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Gingival Diseases

Their Aetiology, Prevention and Treatment

Edited by Fotinos S. Panagakos and Robin M. Davies

GINGIVAL DISEASES – THEIR AETIOLOGY, PREVENTION AND TREATMENT

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Notice

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Meet the editors

Dr. Panagakos received his DMD from UMDNJ-New Jersey Dental School and his PhD in Biochemistry and Molecular Biology from UMDNJ-Graduate School of Biomedical Sciences in 1992. In 1999, he received his Masters in Education from Seton Hall University and in 2007 he received his Masters in Business Administration from Lehigh University. Dr. Panagakos served for 14 years as a faculty member at New Jersey Dental School, serving in a number of administrative positions. In June, 2005, Dr. Panagakos joined the Colgate Palmolive Company. Currently, Dr. Panagakos is the Director of Clinical Research Relations and Strategy within the Research and Development division of Colgate-Palmolive, and is based in Piscataway, NJ, USA.

Dr. Robin Davies recently retired from full time academics and research at the Univeristy of Manchester, UK dental school. During his time at the school, Dr. Davies was a Professor of Clinical Dental Research, and director of the Colgate Palmolive Company Dental Health Unit. Dr. Davies was active in clinical and basic research in periodontology and inflammation, and has published over 1000 research articles, and has made presentations at numerous international dental meetings. More recently, Dr. Davies has served as the Director of National Fluoride Information Centre in the UK, and continues to work with the Colgate Palmolive Company as a consultant.

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Preface

Gingival diseases are a family of distinct pathological entities that involve the gingival tissues. The signs and symptoms of these diseases are so prevalent in populations around the world that they are often considered to be "normal" features. Many attempts have been made to classify gingival diseases, the most recent being that by Mariotti (1999). The diseases are now classified into two main groups namely: Plaque-Induced and Non-Plaque Induced Gingival Diseases. The Plaque-Induced lesions are influenced by a range of factors such as systemic diseases, medications and malnutrition and non-Plaque Induced lesions may be the result of specific bacterial, viral and fungal infections, trauma and dermatological diseases. All these factors have been considered in developing the new classification. Although gingival lesions may occasionally be painful in most instances the signs and symptoms are not perceived by the individual as other than an inconvenience. Gingival lesions do not pose an immediate threat to the dentition and usually respond to relatively simple measures performed by the dental professional and improved levels of oral hygiene performed by the individual at home.

Whilst gingival lesions are essentially reversible if left untreated they can progress to irreversible loss of the periodontal tissues (periodontitis) and threaten the life of the natural dentition. Recent studies have implicated gingival lesions as playing a role in various systemic conditions such as heart disease and have consequently received more attention than before.

This book provides dentists, dental hygienists, dental therapists and students with a comprehensive review of gingival diseases, their aetiology and treatment. We hope that the reader finds this book and its contents a worthy addition to his or her medical and dental library.

Dr. Fotinos S. Panagakos and Robin M. Davies
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Part 1

Gingival Tissues and Plaque-Associated Gingival Disease

1

The Anatomy and Physiology of the Healthy Periodontium

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Stony Brook University
USA

1. Introduction

The anatomy and physiology of the healthy periodontium will be described in its relationship to the natural dentition, jaws, and the oral environment. The periodontium serves as the supporting apparatus for the teeth in function and in occlusal relationships. It consists of the alveolar mucosa, gingiva, cementum, periodontal ligament, and alveolar bone. The embryonic origin, composition, and histological and clinical appearance with normal physiologic variations are presented in order to facilitate an understanding of their relationships in health and to understand the processes that occur in pathology. This will include macroscopic, microscopic, and radiographic details of the components of the periodontium. The knowledge of the details of the tissue compartments, the cells which are involved, and how the cellular products and the cells interact will provide a greater understanding of the functional operation of the periodontium. The response to aging and the normal maturation of the periodontium will also be discussed in relation to its macroscopic, microscopic, and radiographic changes to enhance the appreciation of the changes in the appearance, properties, and responses to functional and physiologic stimuli. A thorough understanding of the components that form the supporting structures of the teeth will provide the necessary starting point from which to establish an appreciation of the interactive and adaptive nature of the system as well as a reference point of how the periodontium changes when pathologic, normal and excessive physiologic, and inflammatory stimuli stress the components.

2. Macroscopic appearance of the periodontium

The periodontium is composed of the gingiva, alveolar mucosa, cementum, periodontal ligament, and alveolar bone (Fig. 1). These components serve to support the teeth in their alveolar bone. The tissues typically seen on clinical inspection are only those of the oral mucosa. The oral mucosa can be divided into three types: the masticatory, lining, and specialized mucosa. The gingiva is firmly bound to the underlying bone and is continuous with the alveolar mucosa that is situated apically and is unbound. The border of these two tissue types is clearly demarcated and is called the mucogingival junction. There is no mucogingival junction on the palatal aspect of the maxilla as the gingiva is continuous with the palatal mucosa. The gingiva consists of a free gingival margin and attached gingiva. The free gingival margin is situated about 2mm coronal to the cementoenamel junction of the tooth and the attached gingiva extends from the base of the free gingiva to the mucogingival

junction (Ainamo and Loe 1966). The gingiva is typically coral pink in color, but may vary due to physiologic pigmentation among some races, whereas the alveolar mucosa is deep red in color (Fig2a/b).

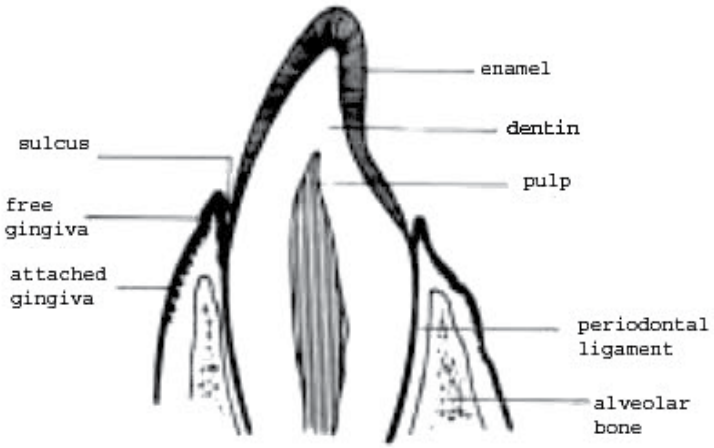
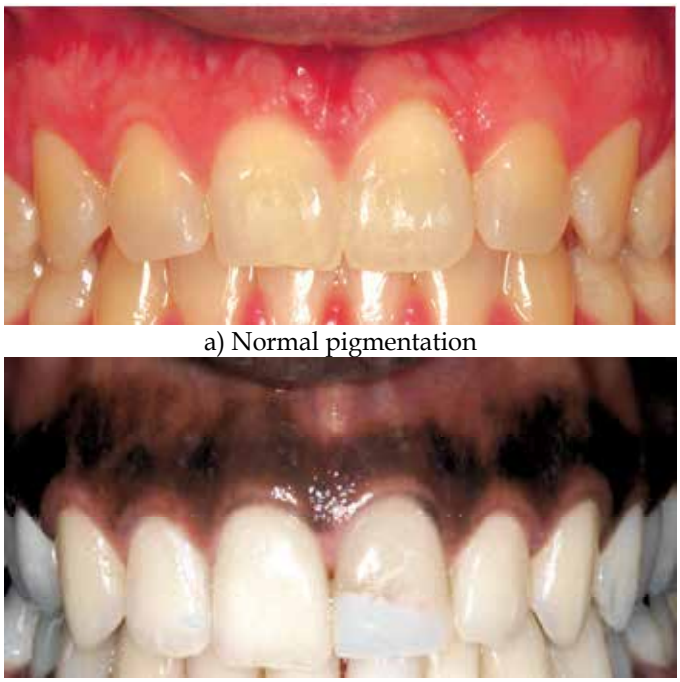


Fig. 1. Components of the periodontium (Garant 2003)



a) Normal pigmentation

b) Physiologic pigmentation

Fig. 2. Normal variation in the appearance of the gingival tissues

The tissue that resides in the interproximal embrasure is called the interproximal papilla. The shape of this tissue is influenced by the shape of the interproximal contact, the width of the

interproximal area, and the position of the cementoenamel junction of the involved teeth. The shape of this papilla varies from triangular and knife-edge in the anterior regions due to point sized contacts of the teeth to broader and more square shaped tissue in the posterior sextants due to the teeth having broad contact areas. Also present in the wider papillary areas is the col. This is a valley-like structure situated apical to the contact area (Fig 3).

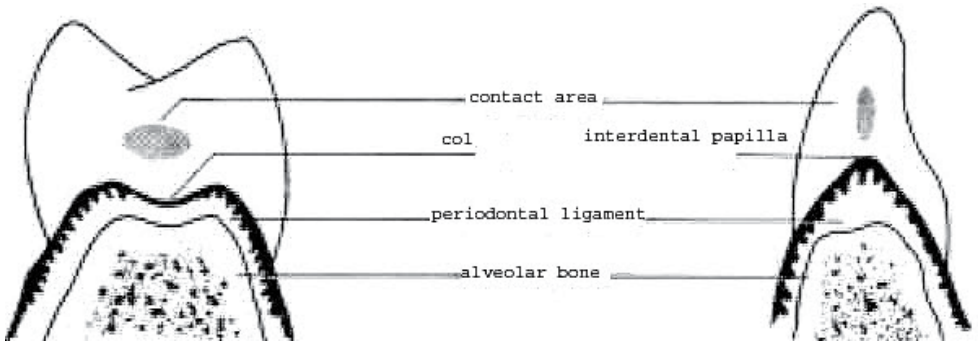


Fig. 3. Interdental tissue shapes (Garant 2003)

The texture of the gingiva varies with age and is typically smooth in youth, stippled in adulthood, and again becomes smoother with advanced age. Stippled tissue has a texture similar to the rind of an orange and its presence does not necessarily mean health. (Fig 4). Another feature that does not appear in all of healthy periodontiums is the free gingival groove. The free gingival groove is a depression that appears in about 50% of population. The groove appears at the border of the free and attached gingiva and usually represents the base of the gingival sulcus. The gingival sulcus is the invagination around a tooth bounded by the free gingival margin.

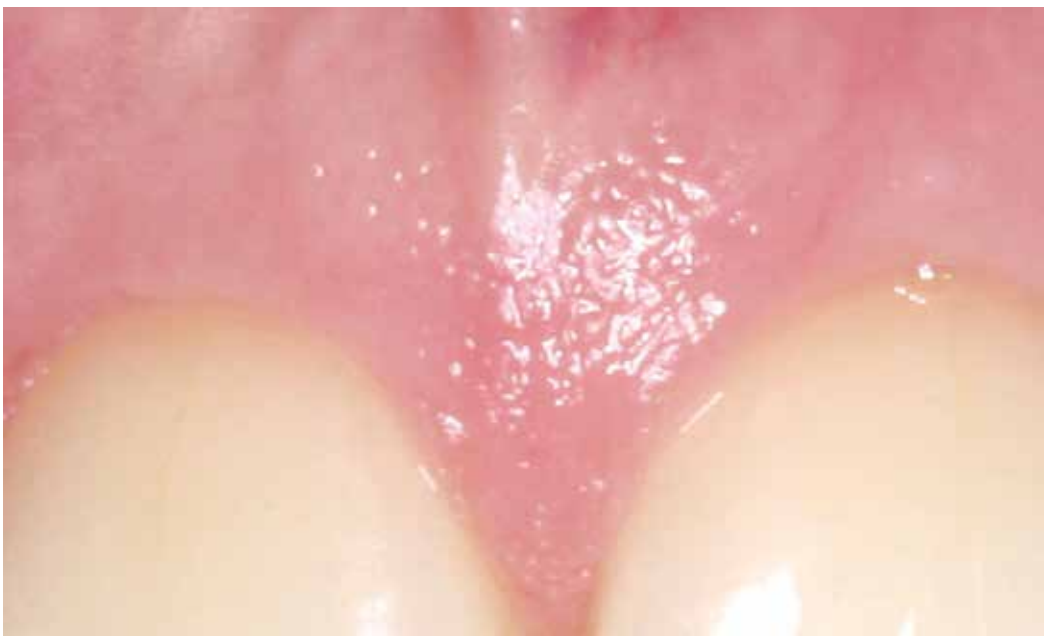


Fig. 4. Stippling of gingival tissue.

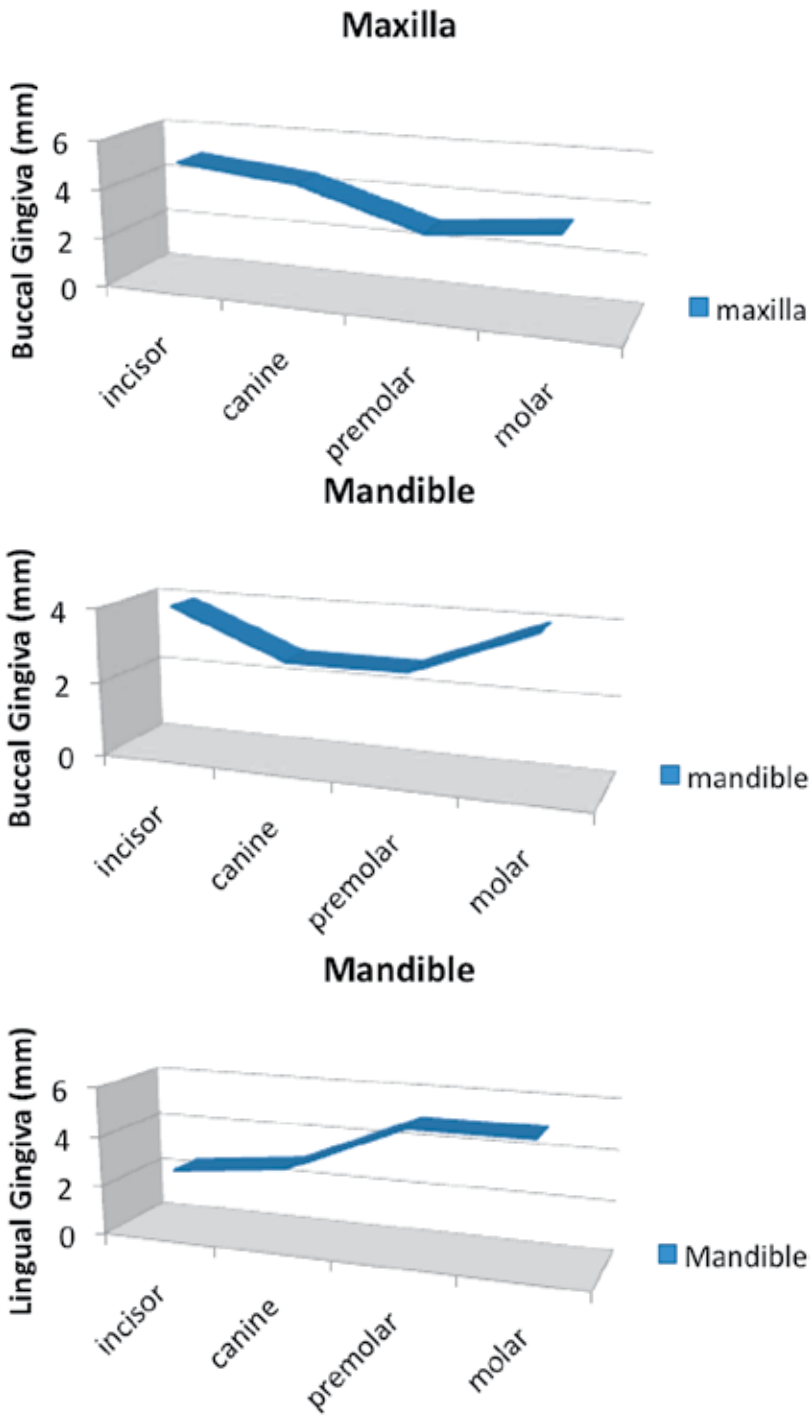


Fig. 5. Width of attached gingiva in specific areas

When a periodontal probe is placed into this space, a measure may be recorded which is very useful for diagnosis. Studies have shown an average depth of 0.7mm but variations may range from 0 to 6mm (Gargiulo 1961)). The width of the attached gingiva varies with the location in the oral cavity as well as with physiologic age. The facial gingiva is typically widest in the incisor region and narrowest in the premolar region for the maxillary arch and ranged from 1-9mm. In the mandible, the facial attached gingiva is narrowest in the premolar and canine regions (Bowers 1963). When the lingual attached gingiva was examined, it was found that the widest areas were on the mandibular molars and the narrowest were on the incisor and canine regions, about 1.8mm (Voigt 1978) (Fig. 5). There is a general increase from the primary to permanent dentition as well as with increasing age (Ainamo and Talari 1976)(Fig. 6).

