the original bone [3]. These two important biological properties allow apposition of new bone formed by osteoprogenitor cells located in the host tissue. It is noteworthy that bovine bone inorganic-phase not only promotes the deposition of calcium and phosphate ions but also is partially remodeled by osteoclasts and osteoblasts of the host [25]. In addition, the large interconnecting pore volume and its composition encourage the formation and ingrowth of new bone at the implantation sites.

BO is a recognized commercial bone defect filling material with osteoconductive properties. Under our experimental conditions, SBM showed similar osteoconductive properties to BO. In this regard, our histological findings showed neovascularization in the area implanted with either SBM or BO. This finding suggests that the new bone graft provided an optimal microenvironment for bone ingrowth. Typically, bone formation starts by bone-forming cells secreting bone matrix (i.e., collagen) into the defect area, followed by mineralization to envelope the implanted graft material [44]. Our results in rats showed that collagen fibers were replaced by mature bone, filling the CSBD, thus confirming active osteogenesis after 24 days postimplantation of the graft.

Similarly to what we observed in rats, histological analysis in rabbits at 4 weeks postimplantation showed the presence of blood vessels and revascularization in the areas implanted with both bone grafts. This finding suggests an optimal microenvironment for bone growth.

Bone formation begins with the appearance of osteoblasts in the defect area that secretes bone matrix (i.e. collagen); this period is followed by mineralization in order to wrap the implanted graft material [44].

Bone is a dynamic tissue that undergoes remodeling. Bone remodeling is a coupled process that starts with osteoclastic bone resorption followed by osteoblastic bone formation [45]. In this sense, the osteoclastic resorption of the graft is affected by both, the particle size as well as the composition and porosity of the material.

Initially, once the graft material is place, it suffers osteoclastic bone resorption followed by bone formation by osteoblastic action. The porosity of the particles enhances new bone formation by allowing the migration and proliferation of osteoblast and mesenchymal cells [46]. In addition, the microporosity of the particles is believed to enhance ionic exchange with body fluids [46]. This characteristic allows each particle of SBM to serve as a 3-D scaffold, in which osteoblast and osteoprogenitor cells migrate and form bone. Consistent with this, during the first period (4 weeks), we observed active osteogenesis evidencing by the presence of bone surfaces covered by osteoblasts around the implanted bone grafts and the formation of mature haversian systems. Toward the end of the experience, the collagen fibers were replaced by mature bone that filled the CSBD region. These findings indicate that the process of bone regeneration induced by SBM was similar to that induced by BO.

It is important to emphasize the quantity and quality of bone formed after placing a bone graft. On this regard, the increase in rat tibiae BMD indicates that the amount of bone increased in the CSBD filled with SBM or BO. In addition, biomechanical test performed in rabbit mandible exhibited an increase in all parameters, suggesting an enhanced quality of the newly formed bone.

Chackarti et al. observed, in a histomorphometric and micro-CT analysis, that granules from different sizes (small or large), produced the same pattern of bone formation: the bone surrounding the graft connects and produces a network of "bone bridges" among the graft particles [47]. The two used bone grafts had a similar granulometry: 1 to 2 mm for BO and 0.84 to 2 mm for SBM. Although BO and SBM are available in small granules as well as blocks, these were considered too small or too large for the rabbit experimental model used. In the present study, the volume of remaining bone graft particles was similar, and the biomechanical results did not show differences between SBM or BO, suggesting that the quality of the bone formed was similar for both products.

The loss of teeth in the posterior area of the maxilla leads to adverse consequences on masticatory function and occlusal balance. These outcomes negatively results in psychophysical conditions associated with temporomandibular joint and muscle diseases. A frequent problem in oral rehabilitation with implant-supported prostheses in the posterior maxilla is the lack of bone volume associated with alveolar ridge resorption or maxillary sinus pneumatization [48]. The reabsorption of the alveolar bone, adjacent to the floor of the maxillary sinus, may be aggravated by the increase in osteoclastic activity that originates in the periosteum of Schneider's membrane, after tooth loss, due to the absence of osteogenesis normally stimulated by the functional load on the bone. In this sense, the bone volume is limited due to the

pneumatization of the maxillary sinus on one hand and the loss of height and width of the alveolar process on the other. In order to increase the bone volume, the maxillary sinus floor elevation technique is used. It consists in elevating the membrane of the floor of the maxillary sinus and filling the intermediate space with bone substitutes [42] to promote bone formation [49]. The results of this procedure can be affected by the surgical techniques used: simultaneous placement versus delayed implantation of the implant, use of barrier membranes on the lateral window, graft material selection and surface characteristics, and length and width of the implants. Depending on the type of graft, the particles are partially reabsorbed and replaced by the patient's own bone during the healing time [50].

In agreement with Shirmohammadi et al. and Wallace et al. on sinus augmentation utilizing BO as bone graft [51, 52], the case report presented here evidences the efficacy of SBM in the bone healing process, showing osteoconductive properties when used as a grafting material for sinus lift elevation. In this regard, biopsies of the grafted areas showed that SBM particles were surrounded by vital new bone, without evidence of inflammation and bone sequestrae after 6 months of implantation. We neither observed inflammation nor thickening of the repaired Schneiderian membrane.

Additional comparative studies with greater number of patients and histomorphometric analysis are needed to determine whether there is any advantage in the use of SBM as opposed to BO in the survival of implants placed in grafted sinuses.

6. Conclusion

The use of bone grafts is important to preserve the alveolar bone ridge height and volume indispensable for dental implant placement. Despite the highly successful outcomes for the implant-supported overdentures, it seems that a majority of edentulous individuals have not pursued implant-based rehabilitation. Among the reasons cited for this discrepancy between highly successful therapy, and its acceptance is the cost of the treatment [53]. Our experimental findings in animal models, as well as the case report, indicate that the bone regeneration process induced by SBM presented similar characteristics in osteoconduction than BO and suggests the use of this material to increase the bone volume of the alveolar crest. The presence of a biomaterial with similar characteristics to those of internationally recognized commercial brands, but developed by the domestic industry, will be an important tool to reduce the high cost of these interventions.

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Conflict of interest

All authors state that they have no conflicts of interest.

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Chapter 7

The Use of Platelet-Rich Fibrin in Bone Grafting

Belir Atalay and Ozge Doganay

Additional information is available at the end of the chapter

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Abstract

In this century, there are two mostly practiced techniques for bone regeneration. These are autogenous bone grafting (ABG) and guided bone regeneration (GBR). It was reported in the late 1970s that platelets have a good regenerative effects. Platelets include growth factors that increase vascularization and collagen production by cell mitosis. Recently, most of the studies have indicated that platelet-rich fibrin (PRF) is a great healing potential for bony and soft tissue that derived from patients own blood. Most beneficial effects of PRF are easily derived directly from patient's venous blood without any ingredients, and it has a great potential for hard and soft tissue regeneration. PRF has no inflammatory effects and can be used with all kind of graft materials. When used as a membrane, it helps protecting the surgical area to stimulate the healing of soft and bone tissues.

Keywords: platelet-rich fibrin, bone healing, oral surgery, regeneration

1. Introduction

Platelet concentrates (PCs) for surgical use are innovative tools of regenerative medicine and dentistry and were widely studied in oral and maxillofacial surgery. PCs are blood extracts, applied to surgical sites, injuries, or wounds, a safe and effective way to promote soft tissue healing and bone growth. The most frequently used substrate, platelet-rich fibrin (PRF) is a member of platelet concentrates developed by Choukroun and colleagues [1]. PRF is an autologous matrix, in which cytokines and cells are released after a short period [2].

The aim of this chapter is to describe the potential functions of PRF for bone grafting in oral and maxillofacial surgery.

There are different types of PRF in local use.