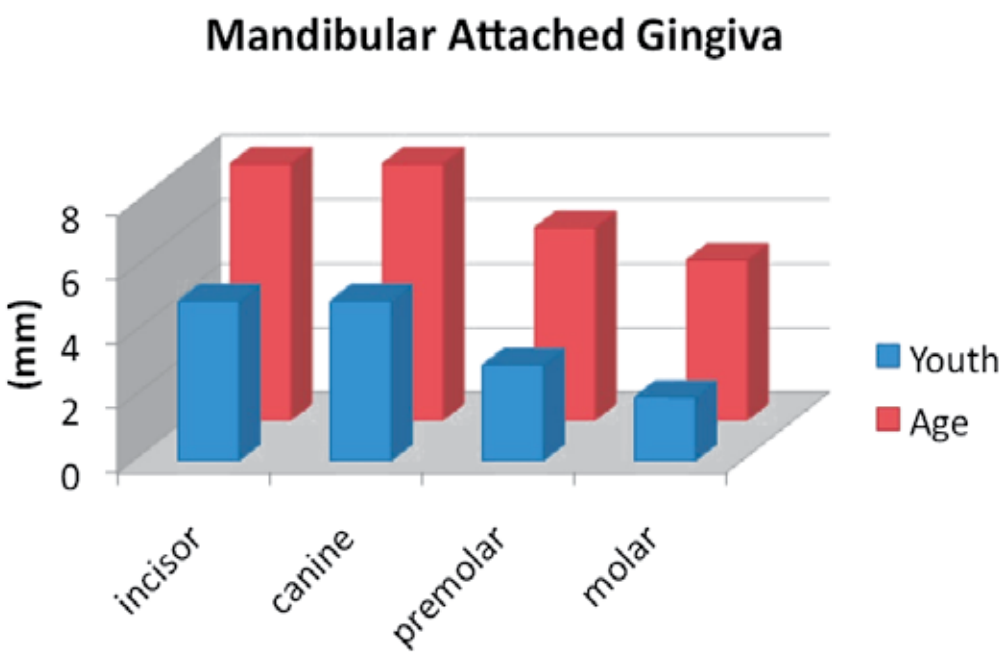


Fig. 6. Changes in the amount of attached tissue with age.

Using radiographs one can visualize several of the components of the periodontium as well as their size and relation to the teeth (Fig. 7). Although the radiograph is a two-dimensional depiction of a three-dimensional object, the location of the alveolar bone crest relative to the cementoenamel junction is seen along with the space occupied by the periodontal ligament. Since the periodontal ligament itself is not a mineralized tissue, the radiograph will show a radiolucent area that it occupies. The cortical bone that houses the teeth is known as the lamina dura. The alveolar bone also follows a path that parallels the positions of the cementoenamel junctions of teeth (Ritchey and Orban 1953). In health the interdental bone is 1.0mm from the cementoenamel junction and increases with age to 2.8mm (Gargiulo 1961).

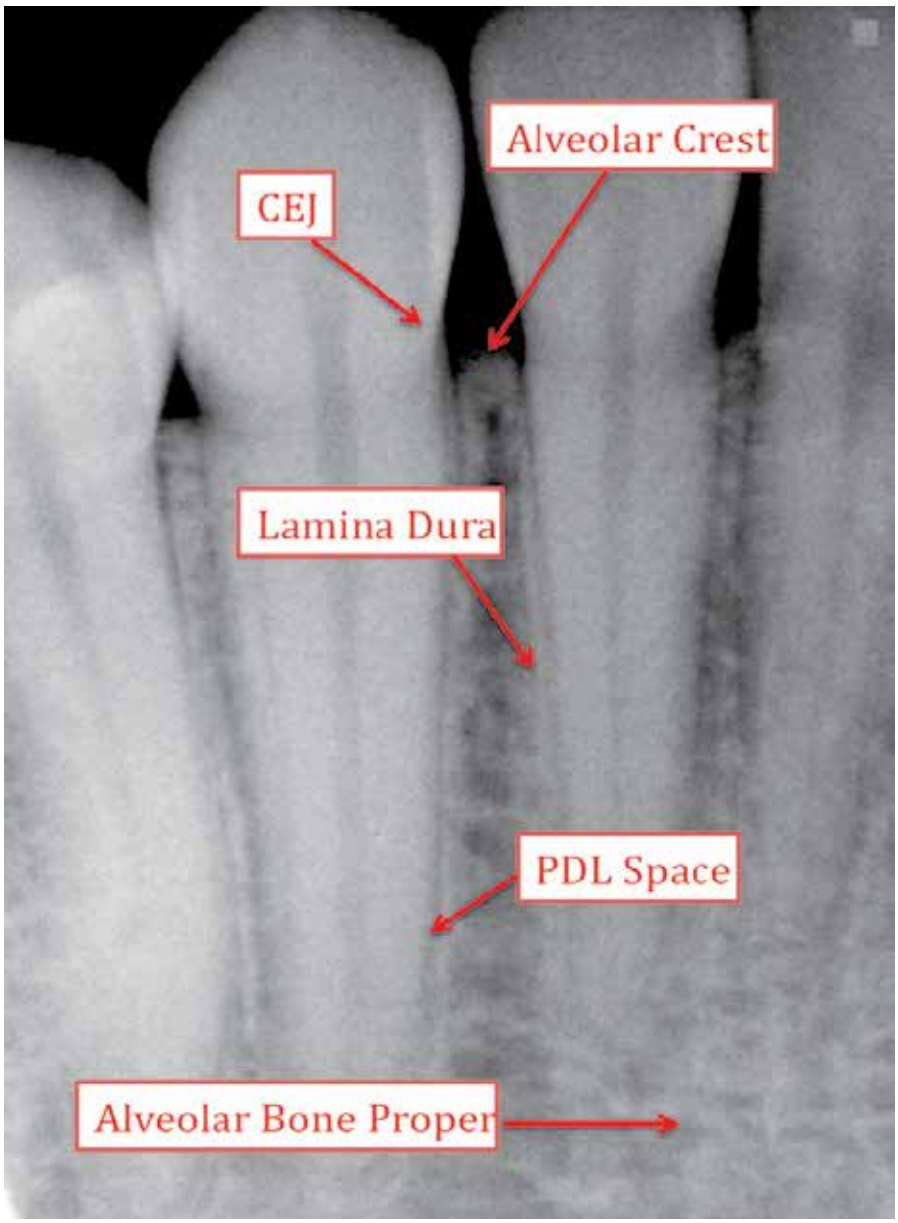


Fig. 7. Appearance and location of periodontal components on a periapical radiograph

3. Microscopic appearance of the periodontium

Greater detail of the periodontium is obtained histologically. The schematic cross-section of the periodontal attachment and components is seen in Fig. 1. The components are again the alveolar bone, gingiva, periodontal ligament, and cementum. The gingiva consists of a surface epithelium and underlying connective tissue termed the lamina propria. There are three types of epithelium present, the oral, sulcular, and the junctional epithelium. The oral

epithelium is continuous with the epithelium of the oral cavity. The sulcular epithelium is adjacent to the tooth but not connected or attached to the tooth surface. The junctional epithelium is at the base of the sulcus and is in direct contact with the tooth (Carranza 2002)(Fig. 8).

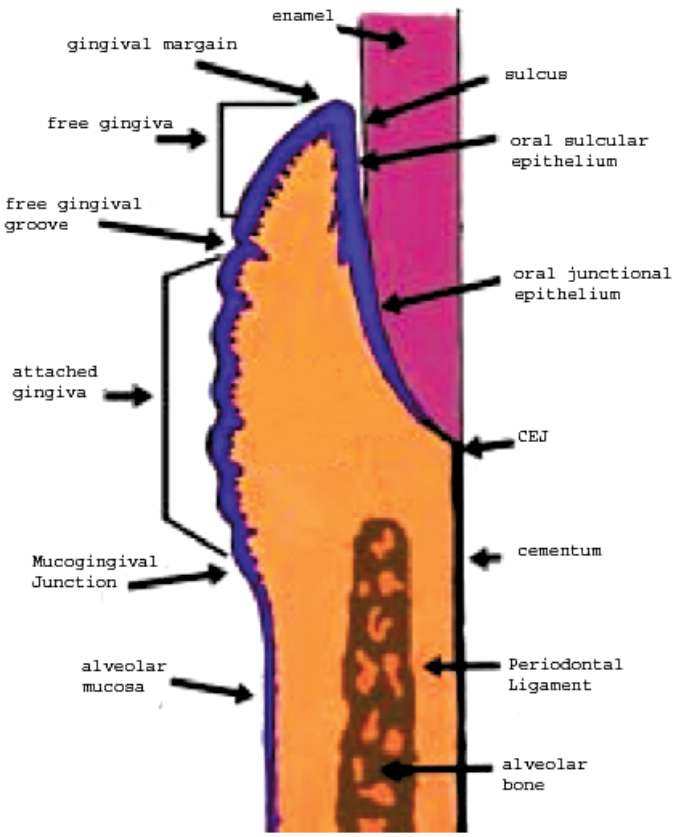


Fig. 8. Types of epithelium in the periodontium (Garant 2003)

The border of the connective tissue and epithelium is undulating (Fig. 9). These epithelial extensions are known as epithelial ridges or rete pegs. The connective tissue layer is also termed the lamina propria or the dental papillae. In health, this is a characteristic finding in the attached gingiva, but are absent in the sulcular and junctional epithelium.

The gingival epithelium is quite similar to the epidermis in its structure. The gingiva consists of keratinized, stratified, squamous epithelium. The major cell type is the keratinocyte. There are four distinct layers; the stratum basale, stratum spinosum, stratum granulosum, and the stratum corneum (Fig. 10). The stratum basale or basal layer consists of one to two layers of cells cuboidal in shape. These are the most undifferentiated of the cells and serve to replenish cells as they are shed during their maturation and exfoliation. The basal cells are immediately adjacent to the connective tissue from which it is separated by a basement membrane. The basement membrane consists of a two zones, the lamina lucida and lamina densa. The lamina lucida contacts the cell surface and has many hemidesmosomes. Hemidesmosomes are specialized structures that connect an epithelial

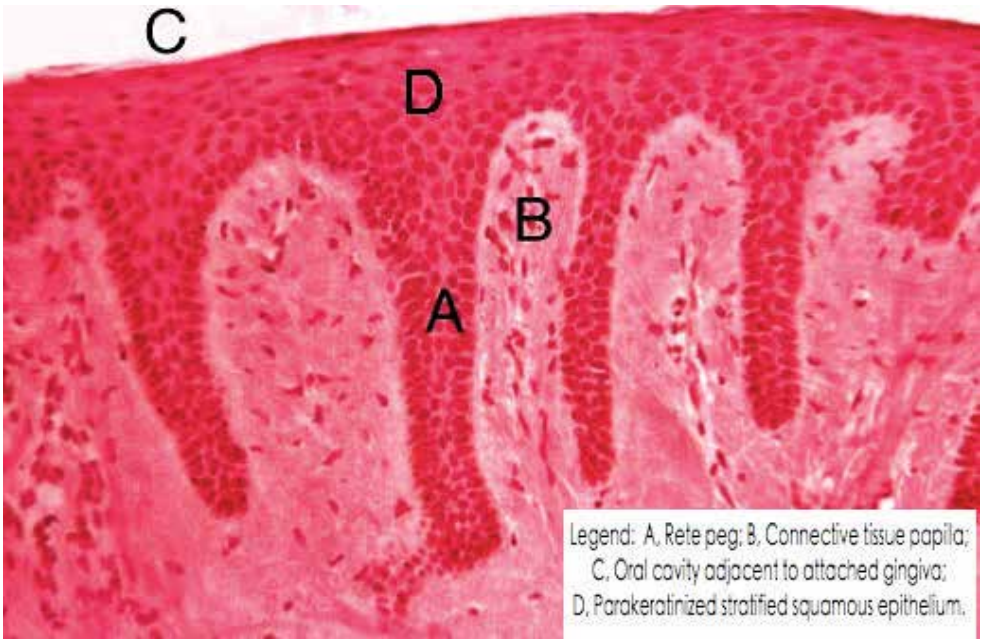


Fig. 9. Rete pegs (Garant 2003)

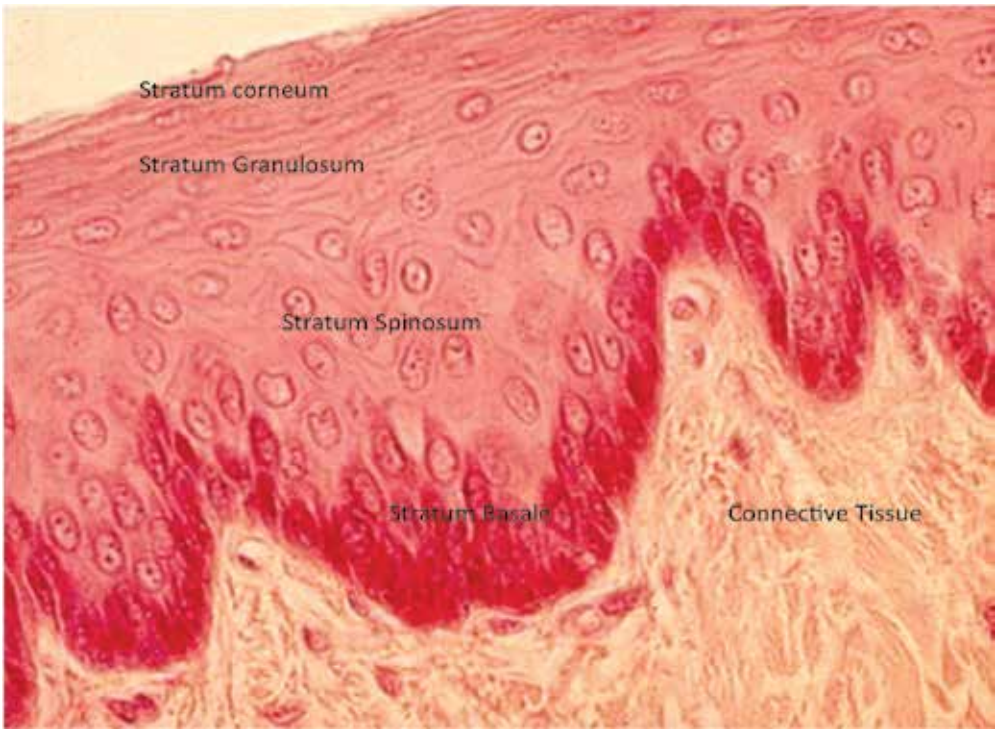


Fig. 10. Cellular layers of epithelium (Garant 2003)

cell to the basement membrane. In the lamina densa, anchoring fibrils formed from Type VII collagen bind to the Type I and III collagen of the extracellular matrix (Listgarten 1972; Schroeder 1997).

The stratum spinosum consists of larger cells with cytoplasmic processes that resemble spines. There are typically 10-20 layers of cell in this stratum. The cells are bound to each other by desmosomes, which are in essence pairs of hemidesmosomes. The cells contain many keratin filament bundles known as tonofibrils. Other cells found in this layer include melanocytes, Langerhan's cells and Merkel cells. Melanocytes produce the pigment melanin which is contained in granules. Langerhan's cells are part of the immune system and serve as antigen presenting cells. The Merkel cells are responsible for the perception of sensation.

In the stratum granulosum, keratohyalin bodies and tonofibrils are seen extensively. As the cells proceed from the basal layer and reach the stratum granulosum, a dramatic decrease in organelles can be observed. The stratum corneum is seen abruptly after the stratum granulosum. It consists of layers of flattened cells that may exhibit different patterns of keratinization depending on location and external stimuli (Carranza 2002).

This keratinization process as the cells mature through the layers is considered differentiation. Orthokeratinized cells are flattened and have no discernible nuclei and cyptoplasmic organelles. Parakeratinized refers to cells that exhibit incomplete keratinization and cells that contain remnants of nuclei and cellular organelles. The location most keratinized is the palate, followed by the gingiva and tongue, and finally the buccal mucosa(Miller 1951). The degree of keratinization of the oral mucosa generally decreases with age and with the onset of menopause (Papic 1950). The sulcular epithelium is thin, non-keratinized epithelium.