2. Pure platelet-rich fibrin (P-PRF)

Pure platelet-rich fibrin (P-PRF) in other terms leukocyte-poor platelet-rich fibrin is a preparation without leukocytes having a high-density fibrin network. This platelet concentrate exists in a strongly activated gel form. It cannot be injected and used like traditional fibrin glues. On the other hand, with its strong fibrin matrix facility, P-PRF can be handled like a real solid material for several applications [3].

During preparation, only very low amounts of leucocytes are collected because of the specific separator gel, which is used to obtain P-PRF. However, the platelet collection level is high, and the preservation of the platelets during the procedure seems to be acceptable [4]. The main disadvantage of this platelet concentrate protocol is its cost and complex procedure if it is compared to the other forms of PRF, which are available nowadays [3].

3. Leukocyte and platelet-rich fibrin (L-PRF)

The second platelet concentrate type is leukocyte and platelet-rich fibrin (L-PRF), which is known as leukocyte and platelet-rich fibrin concentrate, called Choukroun's PRF. This product was invented by Choukroun in 2000 [1, 2]. The main concept of this technique differs from the other protocols. Patient's blood is taken and immediately softly centrifuged. This provides the formation of a fibrin clot in the middle of the tube, between the red blood cell base at the bottom and the acellular plasma at the top. This clot includes nearly all the platelets and more than 50% of the leukocytes from the initial blood harvest [5, 6]. The high quantity of leukocytes provides immune and antibacterial properties, wound healing and growth factor regulation. But, it depends on which leukocytes, in which quantity and in which state the centrifugation process can softly activate the white blood cells [7]. This product therefore only exists in an activated form and cannot be injected like a suspension. Therefore, L-PRF is a practical solid material with strong fibrin scaffold and used in oral and maxillofacial surgery, periodontology, implant dentistry and ear nose throat surgery [6].

4. Advanced platelet-rich fibrin (A-PRF)

The amount of WBCs effect vascularization and bone remodeling. As a result, researchers made new modifications in the centrifugation speeds and times to prevent cell loss within the PRF matrix [8]. These recent modifications of the PRF protocol have led to the improvement of advanced platelet-rich fibrin (A-PRF), which uses lower G-forces to gain higher growth factors compared to PRF [9]. It maintains higher amount of WBCs in the fibrin matrix and has special glass tubes, which are designed to make clotting faster. After the centrifugation, the tubes are removed and placed in their holders and left for 5 min to start clot formation. The fibrin clot which is rich with WBCs provides higher growth factors and recent research emphasizes that A-PRF enhances collagen matrix synthesis and supplement of progenitor cells [8].

5. Injectable platelet-rich fibrin (i-PRF)

One of the recent developments in the PRF technology is injectable PRF (i-PRF). Standard PRF is prepared as a gel which is inconvenient to inject [10]. i-PRF protocol necessitates short centrifugation period in order to produce a liquid platelet concentrate, which includes primarily liquid fibrinogen and thrombin prior to fibrin formation [11]. The plastic tubes with hydrophobic surface are used, and therefore coagulation process does not effectively start. Hence, all the blood components reach the top of the tube under the centrifugation force with short centrifugation time (i.e., 2–4 min). The light yellow-colored layer, which is the combination of plasma and platelets, is aspirated from the top of the tube to obtain i-PRF. Nowadays, it is used with bone grafts to keep graft particles tightly encapsulated in the fibrin matrix. With the coagulation process, i-PRF forms a gel consistency holding bone graft together. Also, the release of growth factor is beneficial for the graft. This has the potential to convert any osteoconductive graft to osteopromotive, which would provide faster and better bone formation. Another type of graft prepared with i-PRF is the PRF block. For its preparation, i-PRF is mixed with a combination of bone graft and shredded PRF clot [10].

6. Titanium prepared platelet-rich fibrin (T-PRF)

Some physicians worry about damage for the patient with glass-evacuated blood collection tubes with silica activators. O'Connell emphasized that silica contact cannot be avoided with glass tubes. These silica particles are dense enough to settle in the sediment and might reach the patient when the product is used during procedures [12]. Recently, Tunali et al. used medical grade titanium tubes to produce titanium prepared platelet-rich fibrin (T-PRF) [10]. This biocompatible material was tried to eliminate the potential negative effects of silica from dry glass or glass-coated plastic tubes [12]. The research showed that T-PRF supply is a more organized network than L-PRF. Furthermore, its fibrin network covers larger area and has thicker fibrin clot. In a human study, wound healing in the palatal mucosa is found better with the T-PRF application [10].

7. Prepared platelet-rich lysate (PRF-L)

A newer application of PRF is the prepared platelet-rich lysate (PRF-L). In this technique, after PRF preparation, it is incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The exudate, which is collected, is referred to as PRF lysate. It is said to be a good source of several growth factors. In a study, it is found that PRF-L can significantly improve the proliferation index, collagen deposition and migration rates in chronically UVA-irradiated human dermal fibroblasts [13]. This is a new technique that requires further studies.

8. Preparation

Venous blood of patient is drawn into 10 mL, anticoagulant-free tubes and then immediately spun in a tabletop centrifuge at 3000 rpm for 10 min. Due to the lack of anticoagulant, platelet activation occurs once the blood contacts the glass tube walls and subsequently initiates the coagulation cascade. By centrifugation, fibrinogen is concentrated in the top and middle of the tube and then circulating thrombin transforms it into fibrin. This action leads to the development of a fibrin clot in the middle of the tube, between the RBCs at the bottom and serum at the top. The upper straw-colored layer is removed from the centrifuge tube and blotted to remove serum and RBC to result in a dense matrix. Timing is also critical to the success of the PRF technique. PRF must be prepared immediately before application to the surgical site (**Figure 1**) [1].

8.1. Clinical applications of PRF in oral surgery

In the past, PRF could be useful for both hemostasis and wound healing. Nowadays, sinus lift technique is considered to be one of the most common and valid procedures for the use of PRF in oral surgery. PRF is also used for augmenting the alveolar ridge, improve soft tissue healing and periodontal regeneration and preserving the alveolar ridge height after extraction. It has been studied that PRF improves wound healing in multiple extractions by reducing healing time [14].

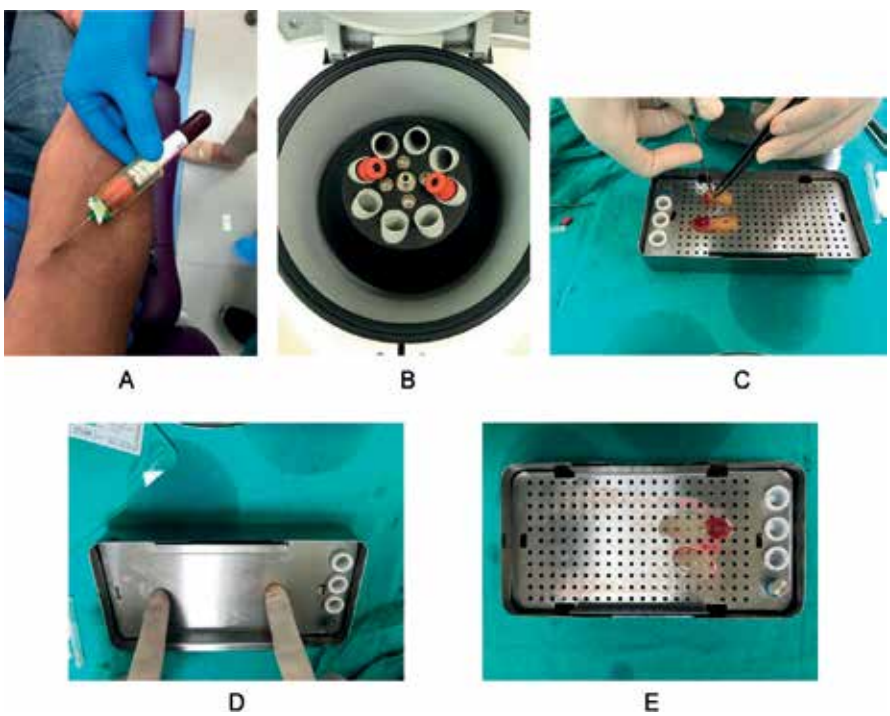

Figure 1. Preparation of PRF membrane. A: Collection of venous blood into the tube; B: centrifuge device; C: platelet-rich fibrin clots; D: PRF clots were pressed to obtain more dense membranes; E: PRF membranes.