In health, the depth of the sulcular epithelium is less than 3mm and ends at the cornoal surface of the junctional epithelium. Cadaver studies found the depth of the sulcus to be an average of 0.69mm (Gargiulo 1961). Rete pegs are not present in the sulcular epithelium. The junctional epithelium contains cells that are directly attached to the tooth surface. An internal basal lamina attaches the cells to the tooth surface through hemidesmosomes and an external basal lamina attaches the cells to the underlying connective tissue. Early in life it typically consists of a few stratified squamous cell layers, but with age the number of layers increases to between 10-20. The average width of the junctional epithelium is 1mm (Gargiulo 1961). The junctional epithelium also has wide intercellular spaces and functions as a permeable barrier. This is an important property since it acts as a semi-permeable barrier through which bacteria and their components and byproducts may pass into and invade the tissue. It also facilitates the passage of leukocytes (e.g. neutrophils) and immune components (e.g. complement), enzymes, and gingival crevicular fluid. Gingival crevicular fluid is a modified inflammatory exudate produced that resembles serum. The col areas share similar characteristics to the junctional epithelium. These areas are also non-keratinized and have a high level of turnover (Garant 2003). In summary, the junctional epithelium differs from the oral epithelium in having cells of smaller size, larger intercellular spaces, and fewer desmosomes.

Beneath the epithelial layer is a connective tissue layer also known as the lamina propria. This layer is composed of a papillary and a reticular layer. The papillary layer is adjacent to the basal cells of the epithelium and their rete pegs. The reticular layer is adjacent to the underlying alveolar bone. Collagen Type I is the predominant component of the lamina propria. Also residing in this layer are cells, nerves, blood vessels, and ground substance. The cells present are fibroblasts, mast cells, and immunologic cells. Mast cells contain vesicles with vasoactive substances such as histamine and proteolytic enzymes. Once

activated by stimuli, the cells can degranulate and induce changes in blood flow to the area and increase tissue permeability. The immunologic cells present are macrophages, neutrophils, lymphocytes, and plasma cells. These cells are present to initiate and maintain a response to a foreign substance or cell present in the area. Ground substance is a gel-like substance composed of glycosaminoglycans and proteoglycans. These substances cause a large amount of water retention which maintains the shape and structure of the area when force is applied. This substance also serves as a medium for the transportation of electrolytes, nutrients, and metabolites (Rose 2004).

The fibroblasts are the predominant cells and function to synthesize collagen and extracellular matrix. These cells are elongated and elliptical in shape and their microscopic appearance is characteristic of a cell producing large amounts of cellular products, a well-developed rough endoplasmic reticulum and Golgi apparatus, and many mitochondria. Collagen is formed by both an intracellular and extracellular process. Intracellularly tropocollagen, the smallest unit of collagen, is produced. Tropocollagen consists of three polypeptide chains of 1000 amino acids in an α-helical formation and is 3000 Å long and has a 15Å diameter. A significant percentage of the amino acid composition is glycine, proline, and hydroxyproline. The later is unique to collagen and when assayed can be used to determine the amount of collagen in the sample. The tropocollagen is excreted into the extracellular environment, where the remainder of the formation takes place. Tropocollagen is arranged into protofibrils and then collagen fibrils. The fibrils are then bundled together

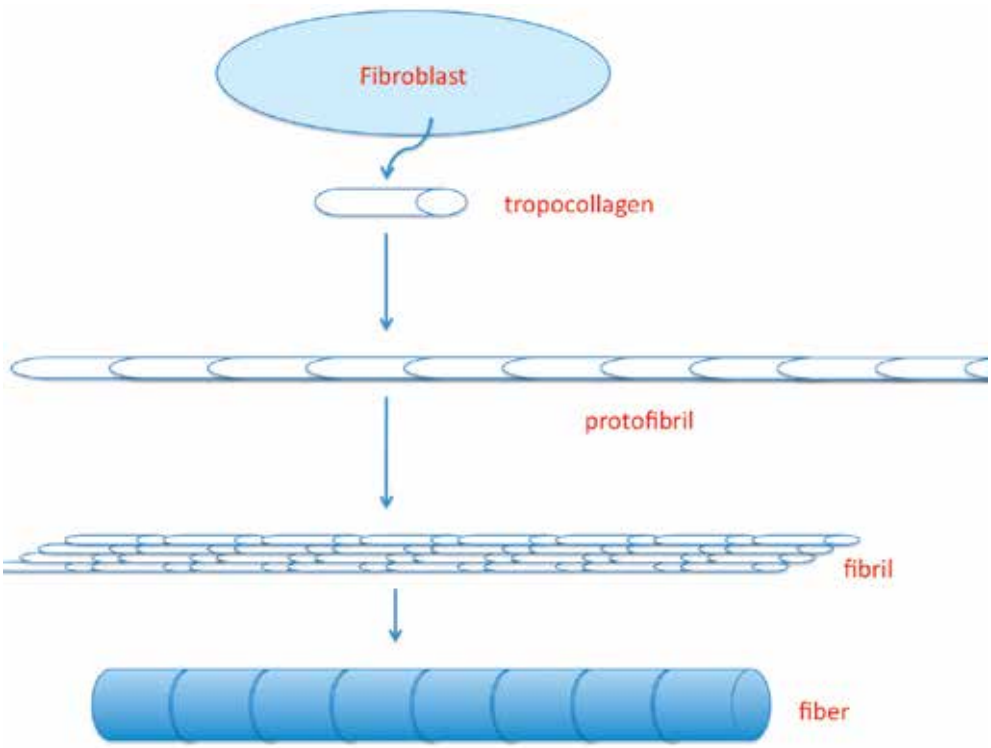
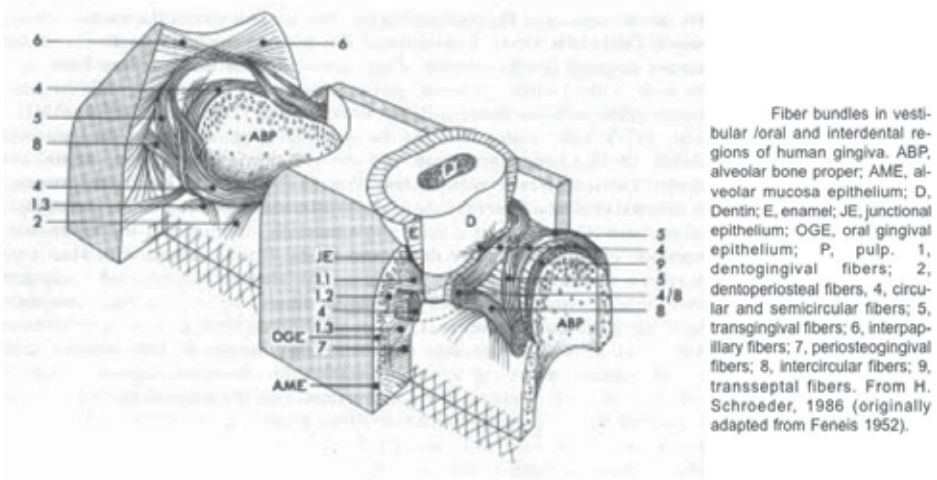


Fig. 11. Formation of collagen. (top to bottom) (tropocollagen, protofibril, collagen fibril, and collagen fiber)

and collagen fibers are created with a typical cross-banding pattern of 700 A. (Fig 11). As collagen matures and ages it develops greater cross-linking making the collagen less soluble and resistant to breakdown (Rose 2004).
Most collagen present in the gingiva and connective tissue is irregularly arranged but some distinct arrangements of fibers can be observed. These include the dentogingival, circular, and transseptal group. The dentogingival group fibers may run from the root surface to the periosteum of the bone, from the root surface to the gingiva, and from the alveolar bone to the gingiva. Circular fibers run circumferentially around the tooth within the gingiva and do not touch the tooth itself. The transseptal group runs from the root surface of one tooth to the root surface of another tooth transversing the alveolar bone (Garant 2003) (Fig. 12).



Fiber bundles in vestibular /oral and interdental regions of human gingiva. ABP, alveolar bone proper; AME, alveolar mucosa epithelium; D, Dentin; E, enamel; JE, junctional epithelium; OGE, oral gingival epithelium; P, pulp. 1, dentogingival fibers; 2, dentoperiosteal fibers, 4, circular and semicircular fibers; 5, transgingival fibers; 6, interpapillary fibers; 7, periosteogingival fibers; 8, intercircular fibers; 9, transseptal fibers. From H. Schroeder, 1986 (originally adapted from Feneis 1952).

Fig. 12. Gingival fiber groups. (Garant 2003)

The periodontal ligament is the connective tissue that connects the tooth to the alveolar bone. The periodontal ligament serves to allow forces to be distributed to the alveolar bone during mastication and occlusal function. The majority of the volume of the ligament is occupied by dense connective tissue and the minority by loose connective tissue with neurovascular structures. Cells present in this tissue include osteoblasts, cementoblasts, osteoclasts, multipotent stem cells, epithelial remnants, and fibroblasts, which are the most abundant (Carranza 2003) Since the periodontal ligament contains such a variety of cells, it plays an important role in healing and repair. This potential is also a focus for to periodontal regenerative procedures (Melcher 1976). The ligament is about 0.15mm to 0.25mm in width and has an hourglass shape with the mid root level having the narrowest width. The width of the ligament can adapt to forces by decreasing in lowered function and a widening of the ligament with increased occlusal load or hyperfunction. With age there is a decrease in vascularity, cell mitotic activity, fiber number and in fibroblasts there is a slight decrease in widthn (Van der Velden 2004).
With root development principle fibers, which are collagenous bundles, insert their terminal ends into the root cementum and alveolar bone and are termed Sharpey's fibers or periodontal ligament fibers. These collagen fibers are produced by fibroblasts, chondroblasts, osteoblasts and other cells in a manner described previously. The fibers are

typically Type I collagen. The fibers can also be arranged by their position and orientation. The six groups are the transseptal, horizontal, alveolar, oblique, apical, and radicular groups (Fig. 13). The transseptal group extends from the cementum of one tooth over the interseptal bone to the cementum of an adjacent tooth. The horizontal group attaches the cementum to the alveolar crest and run perpendicular to the root and alveolar surfaces. The alveolar group attaches the cementum to the alveolar bone and originates apical of the cementoenamel junction. The oblique group constitutes the majority of the fibers and run obliquely from the root cementum to the alveolar bone. These fibers provide support from intrusive forces from mastication. The apical fiber group emerges from the cementum near the apex of the root and connects to the alveolar bone. The radicular group is seen in multirooted teeth near the furcation and connects the cementum of that area to the neighboring bone. Other fibers present include oxytalan fibers, which run parallel to the root surface vertically, and elaunin fibers that are similar to immature elastic fibers (Rose 2004; Garant 2003).

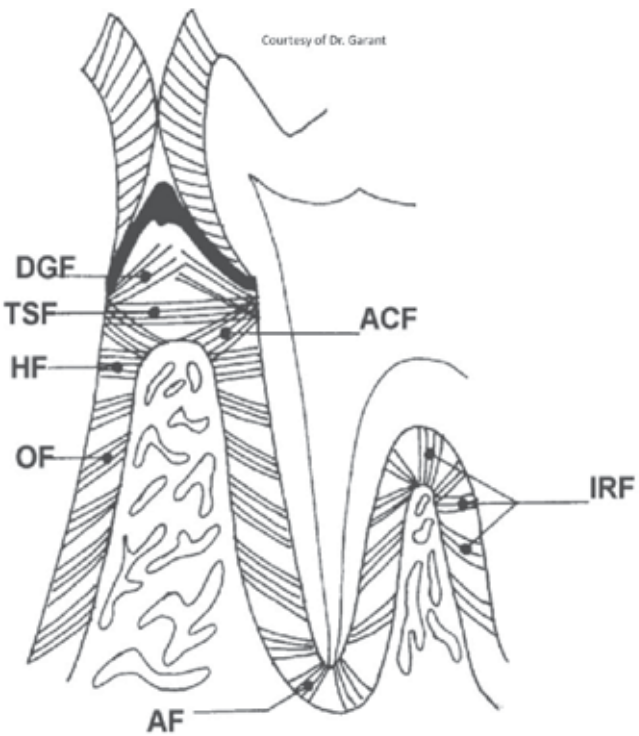


Fig. 13. Periodontal fiber groups (Garant 2003)

The cementum is a mineralized tissue covering the anatomic root of the tooth. Cementum is avascular and has no direct innervation. It is made of collagen fibers within a mineralized matrix. Fibers present in the cementum may be classified as extrinsic or intrinsic. The extrinsic fibers are created by the fibroblasts in the periodontal ligament and the intrinsic fibers are produced by cementoblasts. The mineralized matrix is composed of mainly hydroxyapatite [$Ca_{10}(P0_4)_6(OH)_2$]. Cementum has some characteristics that are both

biochemically and physically similar to bone due to its composition. Cementum is continuously deposited throughout life and the apical third of the root typically has the thickest deposition. In doing so, the deposited cementum compensates for the eruption of teeth from attrition. The thickness of the cementum varies from 15 to 150 microns depending on the location on the root and age of the patient. There is some permeability of cementum to organic substances, ions, and bacterial products. Typically the permeability of cementum diminishes with age. The extent of cementum coronally exhibits different patterns (Fig. 14). In most instances the cementum overlaps the enamel (~60%), and less frequently it has a butt-joint (~30%), and least frequently it ends short of the enamel (5-10%). This anatomical variation among the position the enamel and cementum board is clinically relevant when gingival recession occurs and patients may present with exposed dentin and root hypersensivity (Carranza 2003).

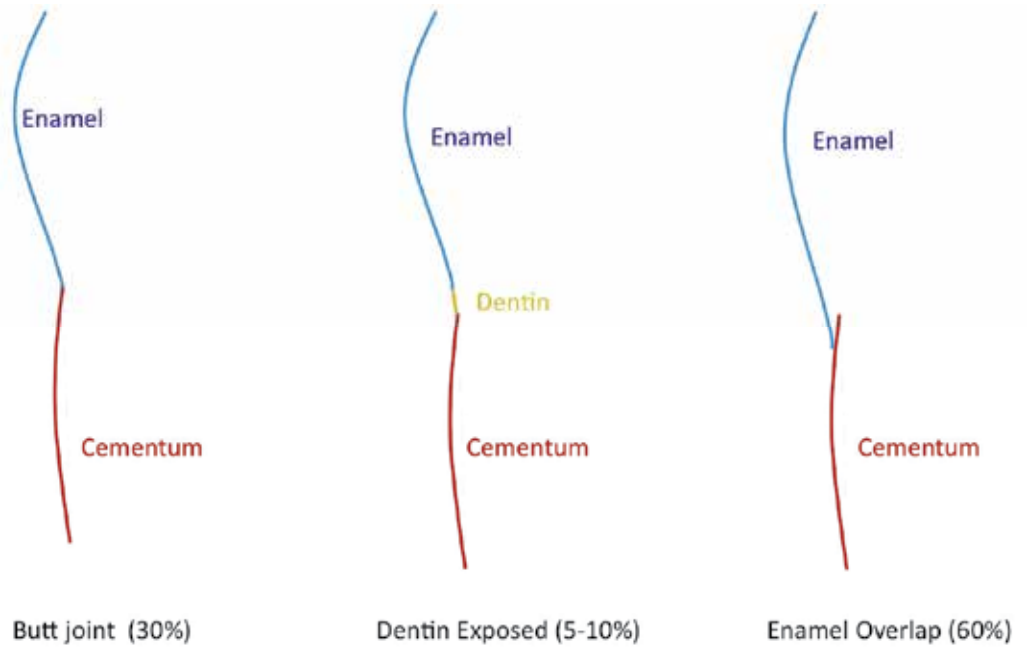


Fig. 14. Configurations of dentin, cementum, and enamel at the cementoenamel junction.

Cementum is characterized into acellular and cellular types. Acellular afibrillar cementum is located near the coronal aspect of the root and has no cells and no extrinsic or intrinsic collagen fibers within it. Acellular extrinsic fibrillar cementum is found in the middle and coronal parts of the root and lacks cells. This type of cementum has Sharpey's fibers, collagen fibers that attach from the cementum to the alveolar bone. Cellular mixed stratified cementum is present in the apical third and in the area of furcations. It also contains Sharpey's fibers and intrinsic fibers. Cellular intrinsic fibrillar cementum has cementocytes, which are cementoblasts trapped within the mineral they deposited, and does not contain extrinsic collagen fibers (Garant 2003).

The alveolar process is the osseous tissue of the maxillary and mandibular jaws which houses and supports the sockets of the teeth. The process consists of an external cortical

plate, the inner socket wall known as the alveolar bone proper and is compact bone, and a cancellous trabecular bone in between the two boney layers. The bone is typically thicker in the palatal and lingual areas when compared to the buccal areas. Some areas may present with defects known as dehiscences and fenestrations. Dehiscences are areas were bone has been lost on a root surface and the root is only covered by periosteum and gingiva. Fenestrations are small areas or "windows" where bone has been lost on a root surface and is only covered by periosteum and gingiva. (Fig. 15). These defects were shown to occur in about 20% of all teeth. Dehiscences were more prevalent in the mandible, whereas fenestrations are more common in the maxilla (Elliot and Bowers 1963). Some areas may be predisposed to these defects by having teeth with prominent root morphology, dental crowding, and a position extending beyond the dental arch. These areas become crucial if periodontal disease occurs or if gingival recession takes place since they may complicate therapy and adversely affect the area's prognosis.

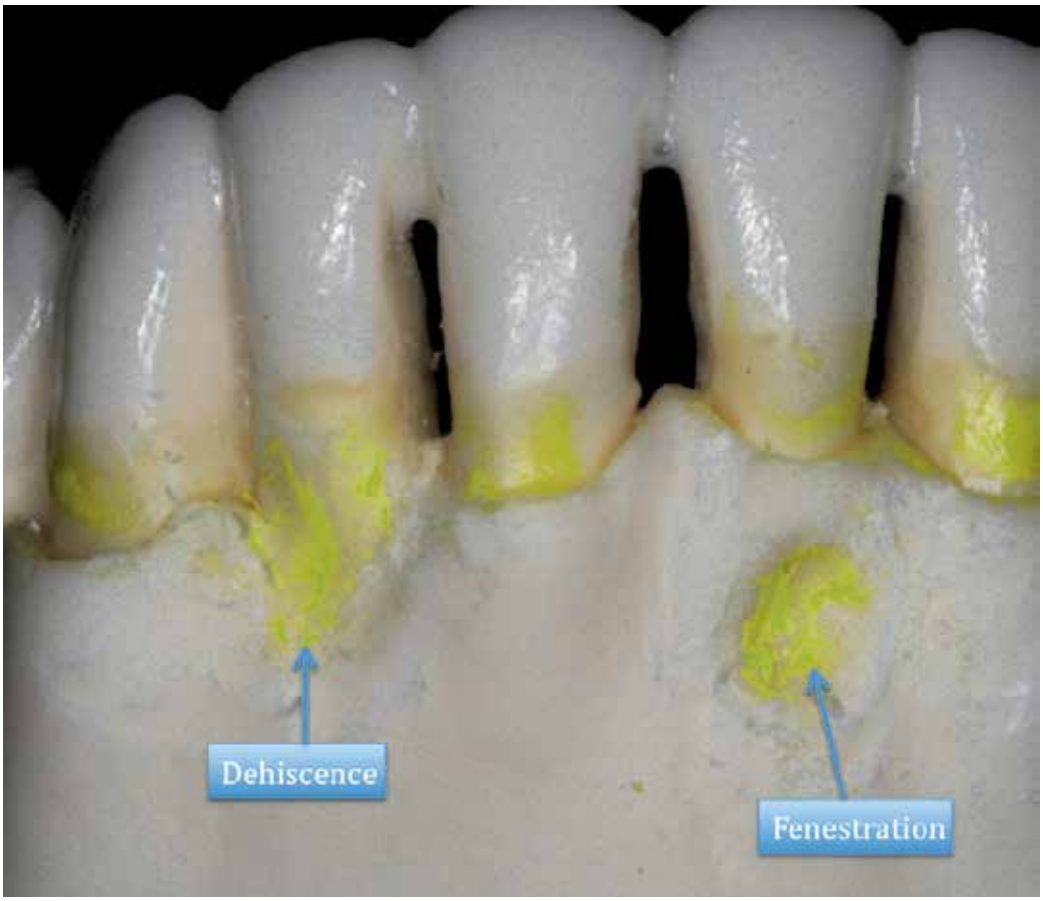


Fig. 15. Diagram of fenestration and dehiscence defects.

The alveolar bone proper is cribiform in appearance and this allows for a connection to the neurovascular structures. The bone is created by osteoblasts during development (modeling) and is constantly remodeled throughout life from the intricate osteoblastic/osteoclastic

relationship. The osteoblasts produce collagen, glycoproteins and proteoglycans to produce the bone matrix that is then mineralized with calcium and phosphate. The mineral is hydroxyapatite and the mineral content is about 60%. When osteoblasts have layed down osseous tissue they become trapped within the tissue and are termed osteocytes. Osteocytes reside in lacunae and connect and communicate with each other through canaliculi. A group of osteocytes surround themselves around the neurovascular bundles (Haversian canals) and are termed osteons. An osteon is the fundamental unit of compact bone and are cylindrical structures. Volkmann's canals which run within osteons, carry nerves and blood vessels, and are perpendicular to the Haversian canals (Fig. 16). An analogy can be that the Haversian canals are elevators of a tall building and the Volkmann's canals are hallways on specific floors. Cancellous bone consists of trabeculae and has irregular marrow spaces. Cancellous or trabecular bone is found interdentally. The bone quality of the maxilla and mandible are generally different and overall the maxilla has more cancellous bone compared to the mandible (Sodek 2000; Rose 2004; Carranza 2003).

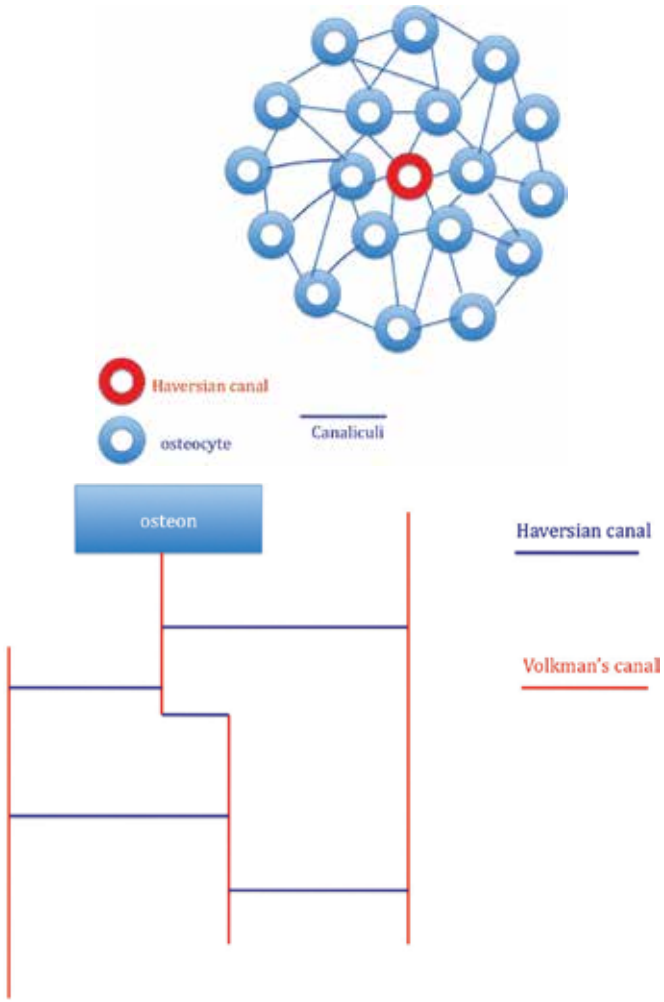


Fig. 16. Microscopic configuration of osseous tissue. A)osteon B)vascular configuration

Osteoclasts are derived from monocytes and resorb bone by a specialized feature called a ruffled border. This structure seals off an area and then vesicles stored within the osteoclast are released to cause the breakdown. These enzymes include acid phosphatase, cathepsins, and matrix metalloproteinases. The interaction between the osteoclasts and osteoblasts is regulated by the RANK pathway (receptor activator of nuclear factor-κβ) which is a balance of the ligand for RANK (RANKL) and a competitive inhibitor osteoprotegerin (OPG). The creation of RANKL involves osteoblasts themselves which activate precursor cells to differentiate, in the presence of macrophage colony stimulating factor, to become osteoclasts. The outer surface of bone is covered by layers of connective tissue called the periosteum. The periosteum contains osteoblasts, stem and progenitor cells, fibroblasts, and vascular and nervous tissue. The inner layer of bone is lined with endosteum which is comprised of connective tissue containing osteoblasts (Garant 2003).
The periodontium's blood supply is derived from the superior and inferior alveolar arteries. These arteries produce branches that extend into the periodontal ligament and into the alveolar bone and periosteum. The gingiva receives its vascular supply from three different sources: the interdental septum, the periodontal ligament, and the connective tissue and periosteum all anastomose and supply the gingiva through a vascular network of capillaries. This vast network enables many periodontal procedures to be performed without depriving the periodontium and dental structures of a vascular supply. Innervation of the periodontium is from branches of the trigeminal nerve. These branches provide sensory function for the periodontal ligament, periosteum, the gingiva, and connective tissue. (Carranza 2003; Rose 2004).

4. Embryonic development of the teeth and periodontium

The development of the dentition and supporting structures begins at about the fifth week embryonically. Cells from the neural tube, known as neural crest cells, migrate to the first branchial arch. Neural crest cells are pluripotent neuroepithelial cells. Neural crest cells give rise to osteoblasts, chondrocytes, fibroblasts, cementoblasts, odontoblasts, and ganglia and other nervous structures. The migrated neural crest form a layer known as ectomesenchyme beneath the oral epithelium. An intricate epithelial-ectomesenchymal interaction takes place led by the ectomesenchyme. Studies have shown that the ectomesenchyme and dental organ contain all the necessary information to create the tooth and its attachment apparatus(Ten Cate 1998). A dental lamina is formed from an in-growth of oral ectoderm surrounded by the ectomysenchyme. A projection off the dental lamina develops which forms the tooth bud. The ectomysenchyme surrounding the tooth bud begins to form immature bone known as woven bone. The tooth bud will differentiate morphologically into the dental organ and proceed through stages known as the bud, cap, and bell stages chronologically (Fig. 17).
During the cap stage, the ectomysenchyme condenses and forms the dental papilla. Also forming are distinct layers during the cap and bell stage within the dental organ know as the inner enamel epithelium, the outer enamel epithelium, the stellate reticulum, and the stratum intermedium (Fig. 18). The outer enamel epithelium is composed of cells cuboidal in shape and contact the star shaped cells of the stellate reticulum. The stratum intermedium lies between stellate reticulum and inner enamel epithelium. One or two layers of cuboidal cells make up this layer. The inner enamel epithelium consists of columnar shaped cells. The

inner enamel epithelium will give rise to ameloblasts that create the enamel of the tooth. A dental follicle also forms from the ectomysenchyme and surrounds the dental organ and dental papilla. The dental papilla will eventually give rise to the crown, root dentin and the cellular elements of the dental pulp. It has been shown that the shape of the dental papilla dictates the shape of the final crown of the tooth (Ten Cate 1998). The cells of the dental follicle will eventually differentiate and become cementoblasts, fibroblasts, and osteoblasts that create the periodontal ligament and alveolar bone proper respectively.

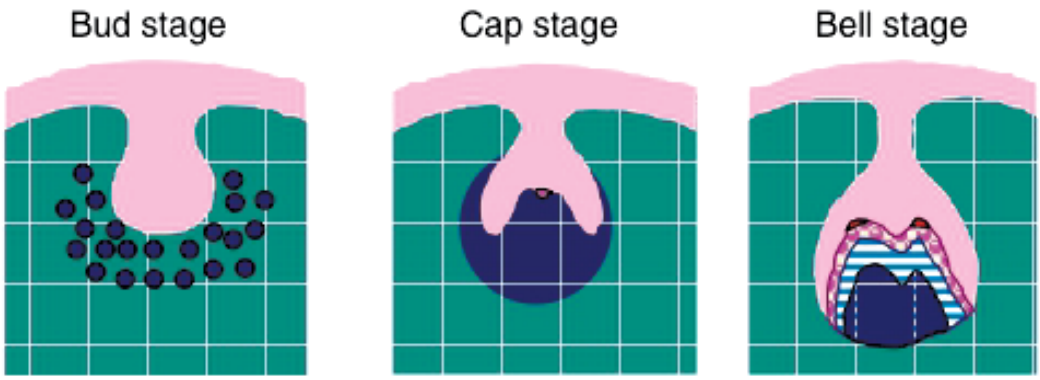


Fig. 17. Initial Stages of tooth development. (Garant 2003)

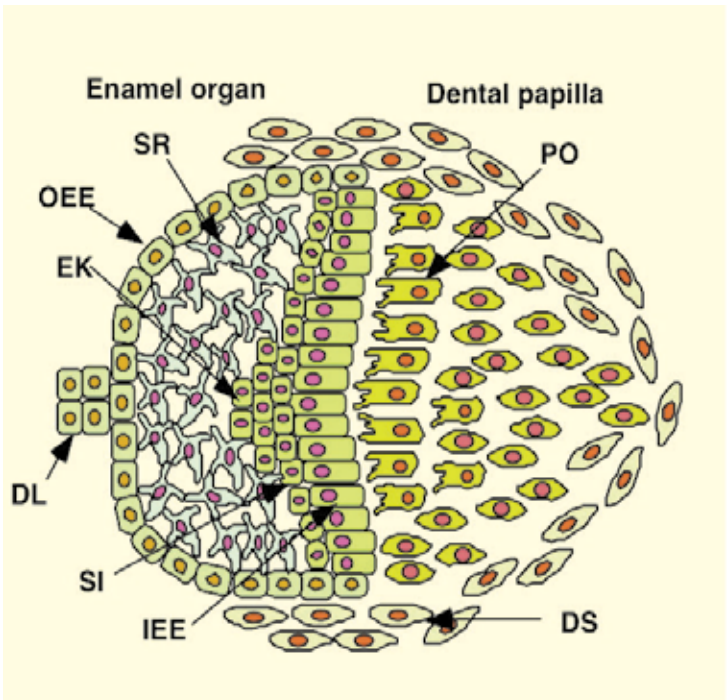


Diagram of the enamel organ and dental papilla. The outer enamel epithelium (OEE) forms the convex surface of the enamel organ and is separated from adjacent dental sac and general mesenchyme (not shown) by a basement membrane. The stellate reticulum (SR) lies between the OEE and the stratum intermedium (SI). The SI cells are closely juxtaposed to the cells of the inner enamel epithelium (IEE). The enamel knot (EK) represents a small group of nondividing cells near the IEE. The IEE is separated from the preodontoblasts of the dental papilla (Fig.6) by a basement membrane (see figure 4). DL, remnant of the dental lamina; DS, dental sac cells.

Fig. 18. Detail of the bell stage (Garant 2003)

As the development of the future crown progresses, the development of the periodontal structures and root takes place. The inner and outer enamel epithelium proliferate and fuse and become the Hertwig's epithelial root sheath at apical end of the root. This structure is composed of a double layer of epithelial cells. Cells of the dental papilla that are adjacent to Hertwig's epithelial root sheath begin to form dentin on the surface of the root. Hertwig's epithelial root sheath begins to disintegrate and epithelial rests of Malassez are created. These are epithelial remnants of the Hertwig's epithelial root sheath and they will reside in the periodontal ligament. Clinically these remnants may become significant, as they are a possible cause of radicular cysts (Garant 2003; Rose 2004).

As the epithelial root sheath becomes discontinuous and the root dentin is exposed, cells from the dental follicle will begin to produce cementoblasts to create cementum over the surface. The first type of cementum formed is acellular fibrillar cementum. The collagen fibers secreted by the cementoblasts are oriented at right angles to the root and will eventually connect with the collagen fibers of the periodontal ligament to form Sharpey's fibers.