8.1.1. PRF in soft tissue regeneration

The use of platelet concentrates may reduce and control the risks associated with delayed bone and soft tissue healing. The success for the healing of a bone graft is certainly related with intact soft tissue coverage.

Patients using antiresorptive therapies exhibit a reduced healing potential in bone and soft tissue that can lead to medication related osteonecrosis. In order to stimulate healing and obtain soft tissue coverage, PRF can be placed to the surgical or exposed area without tension. Even if there is a need for researches in this field, PRF presented valuable solutions till today [15].

The other indication of PRF usage is the method of socket preservation. When implant restoration is considered after extraction of a tooth, this must be kept in mind that the resorption process starts immediately. Thus, the volume of the socket, soft tissue closure and thickness of gingiva must be protected as possible as early, for a complete and quick bone regeneration. If the wound closure and remodeling are achieved properly, implant can be placed in the desired position [15].

8.1.2. Guided bone regeneration (GBR) (as a barrier membrane)

Several oral and maxillofacial surgical procedures have used membranes for guided bone regeneration (GBR). The potential applications of PRF in this field have been shown to be effective in different clinical situations. However, this method may not be superior as a barrier membrane for regeneration when compared to conventional membranes, and therefore appropriate cases should be selected in order to benefit from the properties of PRF in GBR (**Figure 2**) [16, 17].

8.1.3. Sinus lift procedure

Sinus lift is one of the most frequent grafting procedures in implantology and has proven to be a successful and predictable technique for providing bone height for implant placement. In the

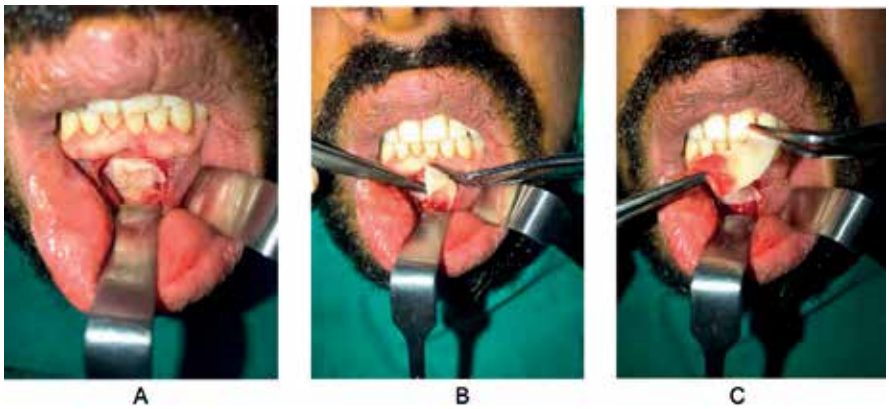

Figure 2. Enucleation of radicular cyst resulted from left central incisor tooth and apical resection were performed at the same time. A: After eliminating the whole lesions, PRF placed into the intrabony defect to accelerate healing and prevent soft tissue collapse. B and C: PRF membrane was used to cover the buccal wall of the cavity as a barrier.

literature, no application using PRF was more studied than the sinus lift procedure. This model generating to obtain bone height, provides a closed, protected and stable environment for healing. Thus, bone healing in sinus cavity can be evaluated more accurate than the other procedures that are in contact with the oral environment [18].

Subsinus cavity floored by the Schneiderian membrane following sinus lift has a capacity of bone regeneration due to the high osteogenic properties, though several bone materials can yield good results in terms of bone healing [19].

Most researches have focused on bone biomaterials to improve bone quality and to accelerate healing for early operations. The main mechanism of subsinus cavity healing is to be filled with blood clot that is promoting neoangiogenesis and proliferation. Similarly, the aim of using PRF into the cavity is to provide space and optimize healing as a biologic agent [20]. The first study reported on PRF by Choukroun et al. represented that the use of PRF in addition to the bone grafts accelerated healing period for sinus floor augmentation [21]. It is important to reduce healing time before implantation.

The incidence of perforation of the membrane that is a common intraoperative finding was reported ranging from 10 to 60% [22]. The rupture of the membrane can lead to postoperative complications, so has to be repaired before grafting. Otherwise, a biomaterial can migrate into the sinus cavity leading to sinus infection and impairment of healing. Some biomaterials such as collagen membrane and platelet concentrates have been commonly used to prevent these complications. In addition to the use of PRF for grafting, it is also used as an adhesion material and frequently preferred for repair of possible perforations of the schneiderian membrane during sinus lift.

8.1.4. Grafting material for bone regeneration

The acceleration of healing in bone defect is of particular interest when dental implants would be inserted in this area. Due to reduction of time, early functional loading and better primary stability of the implant will be obtained after the regeneration [19]. Thus, some biologic agents may be needed to shorten this period. For this purpose, PRF-like substances can be used. The potential of these substances as a biologic agent in oral and maxillofacial surgery relies on the growth factors stored within platelet alpha granules containing platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), platelet-derived angiogenic factor and transforming growth factor-beta (TGF-β). Moreover, PRF acts as a biological connector between different graft elements and serves as a matrix that eases forming new bone tissue into the defect. In fact, due to the lack of standardization and absence of characterization of the studied PRF materials, it is difficult to explain the exact function of platelet concentrates on bone regeneration [23].

8.1.5. PRF for peri-implant bone defects

Peri-implant bone defects are encountered as a result of peri-implantitis and secondly inadequate bone volume for implantation and thirdly immediate implantation. Although the mechanisms of peri-implantitis are considered to be bacterial accumulation or prosthetic overload of

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the implant, it is believed that the main mechanism of peri-implantitis is related to bone/implant interface breakdown [24].

In case of small amount of bone, it is inevitable to make lateral perforation while placing implant or bone resorption around particularly the collar of the implant. In order to reinforce the bone amount around the implant to prevent resorption and gain more esthetic appearance, autogenous and/or synthetic materials can be used to cover the open surface of these implants.

Peri-implant bone defects can be developed after immediate implantation (**Figure 3**). After the insertion of the implant in a fresh avulsion socket, a large peri-implant defect must be filled with a material to prevent soft tissue invagination [25].