Cellular cementum is formed when tooth formation is near completion. The cells on the outer part of the dental follicle will begin to produce osteoblasts and produce alveolar bone. In between the osteoblasts and cementoblasts, cells of the dental follicle will become fibroblasts and produce collagen that will become the periodontal ligament. As the cementum and alveolar bone increase in thickness, the periodontal ligament narrows in size and fibers become fixed within the mineralizing tissues. This process creates the Sharpey's fibers that attach the tooth to the alveolar bone through collagen fibers (Garant 2003). The developmental process is also important for periodontal and oral regenerative procedures. Proteins produced by Hertwig's epithelial root shealth (i.e. enamel matrix derivatives) are used to induce acellular cementum formation and also cause fibroblast differentiation and proliferation in the periodontal ligament. Applications of the developmental process are also of interest with current biologic agents and future stem cell technology to create conditions that would encourage regeneration of oral structures that have been lost.

When the process of enamel formation has concluded, the ameloblasts become reduced in size and become the reduced enamel epithelium. This layer attaches itself to the enamel of the tooth through hemidesmosomes and this is called the primary epithelial attachment. This reduced enamel epithelium exists from the time the enamel is mineralized to the time of tooth eruption. During the process of eruption, the epithelium of the primary epithelial attachment fuses with the oral epithelium. The oral epithelium replaces the cells of the primary attachment and is called the secondary epithelial attachment. This section of multilayer cells is also attached to the tooth through hemidesmosomes (Garant 2003; Rose 2004).

Tooth eruption can be broken down into two phases, active and passive. Active eruption is the physical eruption of the teeth into the oral cavity. Passive eruption takes place when the teeth are in occlusion. During passive eruption, a continued apical movement of the gingival margin and epithelial attachment takes place. Gargiulo and others divided this process into stages. The final stage places the gingival margin at a level slightly coronal of the cementoenamel junction and the sulcus and junctional epithelium are apical of the cementoenamel junction (Gargiulo 1961). This process may take place later in some individuals and caution must be taken when gingival esthetics of young adults is being

evaluated since interventional therapy may not be warranted if the tissues have not settled to the lower apical level.

5. Conclusion

The periodontium is the foundation for the dentition. It is formed by the alveolar bone, periodontal ligament, cementum, and oral mucosa. These components allow for the interaction of the teeth with external forces and helps prevent damage in function. Knowing the structure and origins of the components that constitute the periodontium, the interaction with each other and their biological and physical limits is crucial if one is to understand the changes seen in the periodontium when affected by disease and excessive occlusal forces.

6. Acknowledgements

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2

Plaque Biofilm

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1. Introduction

Periodontal infections are caused by bacteria which colonize the tooth surface and the surrounding gingival tissue to form dental plaque. Dental plaque is a complex polymicrobial biofilm. The term biofilm has been used to describe a well-organized microbial community which adheres to an inanimate or living surface. Bacteria growing in biofilms adhere to a solid surface where they multiply and form microcolonies embedded in an extracellular polymeric matrix, which includes water and nutrient channels. (Costerton et al., 1999) Novel microscopic and molecular techniques have recently been used to investigate environmental biofilms and explore the properties of dental plaque. These studies have shown that dental plaque behaves as a classic biofilm (Socransky & Haffajje 2002, Marsh, 2004). The development of this microbial community is a process that involves cooperation and competition among an extremely diverse community of organisms. (Kolenbrander PE et al., 2002)

2. Definitions

The involvement of "very fine extracellular polymer fibrils" that anchored bacteria to surfaces were observed by Marshall (1976). Communities of attached bacteria in aquatic systems were found to be encased in a "glycocalyx" matrix that was polysaccharide in nature, and mediated adhesion (Costerton et al.,1978.) It was stated that biofilm consists of single cells and microcolonies, all embedded in a highly hydrated, predominantly anionic exopolymer matrix (Costerton et al., 1987.) Other defining aspects of biofilms, such as the characteristics of spatial and temporal heterogeneity and the involvement of inorganic or abiotic substances held together in the biofilm matrix have been described (Characklis and Marshall in 1990).

It was emphasized that biofilms could adhere to surfaces and interfaces and to each other, including in the definition microbial aggregates and floccules and adherent populations within spaces of porous media (Costerton et al., 1995). At the same time it was observed that adhesion triggered expression of genes controlling production of bacterial components necessary for adhesion and biofilm formation, emphasizing that the process of biofilm formation was regulated by specific genes transcribed during initial cell attachment (Costerton and Lappin-Scott, 1995).

More recently a biofilm was defined as a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each

other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. (Donla and Costerton 2002)

3. Significance of biofilms

Epidemiologic evidence indicates that biofilms are a source of several infectious diseases, although the exact mechanisms by which biofilm-associated bacteria induce disease are poorly understood. The pathogenicity of the biofilm in the oral cavity is increased by two biofilm characteristics: increased resistance to antibiotics and to phagocytosis by host inflammatory cells. Current intervention strategies are designed to prevent initial colonization by mechanical removal, minimizing microbial cell attachment to the oral tissues and increasing penetration of the biofilm matrix by antimicrobials. In the future, treatments may inhibit the genes involved in cell attachment and biofilm formation.

4. Formation of dental plaque biofilms

Distinct stages in plaque formation include:

4.1 Acquired pellicle formation

Within minutes of tooth eruption or after professional cleaning of the tooth, the surface rapidly becomes coated with a variety of salivary constituents including albumin, glycoproteins, acidic proline-rich proteins, mucins, cell debris, exoproducts (such as a-amylase and lysozyme), and sialic acid thus providing variety of receptors that are recognized by colonizing bacteria.

4.2 Transport of microorganisms to the pellicle

The primary colonizers of microorganisms attach to these receptors. They are mostly gram-positive cocci , followed by some gram-positive rods and filaments and a small number of gram-negative cocci. The gram-positive cocci species involved in this initial layer include, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus oralis*, *Rothia dentocariosa*, and *Staphylococcus epidermidis*. The gram-positive rod and filament species include *Actinomyces viscosus*, *Actinomyces israelis*. *Actinomyces gerencseriae* and *Corynebacterium* species. *Veillonella parvula* and *Neisseria* sp comprise the gram-negative cocci, which are aerobes or facultative aerobes and are able to adhere to the non-exfoliating hard tooth surfaces (Sbordone, L., Bortolaia.,2003). These early colonizers are able to withstand many of the frequent mechanisms of the oral cavity that contribute to bacterial removal such as swallowing, chewing, and the flow of saliva. The early colonizers are also able to survive in the aerobic conditions present in the oral cavity, without having much protection from other bacteria (Sbordone and Bortolaia 2003). This thin, biofilm is almost always present on the tooth surface as it forms immediately after cleaning.

4.3 Weak, long range physico-chemical interactions between microbes and tooth pellicle

As a consequence of bacterial attachment,a change in gene expression is likely to occur. Consequently, primary colonizers alter the surface not only by their physical presence but by developing a new surface-attached phenotype with distinct metabolic activity and

surface properties, thus altering their surroundings and creating new niches for other bacteria to colonize.(Davey & Costerton, 2006) Reversible adhesion involving weak long-range physicochemical interactions occur between the cell surface and the pellicle. It is reversible because the attraction is weak and the micro organisms can readily detach from the tooth surface.

4.4 Strong, short-range interactions between adhesions of bacteria and receptors on pellicle

This reversible adhesion is followed by a much stronger, irreversible attachment, as short-range interactions between specific molecules (adhesins) on the bacterial cells and the complementary receptor proteins on the pellicle surface occur. Many oral microbial species have multiple adhesion types on their cell surface, and can, therefore, participate in a plethora of interactions both with other bacteria and host surface molecules (Marsh, 2004.)
Theoritically analogs could be synthesized to block adhesin-receptor attachment or co-adhesion thus making them less conducive to bacterial colonization. However, cells can express multiple types of adhesin (Hasty et al., 1992; Zhang et al., 2005) so that even if a major adhesin was blocked, other mechanisms of attachment may be invoked. Furthermore, although adhesion is necessary for colonization, the final proportions of a species within a mixed culture biofilm such as dental plaque will depend ultimately on the ability of an organism to grow and outcompete neighboring cells.

4.5 Co-aggregation

Socransky et al.(1998) examined over 13,000 subgingival plaque samples from 185 adult subjects and used cluster analysis and community ordination techniques to demonstrate the presence of specific microbial groups within dental plaque (Fig. 1).
Six closely associated groups of bacterial species were recognized. These included the *Actinomyces*, a yellow complex consisting of members of the genus *Streptococcus*, a green complex consisting of *Capnocytophaga* species, *Actinobacillus actinomycetemcomitans* serotype a, *Eikenella corrodens* and *Campylobacter concisus* and a purple complex consisting of *Veillonella parvula* and *Actinomyces odontolyticus*. These groups of species are early colonizers of the tooth surface, and their growth usually precedes the multiplication of the predominantly gram negative orange and red complexes. Certain complexes are observed together more frequently than others in subgingival plaque. For example, it is extremely unlikely to find red complex species in the absence of members of the orange complex. In contrast, members of the *Actinomyces*, yellow, green and purple complexes are often observed without members of the red complex or even the red and orange complexes. Most oral bacteria adhere to one another.This cell-to-cell adherence is known as **coaggregation**.

4.6 Multiplication of bacteria and confluent growth

Eventually, the bacterial cells continue to divide until a three-dimensional mixed-culture biofilm forms that is spatially and functionally organized. Polymer production causes the development of the extracellular matrix which is one of the key structural aspects of the plaque biofilm. The bacterial stratification is arranged according to metabolism and aerotolerance, with the number of gram-negative cocci, rods and filaments increasing as more anaerobic bacteria appear (Sbordone and Bortolaia,.2003). As the biofilm thickens and becomes more mature, anaerobic bacteria live deeper within the biofilm which protects them from the aerobic environment within the oral cavity.

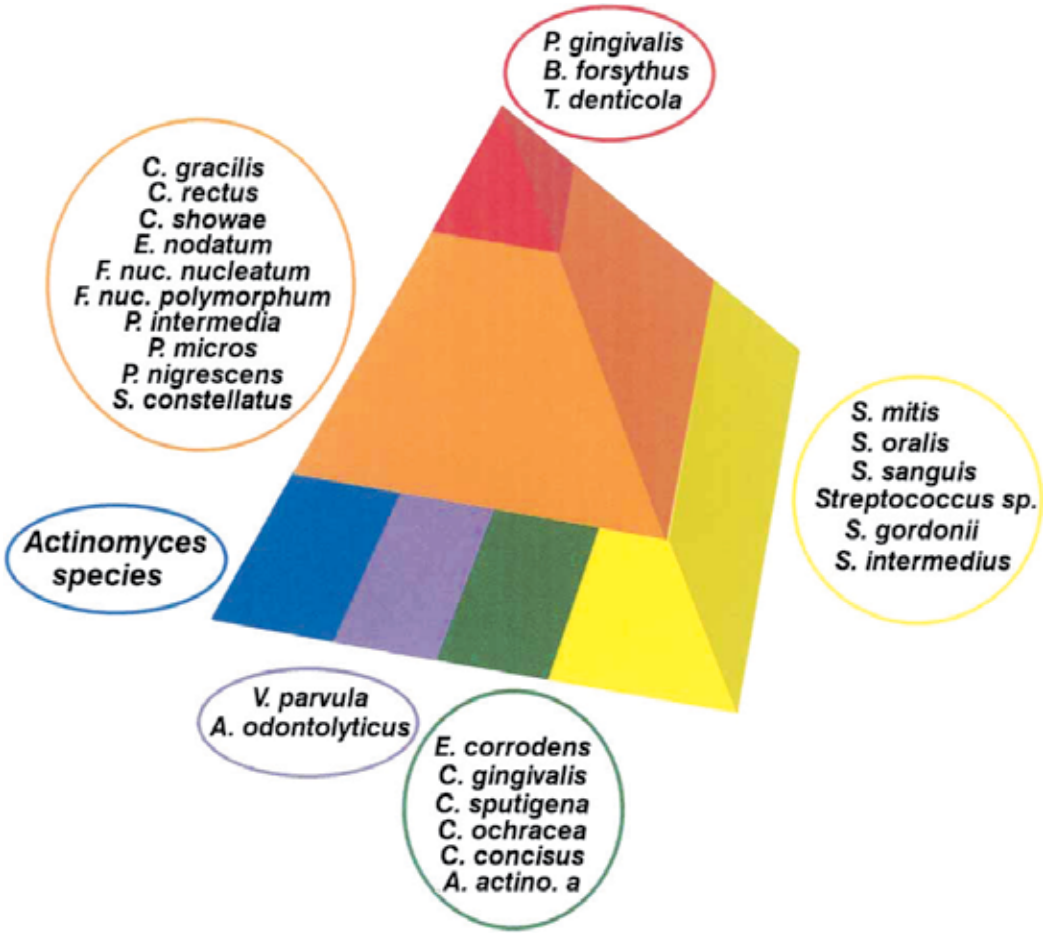

Fig. 1. Diagram of the association among subgingival species. The base of the pyramid is comprised of species thought to colonize the tooth surface and proliferate at an early stage. The orange complex becomes numerically more dominant later and is thought to bridge the early colonizers and the red complex species which become numerically more dominant at late stages in plaque development. (adapted from Socransky et al.,1998)

4.7 Active detachment of bacteria

The composition of the climax community of plaque is diverse, with many species being detected at individual sites. Molecular ecology approaches, in which 16S rRNA genes are amplified from plaque samples, have identified >600 bacterial and *Archae* taxa, of which approximately 50% are currently unculturable. (Wade 1999)

The detachment of bacteria from biofilms is essential to allow colonization of new habitats. It appears from *in vitro* studies that cells detach in different ways. Some of these involve the detachment of single cells in a continuous predictable fashion (erosion), the sporadic detachment of large groups of cells (sloughing) or an intermediate process whereby large pieces of biofilm are shed from the biofilm in a predictable manner. The more predictable intermediate process results in detached clusters consisting of about 10^4 cells.

5. Structure of biofilms

Plaque biofilms are complex three-dimensional structures composed of baterial microcolonies attached to a solid surface like the enamel of the teeth, the surface of the root or dental implants (Socransky and Haffajee 2002) embedded in an exo-polysaccharide matrix.

5.1 Microcolonies

Biofilms are composed of microcolonies of bacterial cells (15–20% by volume) that are non-randomly distributed in a matrix or glycocalyx (75–80%volume). Earlier studies of thick biofilms (.5 mm) that develop in sewage treatment plants indicated the presence of voids or water channels between the microcolonies. These permit the passage of nutrients and other agents throughout the biofilm acting as a primitive ''circulatory'' system. Nutrients make contact with the sessile (attached) microcolonies by diffusion from the water channel to the microcolony rather. (Socransky and Haffajee,2002) Microcolonies occur in different shapes which are governed by shear forces due to the passage of fluid over the biofilm. At low shear force, the colonies are shaped liked towers or mushrooms, while at high shear force, the colonies are elongated and capable of rapid oscillation (Stoodley et al., 1999).

5.2 Exopolysaccharides(EPS) – the backbone of the biofilm

The bulk of the biofilm consists of the matrix which composed predominantly of water and aqueous solutes. The ''dry'' material is a mixture of exopolysaccharides, proteins, salts and cell material.

Exopolysaccharides, which are produced by the bacteria in the biofilm, are the major components of the biofilm, making up 50–95% of the dry weight (Sutherland., 1999). The EPS are largely insoluble and have a complex structure. (Kopec et al., 1997) They play a major role in maintaining the integrity of the biofilm and confer other beneficial properties. Using sucrose primarily as a substrate, the EPS are synthesized mostly by bacterial glucosyltransferases and, to a lesser extent, by fructosyltransferases. (Hamada and Slade, 1980; Bowen, 2002).