For those three clinical situations, bone regeneration between the cavity/defect and implant surface can be achieved by using PRF in addition to bone grafts. With its strong fibrin architecture and specific 3D cell distribution, especially L-PRF may offer positive results when used

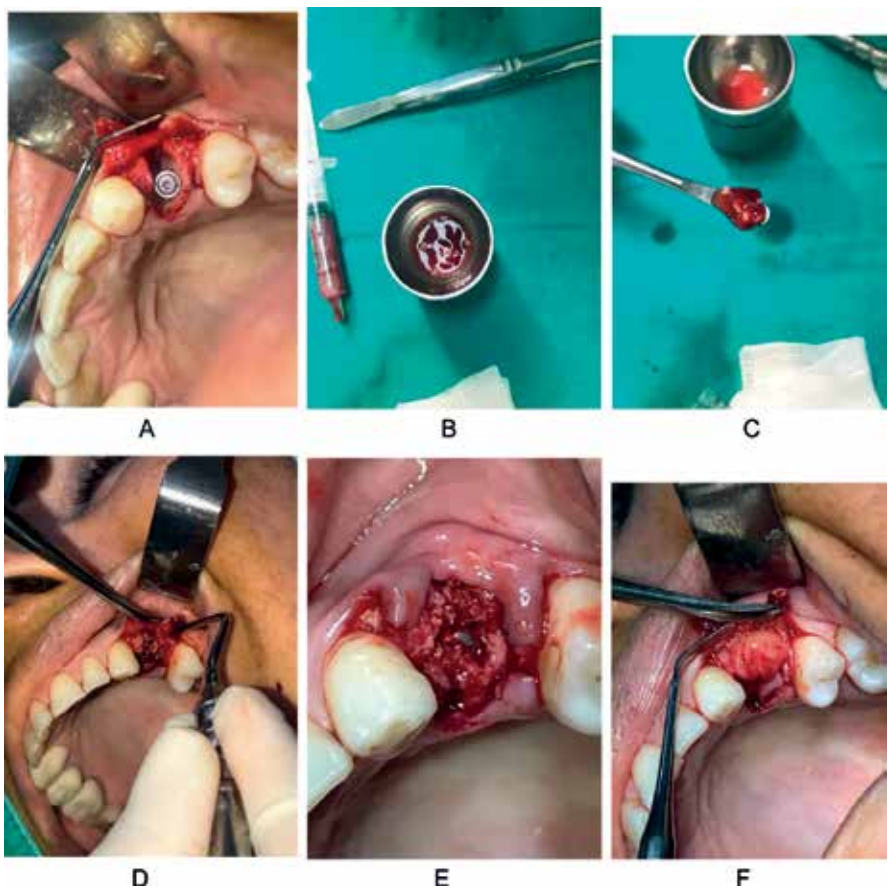

Figure 3. Peri-implant bone defect after immediate implant placement. A: Buccal and palatinal bone defects around the implant; B, C: A mix of PRF and xenogeneic bone graft constituted a sticky mass; D, E: PRF with bone graft placed into the cavity; F: PRF membranes were used to cover the whole defect.

in combination with bone biomaterials for the filling of peri-implant bone defects. On the whole, there are still only few studies published in the literature about the use of L-PRF in peri-implant bone defects [26].

9. In brief: advantages

- Eliminating transmission of diseases (the patient's own blood)
- Promoting soft tissue healing
- Protection extraction socket against resorption and infection
- Minimal invasive and inexpensive method
- Role as a carrier matrix for bone grafts
- Supporting neoangiogenesis
- Minimal immunological reaction
- As a sticky material, holding different substances together
- Growth factors

10. Conclusion

PRF seems to be an accepted minimally invasive approach with good clinical results. With the ease of preparation, low cost and biologic properties, PRF could be considered as a beneficial treatment option. On the other hand, due to the lack of standardization and long-term follow up clinical studies, the major effect of PRF on bone grafting has not been explained yet.

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Chapter 8

L-PRF: A "Super" Biomaterial for Naturally Guided Hard/Soft Tissue Bioengineering and Regeneration of Oro-Dental, Periodontal and Jaw Defects

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Additional information is available at the end of the chapter

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Abstract

Leukocyte and platelet-rich fibrin (L-PRF) is a 3-D autogenous biomaterial derived via simple and rapid centrifugation of whole blood patient samples, in the absence of anti-coagulants, bovine thrombin, additives or any gelifying agents. A relatively new "revolutionary" step in second generation platelet concentrate-based therapeutics, clinical effectiveness of L-PRF remains highly-debatable, whether due to preparation protocol variability, limited evidence-based clinical literature and/or inadequate understanding of its biocomponents. This critical review provides an update on the application of L-PRF during oral surgery procedures, in human Randomized and Controlled Clinical Trials only (up to February 2016). Accordingly, autologous L-PRF is often associated with early bone formation and maturation; accelerated soft-tissue healing; and reduced post-surgical pain and discomfort. L-PRF is a simple, malleable and safe biomaterial suitable for use in oral surgery. An innovative tool in Regenerative Dentistry, L-PRF is a strong alternative and possibly cost-effective biomaterial for oral-tissue regeneration. Preparation protocols require revision and standardization. Furthermore, a good analysis of its rheological properties, biocomponents and their bioactive function would enhance the validity, comprehension and therapeutic potential of the reported findings or observations; a step closer towards a new era of "super" dental biomaterials and bioscaffolds.

Keywords: tissue engineering, regeneration, leukocyte, platelet, fibrin, growth factors, dentistry, oral surgery, periodontology, osteogenesis, grafts

1. Introduction

Despite significant improvements, in reconstruction techniques and materials, during last decades, the regeneration of defects remains a challenge [1]. Indeed, current clinical approaches

used to reconstruct and heal complex defects, including different bone grafting methods, such as autologous bone grafts, allografts, bone-graft substitutes, distraction osteogenesis, and/or guided bone regeneration, are deemed restricted, on a daily basis. This is often multi-factorial; whether due to limited self-renewal capacity of the defect and/or the limited donor supply, increased morbidity, risk of antigenicity and foreign body reactions, associated with the grafts used. Operative-associated time and cost contribute as well. Hence, the art and science of oro-maxillo-facial reconstruction is of great interest for contemporary oral and maxillofacial surgeons; in search for better bioengineering strategies and biomaterials: a core driver for biodental research, today [2].

Briefly, ***Platelet Concentrates*** are autologous blood extracts obtained through centrifugation of whole blood samples. The preparation procedure allows the gathering and concentration of platelets and other therapeutic blood constituents (fibrinogen/fibrin, growth factors, leukocytes and circulating cells), in clinically-usable preparations (surgical adjuvants), which may enhance, accelerate and promote tissue (hard and soft) wound healing and regeneration [3]. Despite promising clinical observations, their overall effectiveness remains debated to date. This is mainly due to: mixed/variable clinical outcomes, limited high-quality evidence-based literature, and poor characterization of end-products (and preparation protocols) used in studies; and - until recently - lack of proper terminology systems to classify these preparations [4]. Indeed, the first "classification" consensus [5] was published in 2009, describing 4 different Platelet Concentrate sub-families, based on variability in biological content (fibrin and cell), properties (gelification) and potential applications: pure platelet-rich plasma (P-PRP), leukocyte and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF) and leukocyte and platelet-rich fibrin (L-PRF) [5]. Today, it can be safely stated that, in oral and maxillofacial surgery, the L-PRF sub-family [4–6] is receiving the most attention, mainly due to simplicity, user-friendliness and cost-effectiveness, when compared to the PRPs.

L-PRF is a second generation autologous platelet concentrate of whole venous blood [4, 6]. A slowly- and strongly-polymerized fibrin gel (**Figure 1**) rich in growth factors, platelets, leukocytes (almost half of the initial blood harvest) and lymphocytes, is collected, following simple and rapid (~10 min) centrifugation (*please note that preparation protocols vary*—**Table 1**) of blood, in vacutainer tubes, without anti-coagulant. The gathered clot (or biomaterial) is stable, resilient, strong, adhesive and malleable, where it can be cut or adapted into different anatomical defects and applications: used directly as filling material, mixed with bone grating material, or compressed into a strong fibrin membrane. Alongside this established clinical ease of use

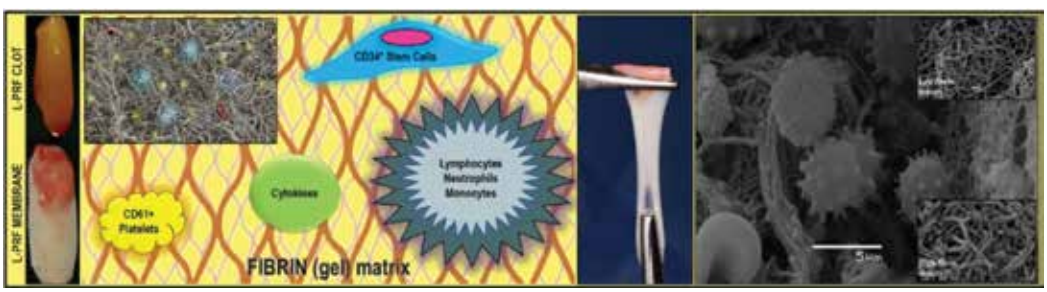

Figure 1. L-PRF clinical presentation and composition/architecture illustration. (A) Clinical presentations of L-PRF clot and membrane. (B) Schematic representation of L-PRF biocomponents. (C) Resiliency and malleability of L-PRF. (D) SEM (scanning Electron microscope) micrograph of the L-PRF membrane displaying its polymerized interconnected fibrin network and large living cell population-content.

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For the most reproducible and clinically-usable L-PRF clots and membranes (and best possible clinical outcome), the following protocol is recommended:	
Collect a 5-9 mL whole venous blood sample into 2-3 sterile 6 mL glass-coated plastic vacutainer tubes (without anti-coagulant).	
Centrifuged immediately for 10-12 minutes at 2700-3000 revolutions per minute (rpm) using a high-quality table centrifuge.	
Collect L-PRF clot carefully from the middle portion of the tubes. Typically, three compartments should be evident in the tube - **UPPER:** straw-colored acellular plasma (***PPP***), **MIDDLE:** yellowish fibrin clot (***FC***); and **LOWER:** red-colored lower fraction containing red blood cells (***RBCs***). Remove the upper layer to collect the middle fraction; 2 mm below to the lower dividing line.	
The collected clot can then be used directly as filling material, mixed with bone grating material (L-PRF plug/block) or compressed into a strong L-PRF membrane, using a surgical box designed to prepare it without damage (**shown in Figure 3A**).	
REMINDER: Quick handling is critical to obtain bioactive L-PRF clots charged with serum and platelets.	

Table 1. Recommendations for L-PRF preparation.

and handling, the biochemical composition of the L-PRF by-products provides it with attractive hemostatic, angiogenic, osteogenic, anti-inflammatory, anti-microbial, pain-inhibitory and wound healing characteristics [3, 7, 8]. This critical review aims to provide the clinical reader with an up-to-date evidence-based presentation on the evaluation of L-PRF use and application for oro-maxillo-facial tissue regeneration, from high-quality Randomized and Controlled Clinical Trials. Thus, *in vitro*, *in vivo* and *case report* studies were excluded from analysis.

2. Materials and methods

A structured literature search (**Figure 2**) was performed on PUBMED (October 2015–February 2016) using MESH terms "Platelet-rich fibrin" and "Platelet-rich plasma" according to the

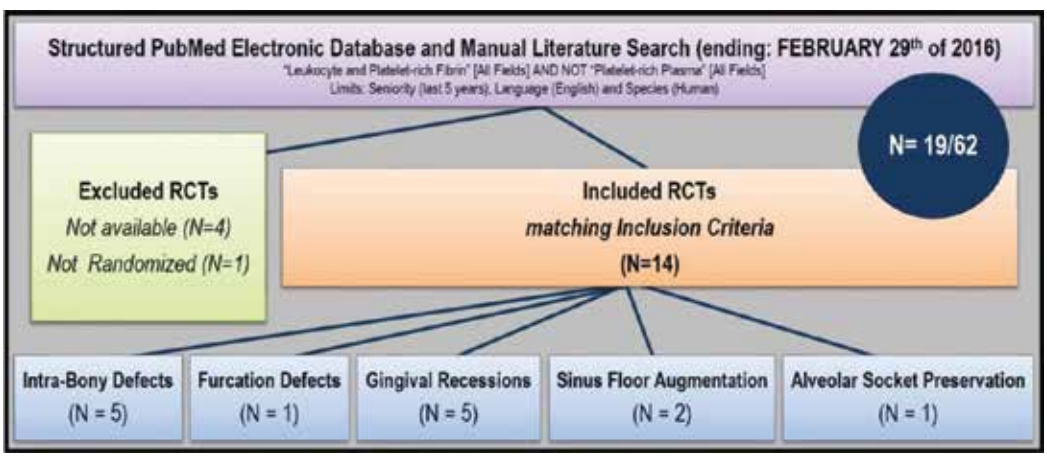

Figure 2. Flow-chart of literature search strategy and results.