Bacteria can produce several different polysaccharides depending on the physiological state of the bacteria and the presence of specific substrates. All biofilms contain exopolysaccharides, which can vary quite markedly in their composition. Some exopolysaccharides are neutral, such as the mutan from *Streptococcus mutans*, whereas others are highly charged polyanionic macromolecules. Different ionic charge and concentrations of exopolysaccharides alter the confirmation and cause rapid changes in the three-dimensional gel network of polysaccharides. Similar effects may also be produced by provision of sucrose or other sugars. The exopolysaccharides can be degraded and utilized by bacteria within the biofilm. One distinguishing feature of oral biofilms is that many of the microorganisms can synthesize and degrade the exopolysaccharides. Exopolysaccharides can exist in both ordered or disordered forms. At high temperatures and often at very low ionic concentrations, the disordered form predominates, although few biofilms exhibit total absence of an ordered structure (Sutherland, 1990). Biofilm matrices are complex structures that contain masses of fibers of varying size, structure, composition and rigidity that interact with each other, with cells and with surface matrices. A wide range of possible

conformations, flexibility and configurations can be expected among different classes of polysaccharides.
The density of the fibrillar masses will affect accessibility of both cells and surfaces to nutrients and other solutes. The chemical composition and tertiary structure of the exopolysaccharides will determine whether it forms an effective adhesive. It will also affect the hydrophilic or hydrophobic nature of the surface. Exopolysaccharides aid in protecting microbial cells within the biofilm by preventing desiccation and attack by harmful agents. They may also bind essential nutrients such as cations to create a local nutritionally rich environment favoring specific microorganisms.The exopolysaccharide matrix could also act as a buffer and assist in retaining extracellular enzymes (and their substrates), enhancing substrate utilization by bacterial cells. They are effective in maintaining biofilm structure through the formation of networked, cross-linked linear macromolecules. In most mixed biofilms, numerous types of polysaccharide are found, complicating the network structure. The quantity of exopolysaccharides in a biofilm does not necessarily reflect the proportion of the bacterial species that produce it. Loss or removal of one type of exopolysaccharide may have a more drastic effect on the biofilm matrix than another even if the removed polymer is not dominant. (Socransky and Haffajee 2002)

6. Cell to cell communication (quorum sensing)

Bacteria are now known to lead highly social lives. (West, et al., 2006) They communicate and respond to local cell density through a process known as *quorum sensing*.
Quorum sensing is widely employed by a variety of gram-positive and gram-negative bacterial species to coordinate communal behavior. Quorum sensingwas originally discovered in the luminescent bacterium *Vibrio fischeri*.
Each individual bacterium is capable of producing a signaling molecule (inducer) and each also has a receptor for the inducer. When the inducer binds to the receptor, it activates the transcription of certain genes, including those responsible for the synthesis of the inducer itself. Imagine that only a few bacteria of the same kind are nearby. Diffusion reduces the concentration of the inducer in the surrounding medium to a negligible amount.
However, as the bacterial population grows, the concentration of the inducer in the surroundings increases, causing more inducer molecules to be synthesized. This forms a positive feedback loop and the concentration of the molecule keeps increasing. Once a threshold concentration is attained, activation of the receptor leads to a signal transduction cascade to switch on specific genes in the bacterial cells, leading to a coordinated population response. As a group, bacteria behave differently if there are few or many bacteria around them. Quorum sensing thus enables bacteria to co-ordinate and respond quickly to environmental changes, such as the availability of nutrients, other microbes or toxins. (Figure II)

6.1 Key players in a quorum-sensing network (table 1)

6.1.1 Autoinducers

Autoinducers are usually small molecules that either diffuse freely across the cell membranes or are actively transported out of the cell.

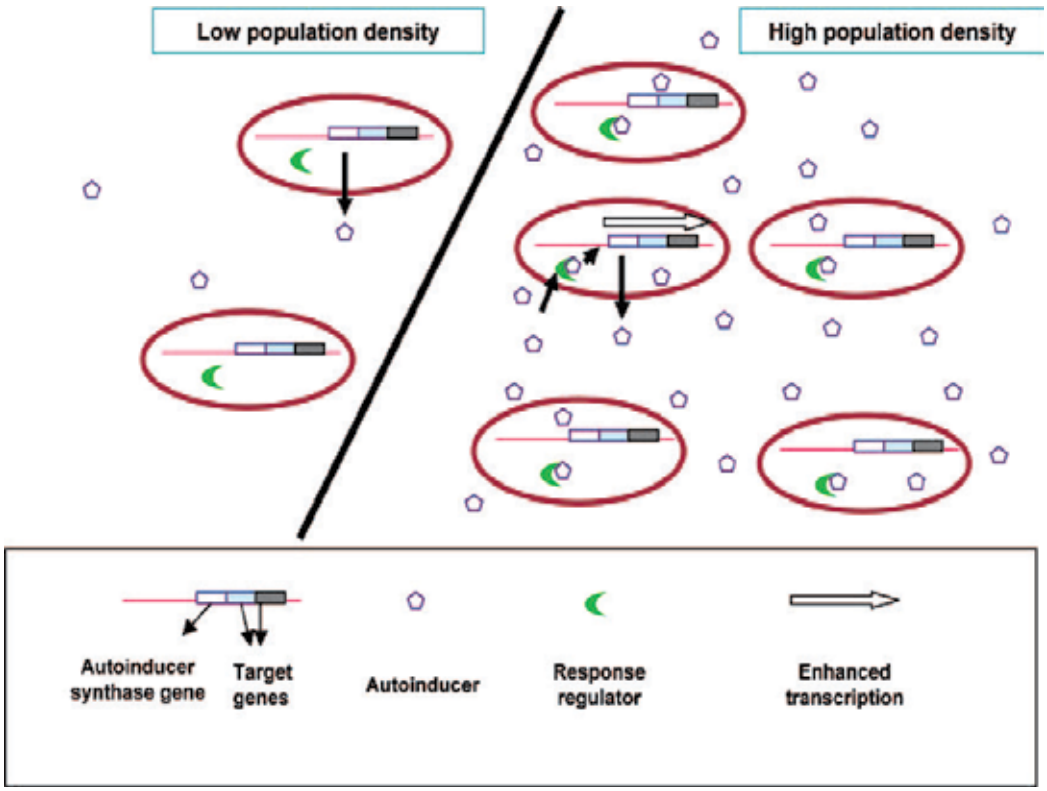

Fig. 2. Schematic representation of bacterial quorum sensing. (Adapted from Gonza´lez and Keshavan, 2006.)

Acyl homoserine lactones(AHL). Acyl homoserine lactones are the major group of autoinducer signals in gram-negative bacteria. They have a conserved homoserine lactone (HSL) ring with a variable acyl side chain. Based on the length of the acyl groups, AHLs can be broadly classified as short- or longchain molecules.

Autoinducer 2. AI-2 was first recognized as a quorum-sensing signal in *Vibrio harveyi* by Bassler et al. (1993). Since then, this type of signaling has been discovered in many gram-negative bacteria. AI-2 is described as a global signal molecule for interspecies communication. It is produced by gram-positive and gram-negative bacteria.

Cyclic dipeptides. A new class of autoinducers was recently identified in strains of *Pseudomonas.*

Bradyoxetin

Other types of autoinducers. In addition to the above-mentioned autoinducers, additional signals have been identified in gram-negative bacteria, including autoinducer (AI-3) in *E. coli* and diffusible signal factor (DSF) in *Xanthomonas campestris*

6.1.2 Autoinducer synthases

AHL synthases.

AI-2 synthase.

Synthases for other types of autoinducers

6.1.3 Quorum-sensing regulators

Quorum-sensing-dependent gene regulation is mediated by transcriptional regulator proteins that are activated upon binding autoinducer molecules.

LuxR-type regulators.

LuxP/Q-type regulators.

6.2 Negative regulation of quorum sensing

Negative regulation in general is the phenomenon of interfering with the bacterial quorum sensing. (Table 2) Of particular interest are the bacterial components used to manipulate quorum sensing called **Quorum Quenchers.**

Several AHL-degrading enzymes identified in various bacteria have the potential to be used as quorum quenchers. Dong et al. initially identified AiiA was isolated from *Bacillus* species and inactivates the AHL signal and attenuates virulence when expressed in *Erwinia carotovora* (Dong et al., 2000)

More than 20 bacteria belonging to the *Bacillus cereus* group are capable of enzymatic inactivation of AHLs. Further genetic analyses revealed that the enzymes responsible for AHL inactivation were homologs of AiiA from *Bacillus* species strain 240B1. This enzyme is an AHL lactonase, known to act by hydrolyzing the lactone bond in the AHL (Dong et al., 2001).

AUTOINDUCERS
Acyl homoserine lactones
Autoinducer 2
Cyclic dipeptides
Bradyoxetin
Other types of autoinducers
AUTOINDUCER SYNTHASES
AHL synthases
AI-2 synthase
Synthases for other types of autoinducers
QUORUM SENSING REGULATORS
LuxR-type regulators
LuxP/Q-type regulators

Table 1. Key Players In A Quorum-Sensing Network

7. Antibiotic resistance

Periodontitis is an infection induced by multiple species of bacteria and the host's response to the bacterial insult. The disease is usually successfully controlled by mechanical debridement, but some cases benefit from adjunctive antibiotic therapy. Antibiotics have been used to treat periodontal infections in the past and they still hold their use today.

The indiscriminate use of antimicrobial agents has the potential of leading to the development of resistant bacteria.(Levy, 1998; Pallasch, 2000).

Antiactivator Proteins
Homologs of Transcriptional Regulators
AHL-Degrading Enzymes
mRNA-Dependent Regulation

EUKARYOTIC INTERFERENCE IN BACTERIAL QUORUM SENSING
Quorum-Sensing Cross Talk between *A. tumefaciens* and Its Host Plant
Furanones: Structural Mimics
L-Canavanine as a Quorum-Sensing Inhibitor
Human Hormones Interfere with Bacterial Quorum Sensing
Other QSI Compounds

USING BACTERIAL COMPONENTS TO MANIPULATE QUORUM SENSING
Quorum Quenchers
Transgenic Plants
Synthetic Analogs for Quorum-Sensing Autoinducers

Table 2. Negative Regulation Of Quorum Sensing

The phenomenon of increased antimicrobial resistances and reduced susceptibilities in biofilms is well recognized. (Walker and Karpinia . 2002; Walker et al., 2004)
Almost without exception, bacteria grown in biofilms are more resistant to antibiotics than are the same cells grown in a planktonic state. Estimates of 1000 to 1500 times greater resistance for biofilm-grown cells than planktonically grown cells have been suggested (Costerton JW. 1999)
One important mechanism of resistance appears to be the slower rate of growth of bacterial species in biofilms,which makes them less susceptible to many, but not all, antibiotics (Ashby MJ et al., 1994; Brooun A et al., 2000; Costerton et al., 1999).
It has been shown in many studies that the resistance of bacteria to antibiotics, biocides or preservatives is affected by their nutritional status, growth rate, temperature, pH and prior exposure to ineffective concentrations of antimicrobial agents (Brown and Williams 1985; Brown et al., 1990; Williams P.1988). Variations in any of these parameters can lead to a varied response to antibiotics within a biofilm.
The matrix performs a ''homeostatic function". Cells deep in the biofilm experience different conditions, such as hydrogen ion concentration or redox potentials, than cells at the periphery or cells growing planktonically. The growth rates of these deeper cells will be decreased allowing them to survive better than faster-growing cells at the periphery when exposed to antimicrobial agents. In addition, the slower- growing bacteria often overexpress ''nonspecific defense mechanisms'' including shock proteins and multi-drug efflux pumps (arcAB) and demonstrate increased exopolymer synthesis. (Gilbert and Allison 1999)
The exopolymer matrix of a biofilm, although not a significant barrier in itself to the diffusion of antibiotics, does have certain properties that can retard diffusion. For example, strongly charged or chemically highly reactive agents can fail to reach the deeper zones of the biofilm because the biofilm acts as an ion-exchange resin removing such molecules from solution . (Gilbert and Allison 1999)

In addition, extracellular enzymes such as b-lactamases, formaldehyde lyase and formaldehyde dehydrogenase may become trapped and concentrated in the extracellular matrix, thus inactivating susceptible, typically positively charged, hydrophilic antibiotics. Some antibiotics such as the macrolides, which are positively charged but hydrophobic, are unaffected by this process. Thus, the ability of the matrix to act as a physical barrier depends on the type of antibiotic, the binding of the matrix to that agent and the levels of the agent employed. (Nichols WW 1993) Since reaction between the agent and the matrix will reduce the levels of the agent, a biofilm with greater bulk will deplete the agent more readily. Further, hydrodynamics (de Beer et al., 1994) and the turnover rate of the microcolonies will also affect antibiotic effectiveness. (Kumon et al 1994)

Alteration of genotype and/or phenotype of the cells growing within a biofilm matrix is receiving increased attention. Such cells express genes that are not observed in the same cells grown in a planktonic state, and they can retain this resistance for some time after being released from the biofilm.

Recently, the notion of a subpopulation of cells within a biofilm that are ''super-resistant'' was proposed. Such cells might explain remarkably elevated levels of resistance to certain antibiotics that have been suggested in the literature. The contribution of multi-drug resistance pumps to antibiotic resistance of organisms grown in biofilms was examined by Brooun et al.(2000). These ''pumps'' can extrude chemically unrelated antimicrobial agents from the cell. Since extrusion places the antibiotics outside the outer membrane, the process offers protection against antibiotics that target cell wall synthesis. They postulated the presence of a ''super-resistant'' subpopulation of cells when grown as biofilms. No ''super-resistant'' subpopulation was detected when the same strains were grown in a planktonic state.

8. Methods of analyzing the biofilm

The Leeds *in situ* device:

Plaque biofilms can be generated using "Leeds *in situ* device" (Robinson et al 1997; Watson et al 2004): Devices are bonded to teeth and worn for seven days, during which time volunteers carried out their normal oral hygiene regime. Devices are then debonded and recovered, with undisturbed plaque in situ.

Direct light and electron microscopic observation:

Direct light and electron microscopic observation clearly showed that biofilm bacteria were enveloped in very large amounts of a fibrous, highly hydrated, exopolysaccharide matrix whose chemical composition was species specific (Sutherland, 1977)

Microelectrodes:

Christiane von Ohle, et al (2010) demonstrated the utility of using microelectrodes to measure the influence of nutrients and antimicrobial agents on the physiology of human dental biofilms nondestructively and in real time. The microelectrode data can be corroborated with microscopy and culture techniques. Microelectrodes with tip diameters of < 10 ,um are useful in the study of microbial biofilms because they allow the in situ measurement of pH, dissolved oxygen (DO), sulfide, and other chemical species with minimal disturbance of the biofilm structure (Lewandowskiet al., 1991; Revsbechn and Ward 1983.)

Chemical probes:

During the examination of eukaryotic tissues by CSL microscopy, a large number of fluorescent chemical probes have been developed. (Haugland, 1992.) These probes can be

introduced into fully hydrated living bacterial biofilms and their fluorescent emissions can be monitored for location and intensity to yield very valuable direct data concerning chemical and physical conditions in virtually all parts of these complex matrix-enclosed adherent populations.(Costerton et al., 1994)

The introduction and application of **"metagenomics"** approach has greatly enhanced and will continue to increase our ability to study microbial community, including dental plaque, in greater detail. The term "Metagenomics" was first invented by Handelsman J,et al (1998), and is defined as "the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and laboratory cultivation of individual species". The advances in refinements of DNA amplification, bioinformatics, and enhanced computational power for analyzing DNA sequences have enabled the adaptation of shotgun sequencing, such as chip-based pyrosequencing, to metagenomic samples. The approach randomly shears DNA, sequences many short sequences, and reconstructs them into a consensus sequence (Breitbart M et al 2002). By performing metabolic function analyses on genes identified via metagenomic approach, researchers are able to retrieve information both on which organisms are present and more importantly, what functions or metabolic processes are possible in that particular community (Gill SR et al 2006). Using comparative genetic studies coupled with expression experiments such as microarray and proteomics, microbiologist will be able to piece together a metabolic network that goes beyond species boundary, and gain valuable insight into the metabolism within the community.

9. Biofilm formation around implant surfaces

Biofilm formation on oral implants can cause inflammation of peri-implant tissues, which endangers the long-term success of osseointegrated implants.

Heuer et al. (2007) examined the crevicular fluid around 14 dental implants/healing abutments over a period of 14 days. Despite massive supragingival biofilm formation, no periodontal pathogens were isolated from the sulcus fluid around the implants/healing abutments during initial bacterial colonization. They concluded that the attachment of peri-implant tissue by means of hemidesmosomal, actin filaments and microvilli, reduced the risk of formation of anaerobic subgingival pockets.

In some studies, *H. actinomycetemcomitans* and *P. gingivalis* were found in greater amounts in peri-implant lesions (George et al., 1994 and Shibli et al., 2003)

No *P. gingivalis* or *H. actinomycetemcomitans* were isolated from stable osseointegrated implant surfaces, in contrast to peri-implant lesions, in which high levels of periodontal pathogens were present. (Botero et al., 2005)

A study of implants in the partially edentulous patient, Quirynen et al. (2006) reported that initial colonization of peri-implant pockets with bacteria associated with periodontitis occured within two weeks. Four subgingival plaque samples were taken from shallow and medium pockets around implants (test sites), and control teeth within the same quadrant one, two, four, 13, 26 and 78 weeks after abutment connection. Checkerboard DNA-DNA hybridization and real-time PCR revealed a complex microbiota (including several pathogenic species) in the peri-implant pockets within two weeks after abutment connection. After seven days, the detection frequency for most species, including the red complex microbiota, was almost identical to samples from the fresh peri-implant pockets (5 per cent and 20 per cent of the microbiota belonging to red and orange complex,

respectively) when compared with samples from the reference teeth. Between weeks 2 and 13, the number of bacteria in peri-implant pockets only slightly increased, with minor changes in the relative proportions of bacteria associated with periodontitis (8 per cent and 33 per cent of the microbiota belonging to red and orange complex, respectively). Although small differences were seen between teeth and implants at week two, a striking similarity in subgingival microbiota was found after three months.

10. Treatment and control of biofilm formation

Due to the structure of biofilms, their physical removal by a professional and the individual remains the most effective means of control. Subgingival debridement of root surfaces is an essential component in the treatment of periodontitis.

The use of antimicrobials can be grouped into two broad categories; those that attempt to kill or affect the metabolism of the organism such as antiseptics and antibiotics and those that affect the environment of the organisms. Other types of therapy are on the horizon, such as possible vaccines against oral pathogens or replacement therapy in which a species is introduced to the biofilm in order to control potentially pathogenic microorganisms. (Socransky and Haffajee 2002)

The main impetus behind the desire to control the bacterial composition of dental plaque is to prevent or reduce the incidence of periodontal diseases. Some potential strategies to achieve these aims were elaborated by Marsh and Bradshaw in 1997.(Table 3)

In a clinical trial, a seven-day treatment regime involving methylene blue led to a decrease in the proportions of Gramnegative anaerobes (including spirochetes) and motile bacteria and a reduction in the flow of GCF, while bacteria associated with gingival health increased (Wilson et al. 1992), suggesting that this approach has genuine potential. Further work on the influence of surface growth on the behavior of plaque communities will also be needed before the full potential of physiological approaches to biofilm control will be realized.

The **bioelectric effect**, in which electric fields are used to enhance the efficacy of biocides and antibiotics in killing biofilm bacteria, has been shown to reduce the very high concentrations of these antibacterial agents needed to kill biofilm bacteria to levels very close to those needed to kill planktonic (floating) bacteria of the same species. Biofilm bacteria are readily killed by an antibiotic on all areas of the active electrodes and on the surfaces of conductive elements that lie within the electric field but do not themselves function as electrodes (Costerton et al. 1994). Considerations of electrode geometry indicate that very low (< 100 μA/cm2) current densities may be effective in this electrical enhancement of antibiotic efficacy against biofilm bacteria, and flow experiments indicate that this bioelectric eflect does not appear to depend entirely on the possible local electrochemical generation of antibacterial molecules or ions. These data are expected to facilitate the use of the bioelectric effect in the prevention and treatment of device-related bacterial infections that are caused by bacteria that grow in biofilms and thereby frustrate antibiotic chemotherapy.

Photodynamic therapy (PDT) has been suggested as an alternative to chemical antimicrobial agents to eliminate subgingival species and treat periodontitis (Wilson. 1993). PDT is based on the concept that non-toxic photosensitizers can be preferentially localized in certain tissues and activated by light of the appropriate wavelength to generate singlet oxygen and free radicals that are cytotoxic to cells of the target tissue (Dougherty et al. 1998). Several studies have shown that oral bacteria are susceptible to PDT in planktonic cultures

(Wilson . 1993 and Wilson et al. 1993) and plaque scrapings (Williams et al. 2003 and Sarkar , Wilson 1993). Recent studies have reported that PDT-induced bacterial cell killing reduced bacterial numbers by more than 10-fold in *Streptococcus mutans, Streptococcus sobrinus* and *Streptococcus sanguinis* (Metcalf et al., 2006; Zanin et al., 2005) biofilms using toluidine blue O or erythrosine as the photosensitizer.

Control of plaque pH
• inhibition of acid production
- fluoride
- sugar substitutes
- antimicrobial agents
• stimulation of base production
- arginine
- urea
- peptides
Control of redox potential
• • redox agents
• oxygenating agents
Control of nutrients
• addition of base-generating nutrients
- arginine
• reduction of GCF flow
• anti-inflammatory agents
• inhibition of key microbial enzymes

Table 3. (Marsh And Bradshaw 1997) Physiological Strategies For The Control Of Oral Biofilms

Efflux pump inhibitors: Bacteria rely on efflux pumps to get rid of toxic substances. It was discovered that efflux pumps are highly active in bacterial biofilms, makingthem attractive targets for antibiofilm measures. A number of efflux pump inhibitors (EPIs) are known. EPIs were shown to reduce biofilm formation, and in combination they could abolish biofilm formation completely. Also, EPIs were able to block the antibiotic tolerance of biofilms. The results of this feasibility study might pave the way for new treatments for biofilm-related infections and may be exploited for prevention of biofilms in general.(Kvist et al., 2008)
The use of **probiotics** (introduction of beneficial bacteria) or **prebiotics** (nutrients that favour the growth of beneficial bacteria)
The role of **nanoscience** in microbiology needs to be assessed. Nanoparticles could be a new delivery mechanism for antimicrobial agents or vaccines that could disrupt biofilms; however, consideration needs to be given to the behavior of nanoparticles in ecosystems and their long-term effects.

11. Conclusion

This chapter attempts to throw light on the nature of plaque biofilms and the strategies towards their control. Biofilms are very complex structures and pose great challenges for

clinicians on a daily basis. Nevertheless, advances in science have made it possible to dissect their complex microbiology and guide the control of plaque biofilm related periodontal and peri implant infections.

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3

Gingival Indices: State of Art

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1. Introduction

Gingivitis, which is prevalent in a large proportion of the child and adult populations, is an inflammatory lesion of the gingival tissues, which usually precedes periodontitis. It has been shown to be reversible (Löe et al., 1967) and, although progression is not predictable, the prevention of gingivitis, in the individual patient or in populations, is still the first step toward preventing periodontitis (Burt et al., 2005). According to Mariotti (1999), the characteristics of biofilm-induced gingivitis are: (1) biofilm present at gingival margin; (2) change in gingival color; (3) change in gingival contour; (4) sulcular temperature change; (5) increased gingival exudate; (6) bleeding upon provocation; (7) absence of attachment loss; (8) absence of bone loss; and (9) histological changes. The intensity of the clinical signs and symptoms will vary among individuals as well as among sites within a dentition.

Surveys in different parts of the world have reported that gingivitis is prevalent in children, adolescents and adults (Baelum & Scheutz, 2002; Gjermo et al., 2002; Oliver et al., 1998; Sheiham & Netuveli, 2002). To assess these data, the state of the gingiva should be accurately defined, in order to be able to compare different population group at a given time, to determine and control risk factors, and to evaluate treatment efficacy (Benamghar et al., 1982). Quantitative measurement of disease most commonly is based on indices systems. An efficient index system should be quick and easy to use, with minimal instrumentation. It must be reproducible and must reflect accurately degrees of pathology (Engelberger, 1983). Several gingival indices have been proposed in literature, all of which have relied on one or more of the following criteria: gingival color (redness), gingival contour, gingival bleeding, gingival stippling and gingival crevicular fluid flow (Ciancio, 1986; Fischman, 1988; Newbrun, 1996). These clinical features can be assessed non-invasively, only visually, (e.g., color, contour, spontaneous bleeding) and/or invasively, with the use of an instrument (e.g., bleeding on provocation). Whereas some of the indices include both visual and invasive components, others are based on either visual features alone or bleeding on provocation alone. Thus, gingivitis can be evaluated by either quantitative clinical indices that are based on a combination of inflammation symptoms or extent of gingival involvement or on bleeding as a single variable (Barnett, 1996; Lorenz et al., 2009). Moreover, several investigators have used variations of "present or absent" indices which do not consider the severity of gingival inflammation. The observation of whether or not inflammation is present in the gingiva might be a useful approach in clinical studies. Such an index would be simple, reproducible with little examiner training and require relatively little time (Hazen, 1974). Although several indices have been proposed, with many different

methodologies, no one has universal application or acceptance. The purpose of this chapter is to describe the main gingivitis indices introduced over the past years, exposing its principles, methods and applicability.

2. Gingival indices

2.1 PMA Index

The PMA index, developed by Schour & Massler (1947) and described by Massler (1967) is probably the first successful attempt to design a numerical system for recording gingival health. The index scores gingival units as separate entities and is based on the premise that inflammation commences in the interdental papilla (P) from where it spreads to the marginal (M) and ultimately the attached gingiva (A). Each gingival unit is scored on the basis of 0-4. Only the labial surfaces are examined. The number of affected Papillary, Marginal and Attached units are counted for each individual and recorded. Its major purpose has been the evaluation of gingival inflammation in children.

2.2 Gingival Index (GI)

The Gingival Index (Löe and Silness, 1963) was created for the assessment of the gingival condition and records qualitative changes in the gingiva. It scores the marginal and interproximal tissues separately on the basis of 0 to 3. The criteria are:
0= Normal gingiva;
1= Mild inflammation – slight change in color and slight edema but no bleeding on probing;
2= Moderate inflammation – redness, edema and glazing, bleeding on probing;
3= Severe inflammation – marked redness and edema, ulceration with tendency to spontaneous bleeding.
The bleeding is assessed by probing gently along the wall of soft tissue of the gingival sulcus. The scores of the four areas of the tooth can be summed and divided by four to give the GI for the tooth. The GI of the individual can be obtained by adding the values of each tooth and dividing by the number of teeth examined. The Gingival Index may be scored for all surfaces of all or selected teeth or for selected areas of all or selected teeth. The GI may be used for the assessment of prevalence and severity of gingivitis in populations, groups and individuals. A score from 0.1-1.0 = mild inflammation; 1.1-2.0 = moderate inflammation from, and 2.1-3.0 signifies severe inflammation. The GI has been used frequently in clinical trials of therapeutic agents The sensitivity and reproducibility is good provided the examiner's knowledge of periodontal biology and pathology is optimal (Löe, 1967).

2.3 Sulcus Bleeding Index (SBI)

An early sign of gingivitis is bleeding on probing and, in 1971, Muhlemann and Son described the Sulcus Bleeding Index (SBI). The criteria for scoring are as follows:
Score 0 – health looking papillary and marginal gingiva no bleeding on probing;
Score 1 – healthy looking gingiva, bleeding on probing;
Score 2 – bleeding on probing, change in color, no edema;
Score 3 – bleeding on probing, change in color, slight edema;
Score 4 –bleeding on probing, change in color, obvious edema;
Score 5 –spontaneous bleeding, change in color, marked edema.

Four gingival units are scored systematically for each tooth: the labial and lingual marginal gingival (M units) and the mesial and distal papillary gingival (P units). Scores for these units are added and divided by four. Adding the scores of the undivided teeth and dividing them by the number of teeth can determine the sulcus bleeding index.

2.4 Gingival Bleeding Index (GBI)

In 1974, Carter and Barnes introduced a Gingival Bleeding Index, which records the presence or absence of gingival inflammation after passing unwaxed dental floss into the proximal sulci. It is readily available, disposable, and can be used by the instructed patient for self-evaluation. The mouth is divided into six segments and flossed in the following order; upper right, upper anterior, upper left, lower left, lower anterior and lower right. Bleeding is generally immediately evident in the area or on the floss; however, thirty seconds is allowed for reinspection of each segment. If copious hemorrhage occurs the patient may be allowed to rinse in between segments. Bleeding is recorded as present or absent. For each patient a Gingival Bleeding Score is obtained by noting the total units of bleeding and the total susceptible areas at risk.

2.5 Gingival Bleeding Index (GBI - Ainamo & Bay, 1975)

This Gingival Bleeding Index (GBI), introduced by Ainamo & Bay (1975), is performed through gentle probing of the orifice of the gingival crevice. If bleeding occurs within 10 seconds a positive finding is recorded and the number of positive sites is recorded and then expressed as a percentage of the number of sites examined. Bleeding can also function as a motivating factor in activating the patient to better oral home care. It has been show that the scores obtained with this index correlate significantly to GI (Löe and Silness, 1963) and has been used in profile studies and short-term clinical trials.

2.6 Papillary Bleeding Index (PBI)

The Papillary Bleeding Index was first introduced by Saxer and Muhlemann (1975), as cited by Muhlemann (1977). This index permits both immediate evaluation of the patient's gingival condition and his motivation, based upon the actual bleeding tendency of the gingival papillae. A periodontal probe is inserted into the gingival sulcus at the base of the papilla on the mesial aspect, and then moved coronally to the papilla tip. This is repeated on the distal aspect of the papilla. The intensity of any bleeding is recorded as:
Score 0 – no bleeding;
Score 1 – A single discreet bleeding point;
Score 2 – Several isolated bleeding points or a single line of blood appears;
Score 3 – The interdental triangle fills with blood shortly after probing;
Score 4 – Profuse bleeding occurs after probing; blood flows immediately into the marginal sulcus.

2.7 Papillary Bleeding Score (PBS)

This is performed using a Stim-U-dent®, which is inserted interproximally (Loesche, 1979). Essentially, the PBS expands the score 2 of the Gingival Index (Löe and Silness, 1963) into three recognized clinical conditions. The criteria are:
0 = healthy gingiva, no bleeding upon insertion of Stim-U-dent® interproximally;

1 = edematous, reddened gingiva, no bleeding upon insertion of Stim-U-Dent® interproximally;
2 = bleeding, without flow, upon insertion of Stim-U-dent® interproximally;
3 = bleeding, with flow, along gingival margin upon insertion of Stim-U-dent® interproximally;
4 = copious bleeding upon insertion of Stim-U-dent® interproximally;
5 = severe inflammation, marked redness and edema, tendency to spontaneous bleeding.
The PBS is determined on all papillae anterior to the second molars.

2.8 Modified Papillary Bleeding Index (MPBI)

Barnett et al. (1980) modified the PBI index (Muhlemann, 1977) by stipulating that the periodontal probe should be gently placed in the gingival sulcus at the mesial line angle of the tooth surface to be examined and carefully swept forward into the mesial papilla. They timed the appearance of bleeding and graded it as follows:
0 = no bleeding within 30 s of probing;
1 = bleeding between 3 and 30 s of probing;
2 = bleeding within 2 s of probing;
3 = bleeding immediately upon probe placement.
The mesial papillae of all teeth present from the second molar to the lateral incisor were assessed. Indices were derived for the maxillary left and mandibular right buccal segments, and the maxillary right and mandibular left lingual segments, and from these a full-mouth index was calculated. This distribution of test sites was utilized since each mesial papilla could only be tested once, i.e. from either the buccal or lingual side. They showed that the modified PBI may be more sensitive than the visual aspects of the GI in assessing changes in gingival health.

2.9 Bleeding Time Index (BTI)

Nowicki et al. (1981) concluded that a gingival index bleeding would be useful for detecting the first clinical evidence of gingival inflammation. The method consisted of inserting a Michigan "0" probe in the sulcus until slight resistance was felt and then the gingiva was stroked back and forth once over an area of approximately 2 mm. The following scores are applied:
0= no bleeding within 15 seconds of second probing (i.e. 30 seconds total time);
1= bleeding within 6 to 15 seconds of second probing;
2= bleeding within 11 to 15 of seconds of first probing or 5 seconds after second probing;
3= bleeding within 10 seconds after initial probing
4= spontaneous bleeding.

2.10 Eastman Interdental Bleeding Index (EIBI)

Caton & Polson (1985) developed the Eastman Interdental Bleeding Index (EIB). A wooden interdental cleaner is inserted between the teeth from the facial aspect, depressing the interdental tissues 1 to 2 mm. This is repeated four times and the presence or absence of bleeding within 15 s is recorded. Considering the over-all high levels of reliability between and within examiners, this method would be suitable for use in clinical trials and epidemiological studies (Blieden et al., 1992).

2.11 Quantitative Gingival Bleeding Index (QGBI)

In 1985, Garg & Kapoor formulated a quantitative gingival bleeding index. This index takes into consideration the magnitude of blood stains covering tooth brush bristles on brushing and squeezing gingival tissue units in a segment, with one score for entire one segment (canine to canine, or left or right pre-molars and molars in maxillary or mandibular arches – six segments in all). The criteria scores are:
0 – no bleeding on brushing; bristles free from blood stains;
1 - slight bleeding on brushing; bristle tips stained with blood;
2 - moderate bleeding on brushing; about half of bristle length from tip downwards stained with blood;
3 – Severe bleeding on brushing; entire bristle length of all bristles including brush head covered with blood.
Bleeding is generally immediately evident on the bristles of the brush; however, 30 seconds were allowed for reinspection of each segment. According with authors, this index has good reproducibility, reliability, objectivity and simplicity of use.