Application	N° Patients/Defects	Groups	Follow-up (month)	Main Result	REF
IBD	32/32	1) L-PRF + open flap surgery 2) Open flap surgery	9	All sites healed uneventfully. PD reduction, CAL gain, defect fill, percentage defect fill and post-treatment gingival margin stability were significantly greater in the L-PRF treated group. (P<0.05).	(11)
IBD	15/30	1) L-PRF + open flap surgery 2) Open flap surgery	12	All sites healed uneventfully. PD reduction, CAL gain, radiographic IBD depth reduction and post-treatment gingival margin stability were significantly higher in the L-PRF group. Statistically significant higher patient acceptance and healing index in L-PRF vs. control.	(10)
IBD	35/56	1) L-PRF + open flap surgery 2) Open flap surgery	9	All sites healed uneventfully. PD reduction, CAL gain and radiographic IBD defect fill were significantly higher in the L-PRF group. GMS higher in L-PRF group.	(12)
IBD	17/34	1) L-PRF + Bio-Oss® 2) L-PRF	6	All sites healed uneventfully. Both groups showed significant PD reduction, CAL gain and IBD fill. Intergroup differences were also significant and favored the L-PRF/Bio-Oss group.	(13)
IBD	10/20	1) L-PRF + DFDBA 2) DFDBA	6	Both groups experienced significant PD reduction, CAL gain, IBD fill and IBD resolution. Intergroup differences were statistically significant only for PD reduction and CAL gain favoring the L-PRF/DFDBA group.	(14)
PFD	18/38	1) L-PRF + Open flap surgery 2) Open Flap surgery	9	All sites healed uneventfully. No significant visual differences between groups were noticed. Complete clinical closure was achieved in 66.7% of the defects in the L-PRF group. Within residual furcation defects, 5/6 were reduced from grade II to grade I, and one defect remained grade II. Significantly greater PD reduction, CAL gain and defect-fill was noticed in the L-PRF treated group vs. control.	(16)
Gingival Recession	15/30	1) L-PRF + CAF 2) CAF	6	Both groups experienced statistically significant RD reduction, CAL gain and KTW increase at all time intervals (P<0.05). Intergroup differences were statistically significant and favored the L-PRF group. Mean percentage of root coverage for the test and control group were 100% and 68.44%, respectively. Differences between groups were statistically significant and favored the L-PRF group.	(18)
Gingival Recession	20/67	1) L-PRF + CAF 2) CAF	6	With exception of CAL gain and Gingival Tissue Thickness (GTH) increase, the addition of L-PRF to CAF failed to produce significant additional clinical benefits (vs. CAF-alone). Percentage root coverage, full root coverage, GMS and recession width (RW) reduction were significantly higher in CAF controls than L-PRF treated sites, after 6 months.	(19)
Gingival Recession	20/40	1) L-PRF + CAF 2) EMD + CAF	12	Both groups experienced statistically significant RD reduction, PD reduction and KTW increase. Intergroup differences were significant only for KTW increase and favored the EMD group. Mean root coverage was 70.5 ± 11.78% in the EMD group and 72.1 ± 9.55% in the L-PRF group. Complete root coverage was achieved in 80% of the EMD sites and 65% of the L-PRF sites. *No intergroup comparison was carried out.* Healing index of the L-PRF group after the first week was significantly superior to that of EMD. Non-significant differences between groups were found after 2 weeks post surgery. Three patients of the EMD group and 1 of the L-PRF group experienced severe pain. All patients in the EMD group reported greater discomfort. Analysis of the first five days post-surgery revealed statistically significant differences between both groups favoring L-PRF (less pain).	(20)
Gingival Recession	22/44	1) L-PRF + CAF 2) SCTG + CAF	6	Both groups experienced a statistically significant decrease in RD, RW, RA plus an increase in CAL gain, KTW and GTH. Intergroup differences were non-significant. Higher yet non-significant gingival margin stability was reported for the L-PRF group. Percentage of root coverage and complete root coverage were 92.7% and 72.7% in the test group and 94.2% and 77.3% in the control group. No statistical significant differences between both groups were found (P>0.05). All sites healed uneventfully however control group reported complications (i.e., pain) related to the palate donor site.	(22)
Gingival Recession	15/30	1) L-PRF + CAF 2) CTG + CAF	6	Both groups experienced a significant CAL gain, RD reduction and GMS. Intergroup differences were non-significant. Both groups experienced a statistically significant increase in KTW. Intergroup differences were statistically significant and favored the CTG group. Mean root coverage for the L-PRF group was 88.66 ± 10.66% and 91.96 ± 15.46% for the control group. Complete root coverage was achieved in 75.85% of cases in the L-PRF group and 79.56% of cases in the control group. Inter-group differences were non-significant. Healing Index values of the L-PRF group during the first two weeks were statistically superior to those of CTG control. One patient from the L-PRF group and 7 of the CTG group experienced severe pain. Also, all patients of the control group reported some discomfort. Pain intensity was statistically superior in the CTG during the first week.	(21)
Maxillary Sinus Augmentation (Graft)	10/11	1) L-PRF + Bio-Oss® 2) Bio-Oss®	6	Healing was uneventful for all patients. Both groups exhibited adequate amount and density of radiographic mineralized tissue plus similar composition, distribution and inflammation of histological structures. Intergroup differences were non-significant. Percentage of newly formed bone was about 1.4 times greater in L-PRF group (18.35 ± 5.62% vs. 12.95 ± 5.33% of control). Percentage of residual bone substitute material was about 1.5 times greater in control group (28.54% ± 12.01% vs. 19.16 ± 6.89% of L-PRF). The bone-to-bone substitute contact was 21.45 ± 14.57% and 18.75 ± 5.39% in L-PRF and control group. Intergroup differences were non-significant.	(26)
Maxillary Sinus Augmentation (Membrane)	6/12	1) L-PRF 2) Bio-Gide®	5	Wound healing was uneventful for all patients. No soft tissue in-growths were observed in both groups. Surfaces seemed homogenous with visible bone-substitute material embedded into newly-formed bone. Average amount of vital bone and bone substitute were 17.0% and 15.9% in the L-PRF group. Control group had 17.2% and 17.3%. *No intergroup comparisons were carried out.*	(27)
Alveolar Socket Preservation	20/40	1) L-PRF 2) Empty (blood clot)	3	Soft tissue healing was significantly better in L-PRF group vs. controls (Laundry, Turnbell and Howley Soft Tissue Healing Index). Early bone formation/maturation was noticed for experimental sites vs. controls. Differences were significant only at 8 weeks post-extraction and favored L-PRF group. Higher bone density was noticed in L-PRF group vs. controls. Intergroup differences were non-significant. Mean post-surgical pain (measured by VAS score) was reduced in L-PRF group vs. non-L-PRF controls, at day 1. By day 7 no intergroup differences were noticed.	(31)

Table 2. Summary of clinical literature (RCTs) on L-PRF use in dentistry.

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following search strategy: "Platelet-rich fibrin" [All Fields] NOT "Platelet-rich plasma" [All Fields]. Results were limited by: Seniority (5 years since publication), Language (English), Availability (Full-text) and Species (Human). Inclusion criteria were: (a) randomized clinical trials (RCTs) using (b) Choukroun's L-PRF (not PRF) in (c) Oro-Maxillo-Facial procedures. Initial search resulted in 62 articles, 19 of which met the inclusion criteria (**Table 2**). Five articles were excluded due to quality/availability lack of randomization. Due to the high heterogeneity, results are presented in a narrative format.

3. Results and discussion

3.1. L-PRF in the treatment of periodontal intrabony defects (IBDs)

Periodontal tissue regeneration has been defined as the formation of new cementum, alveolar bone, and a functional periodontal ligament on a previously-diseased tooth-supporting root surface. Due to limited intrinsic regenerative potential, IBDs are a common and challenging sequel of periodontal disease. Meta-analyses demonstrated that, treatment with conservative open flap debridement, produces an Average Clinical Attachment (CAL) gain of 2.0 mm [9]. While about 1.5 mm may be attributed to newly formed bone; bone-fill does not implicate the regeneration of new attachment to the root [9]. In this context, L-PRF appears promising for regeneration of the whole periodontal attachment system (**Figure 3A** and **B**). Five RCTs addressing the prospective application of L-PRF in the treatment of Periodontal IBD were found. The identified studies allowed for the following comparisons: (a) L-PRF/Open flap surgery vs. Open flap surgery [10–12], (b) L-PRF/Bio-Oss® constructs (Bio-Oss®, Geistlich Pharma North America, Inc.) vs. L-PRF [13] and (c) L-PRF/DFDBA constructs vs. DFDBA (Demineralized Freeze-Dried Bone Allograft) [14]. All patients included in those studies were periodontally stable and systemically healthy individuals who presented: similar bilateral IBD of at least 5 mm probing depth, located in vital asymptomatic teeth with no furcation involvement. Studies evaluating the addition of L-PRF to conventional open flap procedure reported the biomaterial notably improving both, clinical and radiographic parameters of IBDs, after 9 [11, 12] and 12 months [10]. A significant increase in probing depth (PD) reduction, CAL gain, post-treatment.

Gingival Margin Stability [(GMS) *less post-treatment gingival recession*], bone defect fill and percentage bone defect fill were noticed in all L-PRF-treated sites vs. controls [10–12]. Interestingly, higher patient acceptance was also associated with use of L-PRF. Most probably, this is attributed to the accelerated wound healing and pain-inhibitory properties [10, 11]. The presented PD reduction and CAL gain values were superior to previously-reported values in meta-analysis performed for open flap surgery [9], suggesting the additional benefits of L-PRF over the conventional approach. Treatment with L-PRF/particulate bone-graft substitutes (Bio-Oss® [13] and DFDBA [14]) provided additional statistically-significant benefits, in terms of PD reduction, CAL gain and bone defect fill vs. graft substitutes, after 6 months. Nonetheless, the absence of "simultaneously-run" L-PRF-alone control renders it difficult to

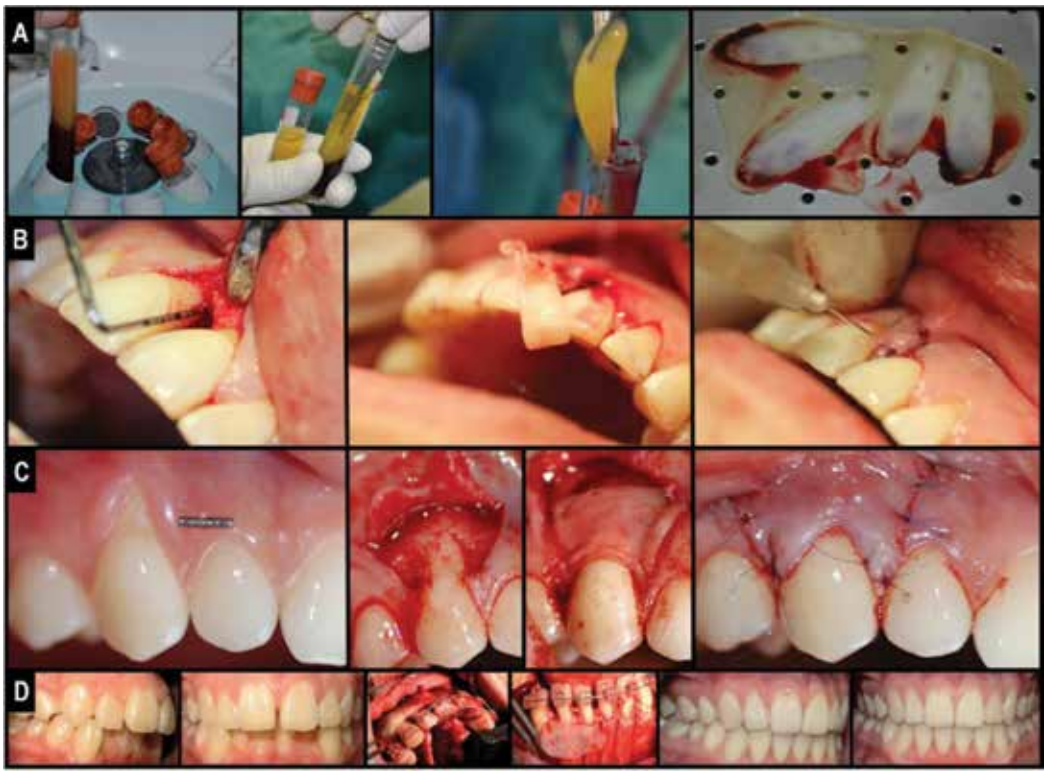

Figure 3. Clinical illustration of L-PRF application in Oro-Maxillo-facial surgery defect regeneration: Natural guided tissue bioengineering using L-PRF as a "bio-scaffold". (A) L-PRF membrane preparation. (B) Clinical application in IBD. (C) Clinical application under CAF. (D) Clinical application in PAOO.

distinguish between the effects of L-PRF and other potential variables in the study. Thus, while promising, additional studies are deemed essential in order to appropriately determine (quantifiably) the effectiveness and advantages of L-PRF application over particulate bone-grafts use.

3.2. L-PRF in the treatment of periodontal furcation defects (PFDs)

Molars with furcation involvement (resulting from periodontitis) have higher rates of periodontal breakdown and poorer prognosis, than single-rooted teeth [15]. Contemporary treatment options often include the use of regenerative materials and bone grafts; however, the introduction of L-PRF seems promising for better therapeutic outcomes. In our analyses, 1 RCT addressing the therapeutic use of L-PRF in PFDs was found [16]. The study compared L-PRF/open flap vs. open flap debridement alone, in the treatment of grade II mandibular defects. Included patients were periodontally-stable and systemically healthy, with similar bi-lateral grade II buccal furcation defects (at least 5 mm probing depth and ≥ 3 mm horizontal probing depth), in vital asymptomatic mandibular first molars.

L-PRF use significantly improved clinical and radiographic parameters of conventional open flap debridement. After 9 months, complete clinical closure of the defect was achieved in

66.7% of L-PRF-treated sites. Severity within residual defects was reduced in 5/6 sites (degree I), whereas one defect remained in degree II. Significantly greater PD reduction, CAL gain and radiographic vertical defect fill was reported on experimental sites vs. controls. L-PRF use was also associated with a greater post-treatment GMS [16].

3.3. L-PRF in the treatment of miller class I and II gingival recessions

Gingival recessions are characterized by the apical migration of the gingival margin with subsequent root surface exposure. If left untreated, the condition may lead to other problems including: deficient esthetics, dentine hypersensitivity and higher risk of dental caries [17]. Available treatment options include the use of: (a) Coronally Advanced Flaps (CAF); (b) Connective Tissue Grafts (CTG); and (c) Sub-epithelial Connective Tissue Grafts (SCTG). On their own, the aforementioned techniques have important limitations such as (a) unpredictable long-term root coverage (i.e. CAF decreases from 89% to 58.8% after 6 months), (b) limited gain of keratinized tissue width (KTW); important to prevent recurrence, and (c) adverse post-surgical effects such as pain/discomfort, swelling, flap necrosis, etc. [17]. In this review, five RCTs evaluating the application of L-PRF in the treatment of gingival recessions were identified and included. The studies allowed for the following comparisons: (a) L-PRF/CAF vs. CAF [18–19]; (b) L-PRF/CAF vs. EMD (Enamel Matrix Derivate)/CAF [20]; (c) L-PRF/CAF vs. CTG [21]; and (d) L-PRF/CAF vs. SCTG [22]. Similar to previous RCTs, all patients included herein were periodontally-stable and systemically healthy; presented with: similar bi-lateral Miller Class I or II gingival recessions (>2 mm depth) localized on vital teeth, without restorations. According to Padma et al., the addition of L-PRF to CAF improved both, clinical outcomes and post-treatment stability of CAF [18]. After 6 months, the authors reported (significantly) more Recession Depth (RD) reduction, CAL gain and KTW increase in all L-PRF-treated sites vs. controls. Interestingly, post-treatment GMS was also higher in the test group with 100% root coverage after 6 months vs. 64.88% in controls [18]. However, in contrary with this RCT, Aroca et al. reported limited clinical benefits when using the L-PRF/CAF approach [19]. Herein, CAL gain and Gingival Tissue Thickness (GTH) were the only benefiters of the combination; whereas percentage root coverage, full root coverage, GMS and Recession Width (RW) reduction were significantly higher in CAF-alone controls than the test group [19]. Such "*contradictory*" results may be partially explained by deficient study design, which, not only failed to adequately include blind examiners (leading to potential bias in favor of the "traditional" approach), yet also included: multiple adjacent gingival recessions (with poorer prognosis than single/localized recessions); heavy smokers (in which healing response is usually altered); and the L-PRF were stored in a 4°C refrigerator until use (L-PRF protocols often recommend immediate/fresh use). Indeed, emerging evidence states that growth factor release from L-PRF initiates as early as 5 min from preparation/centrifugation. Hence, storage could have altered its properties and thereby diminished or deteriorated its clinical potential. When compared to other root coverage procedures (EMD/CAF, CTG and SCTG), the L-PRF/CAF approach showed similar clinical outcomes regarding RD reduction, CAL gain, mean root coverage (%) and complete root coverage (%). KTW increase was the only exception, with both EMD/CAF and CTG controls showing higher KTW than L-PRF-treated groups [20–22]. Interestingly, all studies reported significantly faster healing and fewer complications (pain and discomfort) when L-PRF was used [20–22]. Findings are notable, especially when

comparing with SCTG (the current "*gold standard*" technique for treating Miller Class I and II gingival recessions); indicating that L-PRF/CAF could be a safer and less invasive alternative to current grafting techniques, and a more cost-effective strategy or approach than EMD is in treating Miller Class I and II gingival recessions (**Figure 3C**).

3.4. L-PRF in sinus floor augmentation

Resorption of the upper jaw bone after tooth loss is a frequent problem faced in posterior maxillary implant placement due to lack of sufficient bone mass for anchorage. Common maxillary sinus augmentation techniques provide a solution via increasing the available bone height at the expense of sacrificing volume of the maxillary sinus [23]. Traditionally, autologous bone grafts and resorbable membranes are used to promote osteogenesis and avoid soft tissue in-growth into the surgical site. However, donor site morbidity and size restrictions, latter resorption of the graft and high-cost of membranes, are main disadvantages [24, 25]. In this context, L-PRF appears to provide a promising alternative overcoming such limitations. In this review, two RCTs evaluating the use of L-PRF in lateral window sinus augmentation were found. Applications were performed either as: (a) grafting material (L-PRF/Bio-Oss® constructs vs. Bio-Oss®) [26] or (b) absorbable covering membrane for the lateral osteotomy window (L-PRF vs. Geistlich Bio-Gide®) [27]. In both studies, included subjects were systemically healthy adults with maxillary atrophy (defined as <5 mm residual bone crest height measured in OPG/orthopantomogram). Smoking status was not assessed. The addition of L-PRF to Bio-Oss® bone-substitute revealed neither advantages nor disadvantages over Bio-Oss®-alone controls [26]. After 6 months, clinical and radiographic examinations revealed both groups exhibiting similar amounts and density of mineralized tissues, with no signs of material resorption. Histological evaluations also showed non-significant differences regarding: (a) percentage of newly formed bone, (b) percentage of residual Bio-Oss®, (c) bone-to-bone-substitute contact, and (d) post-treatment inflammatory reactions [26]. Regarding coverage of lateral osteotomy sinus window, L-PRF use resulted in a similar amount of vital bone formation (%) and residual bone-substitute when compared to Bio-Gide® controls (L-PRF: 17.0 and 15.9%, Bio-Gide®: 17.2 and 17.3%, differences are not statistically-significant). Overall, despite a slightly superior to no coverage at all (12.1%), it can be stated that results were similar to those reported using other conventional membranes (collagen: 17.6%; e-PTFE: 16.9%) [27]. Within the presented limitations in both RCTs, evidence suggests that L-PRF is a safe, simple to use and handle, and cost-effective alternative to traditional bone grafts and absorbable membranes; in low-income patients, pursuing maxillary sinus augmentation procedures.

3.5. L-PRF in alveolar ridge preservation

Post-extraction changes in alveolar bone compromise prosthodontic rehabilitation with fixed, removable and/or implant-supported prosthesis. Alveolar Ridge Preservation (ARP) is a technique which involves the use of grafting and barrier materials in order to significantly reduce post-extraction bone loss [28]. L-PRF has been demonstrated to accelerate/enhance bone repair [29, 30], promote fibroblast proliferation [3, 30] and increase vascularity [31]; thereby potentially favoring the post-extraction healing process and the ARP approach. Yet, a single

RCT evaluating the use of L-PRF in ARP was identified, according to the inclusion criteria set herein [32]. This sole study compared the application of L-PRF vs. natural blood clots in post-extraction sockets of third molars. Patients were systemically healthy and non-smoking adults requiring bi-lateral mandibular third molar removal. The use of L-PRF significantly improved post-extraction soft tissue healing after 7 days [32]. Early and significantly-higher radiographic bone formation/maturation was noticed in the L-PRF treated sites vs. controls, at 8 weeks. By 12 weeks, inter-group differences were non-significant. Radiographic bone density (measured by gray scale value) at 12 weeks increased in the biomaterial group compared to controls, nonetheless, the differences were not significant [32]. Similar to other studies, L-PRF reduced early post-surgical pain (VAS scale) on day 1; however inter-group differences were not significant by day 7 [32].

3.6. Personal expertise: L-PRF in periodontally accelerated osteogenic orthodontics

In our own pilot prospective observational study [30] involving a cohort of 11 patients (with informed consent) receiving a Wilcko's modified **PAOO** (Periodontally Accelerated Osteogenic Orthodontics—*a somewhat new surgical procedure which allows faster tooth movement via combining orthodontic forces with corticotomy and grafting of alveolar bone plates*) technique with L-PRF (incorporated into the graft and as covering membrane), accelerated wound healing with no signs of infection or adverse reactions was evident (**Figure 3D**). Post-surgical pain, inflammation and infection were recorded for 10 days post-operatively, while the overall orthodontic treatment and post-treatment stability were followed up to 2 years. In our data analysis, post-surgical pain was found to be either "mild" (45.5%) or "moderate" (54.5%); immediate post-surgical inflammation was recorded as either "mild" (89.9%) or "moderate" (9.1%); and, resolution was marked to begin on day 4 where most patients experienced either "mild" or no inflammation (72.7 and 9.1%, respectively). Interestingly, complete resolution was achieved in all patients by day 8, the average orthodontic treatment time was calculated at 9.3 months and all cases were stable throughout. Thus, we concluded that combining L-PRF with traditional bone grafts (L-PRF plug or block) potentially accelerates wound healing and reduces post-surgical pain, inflammation and infection without interfering with tooth movement and/or post-orthodontic stability, over an extended 2-year observational period; thereby alleviating the need for analgesics and anti-inflammatory medications [30].

4. Conclusions and closing remarks

Tissue regeneration and anatomical reconstruction in defects of the oro-maxillo-facial complex have been always a critical and controversial issue. Both, quality and quantity of the regenerated tissues are important to consider, esthetically and functionally. Practically, the surgeon is faced with an ample collection of regenerative techniques and materials to choose from. *How can one select the "ideal" or "best-fit" strategy and procedure for an optimal clinical outcome? Evidence-based studies? Level of evidence?* To the best of our knowledge, this is the first review of Randomized Controlled Clinical Trials on L-PRF use and application in Oral

Surgery. While the available literature is found to be highly-limited, L-PRF *can be* indicated as an innovative *tool* for contemporary oro-maxillo-facial tissue regeneration and bioengineering. Indeed, existing evidence suggests that L-PRF improves early wound healing and promotes post-surgical bone formation/maturation. However, it is noteworthy that a clearer consensus seems to be present regarding its significant beneficial impact on post-surgical pain and discomfort control, regardless the type of procedure. Unlike its predecessors, new L-PRF preparations tend to function more as biologically-active biomaterials and scaffolds for the delivery of autologous cells, cytokines and growth factors.

Thus, L-PRF should be considered a "living tissue" preparation for natural guided tissue regeneration and not simply a "growth factor-rich" surgical adjuvant. Yet, it is safe to say that this remains an un-explored territory in Dental Biomaterial (Dental Bioengineering) Research, in general.

Our group is currently investigating the potential of incorporating oral-derived mesenchymal stem cells or growth-factor embedded nanoparticles within the L-PRF, as "*super*" or "*smart*" bio-scaffolds, to further boost, with predictability, bone formation, soft tissue healing, treatment time and post-surgical stability, in advanced oro-maxillo-facial surgical procedures such as Periodontally-Accelerated Osteogenic Orthodontics.

Our research extends to investigate the potential of L-PRF in reducing the need for prescription drugs following invasive surgical procedures such as third molar extraction and cysts resections.

Finally, we are vigorously working on characterizing the rheological and biological variations of L-PRF, alongside partnering up with nurses, physicians and dentists to standardize the preparation protocol, for use in other therapeutic indications.

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Conflicts of interest

The author of this review article declares having no conflict of interest of any form or nature with any platelet concentrate product, protocol, technique or company.

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Edited by Raja Kummoona

This valuable book presents clinical experiences and research of bone grafting. Bone grafting is an essential technique practiced by craniofacial, maxillofacial, orthopedic, neuro, reconstructive and oral surgeons.

Bone grafting can be used for reconstruction and restoring missing bone in trauma and tumor surgery of the facial bone or in road traffic accidents with multiple injuries and in post-traumatic missile war injuries to the face or limbs.

Bone grafts, in the form of Kummoona Chondro-Ossous graft or Costo-Chondral graft, are used for reconstruction of damage TMJ for restoration of growth, function, and repair. Bone grafting is a surgical procedure where the iliac crest or rib or tibia is used to perform grafting.

In this book, we examine the experimental studies on rabbits to understand the cellular changes associated with bone grafting. From this, we noticed that mesenchymal stem cells and growth factor are released from platelets and these play an important role in healing the bone graft.

We recommend this valuable book to all cranio-maxillofacial, orthopedic, plastic, reconstructive, neuro and oral surgeons and to all postgraduate students studying bone grafting.

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