2.12 Modified Gingival Index (MGI)

The Modified Gingival Index (MGI), devised by Lobene et al. (1986), introduced changes in the criteria of the Gingival Index (Löe and Silness, 1963) through a non-invasive (no probing) and resetting the rating for mild and moderate inflammation. This way, the following criteria are adopted:
0 = absence of inflammation;
1 = mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary;
2 = mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary;
3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary;
4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration.
Gingival units as well as the calculation of the index follow the same criteria described in GI.

2.13 Bleeding on Interdental Brushing Index (BOIB)

Whereas measures of gingival inflammation through indices of bleeding with polling can be influenced by factors such as angulation of the probe, the probe insertion depth, direction, and motion of the probe and probing force and indices that use wooden spatulas, according to its shape and rigidity, may represent a potential for trauma, Hofer et al. (2010) developed the Bleeding on Interdental Brushing Index (BOIB). This index is performed by inserting a light interdental brush placed buccally, just under the contact point and guided between the teeth with a jiggling motion, without force. Bleeding is scored as either present or absent, for each interdental site, after 30 s. The authors describe like advantages: atraumatic manipulation of the papillae, ease of application, integration into existing oral hygiene instruction and motivating patients to monitor their own progress at home, while at the same time performing a beneficial oral hygiene procedure and removing any interdental plaque that may be present.

Index Name (Abbreviation)	Author (s)	Year	Instrument	Graded response	Time delay (seconds)
PMA Index	Schour and Massler	1947	Probe	0-5	Not stated
Gingival Index (GI)	Löe and Silness	1963	Probe	0-3	Not stated
Sulcus Bleeding Index (SBI)	Muhlemann and Son	1971	Probe	0-5	Not stated
Gingival Bleeding Index (GBI)	Carter and Barnes	1974	Unwaxed dental floss	Dichotomous (yes/no bleeding)	Not stated; 30 s is allowed for reinspection
Gingival Bleeding Index (GBI)	Ainamo and Bay	1975	Probe	Dichotomous (yes/no bleeding)	10
Pappilary Bleeding Index (PBI)	Muhlemann	1977	Probe	0-4	Not stated
Papillary Bleeding Score (PBS)	Loesche	1979	Wooden interdental cleaner	0-5	Not stated
Modified Papillary Bleeding Index (MPBI)	Barnett et al.	1980	Probe	0-3	0-30
Bleeding Time Index (BTI)	Nowicki et al.	1981	Probe	0-4	0-15
Eastman Interdental Bleeding Index (EIBI)	Caton and Polson	1985	Wooden interdental cleaner	Dichotomous (yes/no bleeding)	0-15
Quantitative Gingival Bleeding Index (QGBI)	Garg and Kapoor	1985	Tooth brush	0-3	Not stated
Modified Gingival Index (MGI)	Lobene et al.	1986	No instrument (visual)	0-4	Not applicable
Bleeding on Interdental Brushing Index (BOIB)	Hofer et al.	2010	Interdental brush	Dichotomous (yes/no bleeding)	30

Table 1. Gingival Indices

3. Discussion

Since periodontal diseases are primarily inflammatory in nature, the ability to detect inflammatory lesions in gingival tissues is essential for the diagnosis and monitoring of changes in gingival status. Clinical indices provide a means of converting observed clinical data into numerical data for statistical analysis. Gingivitis indices have been based on clinical features of inflammation, and they contain components that are assessed non-invasively, by visual examination (e.g., color, texture, changes in form, spontaneous bleeding) and components that are assessed invasively (e.g., bleeding on stimulation or provocation) (Armitage, 1996).

Hazen et al. (1974) set guidelines for the choice of "most suitable or ideal" index. These were that (1) an index should be simple to use and low cost; (2) the criteria that describes the components of the index must be clear and easily understood; (3) the index should be equally sensitive across its range indicating the clinical phases of the disease; (4) the index must be amenable to statistical analysis. In addition, Carter and Barnes (1974) considered that a good index should measure those things that it purports to measure and at the same time be sensitive enough to recognize small degrees of change. For clinical trials, the precision, accuracy, reliability and the validity of the measurements produced must be evaluated. The validity of the indices, which can be estimated by calculating its sensitivity and specificity, must be considered when comparing different indices. The sensitivity of a diagnostic test refers to the probability of the test being positive when the disease is truly present. A perfect test would be able to detect the disease in all cases without registering a false negative. The specificity of a diagnostic test refers to the probability of the test being negative when the disease is not present. A perfect test would be able to correctly identify all instances in which the disease was absent without registering a false positive. The positive predictive value of a test refers to the probability that the disease is present when the test is positive. The negative predictive value refers to the probability that the disease is absent when the test is negative (Armitage, 2003).

One of the first clinical signs of gingival inflammation, besides the exudation of gingival fluid, is the redness of the gingival margin. It arises partly from the aggregation and enlargement of blood vessels in the immediate subepithelial connective tissue and the loss of keratinization of the facial aspects of gingiva. Swelling and loss of texture of the free gingiva reflect the loss of fibrous connective tissue and the semi liquidity of the interfibrillar substance. Bleeding occurs because of frequent micro-ulcerations in the epithelium that lines the gingival sulcus/periodontal pocket. Gingival bleeding has been used as a key parameter in the evaluation of gingivitis because of its objectivity and ease of clinical access. The fact that the gingival tissues can be provoked to bleed just by touching the gingival margin with a blunt instrument suggests that the epithelial changes and the vascular changes are well established. These findings support the importance and applicability of using indices of visual and bleeding changes (Chaves et al., 1993; Newbrun, 1996; Lang et al., 2009).

In one sense, gingival indices may be considered arbitrary in that any choice of criteria represents only one of many possible representations of the reality of the disease (Barnett, 1996). Nevertheless, in order to be useful, an index must have a substantiated relationship between signs, as defined by the index criteria, and actual clinical changes accompanying the progression of disease. Analyses of gingival biopsies with an inflammatory cell infiltrate in the gingival tissues are correlated with visual signs of inflammation and bleeding on

probing (Barendregt et al., 2002). This validation can also be done by correlating results using a new or modified index with results obtained using a previously accepted and validated index when both are included in the same study (Barnett, 1996).

Most of the indices include an invasive component, that is, they demand the use of an instrument. Periodontal probes (Massler, 1967; Löe & Silness, 1963; Muhlemann & Son, 1971; Ainamo & Bay, 1975; Muhlemann, 1977; Nowicki et al., 1981; Barnett, 1980), wooden interdental cleaners (Loesche, 1979; Caton & Polson, 1985), dental floss (Carter & Barnes, 1974) and tooth brushes (Garg & Kapoor, 1985; Hoffer et al., 2010) have all been utilized.. Unwaxed floss is simple to use and may be used by the dentist and patient. The GBI, which uses unwaxed floss, has good validity and reliability and can register subtle gingival changes. However, it has the disadvantage of not being immediately reproducible (Carter and Barnes, 1974).

Comparisons between clinical indices are difficult to assess. When probing is used for bleeding response, the force used, the probe size and probe position are factors that must be taken into consideration (reviewed in Listgarten, 1980). Regarding "bleeding on pressure", Bollmer et al. (1986) compared the intrusive gingival index (GI) for estimating gingivitis with a nonintrusive visual index. The results indicated that the methods were similar and the number of bleeding sites per subject did not diminish after manipulation. Thus, for those studies where it is desirable to measure bleeding sites, the GI index is recommended. The pressure used for probing must be standardized since the percentage of sites with bleeding on probing increases linearly with an increase in probing force. A maximum force of 0.25 N has been suggested in order to limit the number of false positive readings. According to Armitage (2003), bleeding on probing could, in several instances, be the effect of mechanical trauma of healthy sites. However, to date the appropriate probing pressure to be applied to minimize false positives and hence, distinguish between health and disease in the gingival tissues has not been definitely determined.

Another common variation is whether probing is performed in the marginal gingiva or in the bottom of the pocket. From a diagnostic perspective, it is unclear as to which of these is the more sensitive indicator of early gingival pathology. Van der Weijden et al. (1994) assessed gingival bleeding by running a probe along the marginal gingiva, at an angle of approximal 60˚ to the longitudinal axis of the tooth. This method was compared to probing to the bottom of the pocket. They considered that marginal probing more accurately evaluated a healthy gingival condition and was the most appropriate method to detect differences in the development of gingivitis between experimental groups.

The following objections to the use of invasive procedures for indices used in clinical trials are (1) the effect of probing on disrupting plaque at the gingival margin and on producing trauma to the gingiva; (2) the impediment to calibrating examiners or assessing the reliability of a single examiner using the same subjects and gingival areas; (3) the obscuring of specific bleeding sites by blood oozing from previously probed areas on the opposite or adjacent tooth surfaces. Divergent schools of thought have developed with regard to selecting the appropriate gingival index. One opinion maintains that only invasive indices should be utilized, since indices that include a bleeding-on-provocation component are, by definition, the most objective. These clinical investigators hold the view that visual indices are not appropriate because of their "subjectiveness" (Barnett, 1996). An alternative opinion maintains that, for longitudinal plaque and gingivitis studies, non-invasive indices are the most appropriate because invasive procedures will not only disrupt the plaque but also

could mildly traumatize the tissue and present an impediment to assessing examiner standardization and reproducibility. Also, a variety of measures are used to elicit bleeding and can vary a lot between studies. Variable factors include the time between provocation and bleeding, the depth of sulcular insertion of the probe, the probing technique, the angle of insertion, and the probing force (Lorenz et al., 2009). The non-invasive index developed by Lobene et al. in 1986 (MGI) satisfies the criteria for a gingival index specified in the A.D.A. Council on Dental Therapeutics guidelines and has been generally accepted for use in clinical trials (Barnett, 1996). The authors showed that MGI increases the sensitivity of assessing early visual changes, which occur during the onset or regression of gingivitis. According to Lorenz et al. (2009), there is no doubt that indices containing a bleeding component can successfully be used in clinical trials. On the other hand, as discussed by Barnett (1996), the data presented indicate that non-invasive and invasive gingival indices contain both subjective and objective aspects to their use and the evidence does not support the assumption that invasive indices are truly objective. Therefore, utilizing a pure visual index in assessing gingivitis can be an alternative to an invasive index.

Some of the bleeding indices described are dichotomous; they record the presence or absence of bleeding (Carter & Barnes, 1974; Ainamo & Bay, 1975; Caton & Polson, 1985; Hoffer et al. 2010). However, clinicians who perform periodontal examinations recognize that a range of bleeding responses occurs in relation to extent and time bleeding occurs after provocation (Newbrun, 1996). Several bleeding indices, described previously, use different scales of bleeding response (Schour & Massler, 1947; Löe & Silness, 1963; Muhlemann & Son, 1971; Muhlemann, 1977; Nowicki et al., 1981; Barnett, 1980; Loesche, 1979; Garg & Kapoor, 1985; Lobene et al., 1986). The results of GBI (Ainamo and Bay, 1975) showed that the score obtained correlate significantly with the GI index (Löe and Silness, 1963) scores of the same persons and the simplification of GI (with a simple dichotomous score) did not seem to reduce the accuracy of the results obtained.

A striking problem in developing a gingivitis index is the lack of agreement as to the measurement criteria to be used and the evaluation standards to be employed. Examiner subjectivity as to what constitutes inflammation and the difficulty in accurately registering the related signs of gingival disease are among the major obstacles. Reports described in the literature have suggested that successive measures of gingival inflammation, evaluated by bleeding, performed by one or more examiners may not be reproducible (Feldman et al., 1982). Consequently the reproducibility of bleeding measurements has been a problem. It is important to note that the degree of intra- and inter-examiner reliability achievable is fundamental in deciding which index is appropriate for use in clinical trials and epidemiological studies (Kingman, 1986). The EIBI have been shown to have high levels of examiner agreement and a reason for this reliability may be due to the method of stimulation used for bleeding and the location (mid-interproximal tissue) of the inflammatory lesions that were examined (Blieden et al., 1992).

The choice of index should depend on the purpose of a study. For epidemiological surveys, partial recording of selected teeth or sites may be sufficient. On the other hand, for research and clinical trials, a quantitative measurement of bleeding is more informative than a dichotomous index of presence or absence of bleeding on stimulation. For patient education and motivation, a dichotomous index will suffice (Newbrun, 1996; Barnett, 1996). Although some clinical investigators may favor a given index to the exclusion of all others, a variety of indices could be appropriate for use in clinical trials

(Barnett 1996). As argued by Lobene (1986), it is clear from the number of indices that no one index universal application. The most important quality that an index must have is validity; does the scoring system measure what it purports to measure? If it does, then the index selected is the appropriate measurement for evaluating the outcome of the study. Another point that must be considered is the examiner's training. McClanahan et al. (2001) demonstrated that clinicians develop a specific style when recording gingival indices. This behavior strongly influences the recorded data and impacts on a number of important outcomes, which include measurement of disease levels, examiner calibration, power calculations, treatment differences, and determination of clinical significance. It is tempting to try to identify one particular style of examining as "correct." However, a judgment of this kind would be arbitrary in the absence of a common objective standard for examiner calibration. Therefore, it should not be assumed that an individual, untrained in either the conduct of clinical investigations or in the use of a given index, can successfully conduct a clinical trial without prior training. Thus, irrespective of the index utilized, the rigorous calibration and standardization of examiners by an investigator experienced in the use of the index is essential for its successful use in a clinical trial (Barnett, 1996). An absence of interexaminer calibration will impact on the structure of the resulting data set. It is particularly relevant to dental researchers, practicing dentists, and organizations such as the FDA and ADA where global assessments of the clinical effectiveness of products and treatments are routinely made by examining the results of independent clinical trials conducted by different sponsoring organizations. In these situations, interexaminer calibration of examiners is not routinely possible (McClanahan et al., 2001)

Periodontal diagnoses are determined by analyzing the information collected during a clinical examination. The information collected during such an examination includes demographic data (e.g., age, gender, etc.), medical history, history of previous and current periodontal problems, periodontal probe measurements (i.e., probing depths, clinical attachment loss, etc.), radiographic findings, and miscellaneous clinical features or observations (e.g., gingival inflammation, biofilm/calculus, mobility, occlusal problems). In some situations, supplemental qualitative or quantitative assessments of the gingival crevicular fluid (GCF) and subgingival microflora are performed. In addition, a genetic test for susceptibility to chronic periodontitis has become commercially available (Armitage, 2003). According to the American Academy of Periodontology (Burt et al., 2005), the most promising disease markers are the inflammatory cytokines that are expressed in gingival crevicular fluid (GCF) as part of the host response to inflammation. These cytokines include prostaglandin E2 (PGE2), tumor necrosis factor-alpha (TNF-α), IL-1 alpha (IL-1α), IL-1 beta (IL-1β), and others. While it has been documented that these and other constituents of GCF are associated with inflammatory response, actually quantifying these associations and determining the sensitivity of the measures (i.e., the extent to which the quantity of expressed cytokine goes up or down as inflammation goes up and down) is proving more difficult. New methods for assessing early gingival changes are being investigated. Gleissner et al. (2006) used the laser Doppler flowmeter to evaluate non-invasively changes in gingival blood flow (GBF). The authors observed that although it is a valuable, non-invasive method for clinical research of gingival microcirculation modifications of the probe are needed to improve its clinical applicability.

At the present time, supplemental information on GCF components, the subgingival microflora, and genetic susceptibility and other methods, such as laser Doppler flowmeter, are are still being evaluated (Armitage, 2003; Burt et al., 2005). Until these and other new, valid and reliable measures of disease are available the clinical visual-tactile indices will continue to be the most widely used and accepted methods of assessment.

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4

Etiology of Gingivitis

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1. Introduction

Gingival diseases are a group of different disease entities that are localized to the gingiva. They all manifest clinical signs of inflammation and are classified into two main groups: plaque-induced and non-plaque-induced gingival diseases.

2. Non-plaque-induced gingival diseases

Gingival lesions not induced by plaque are common and can help explain the many different periodontal tissue reactions observed. Gingival inflammation sometimes differs from that of routine plaque-associated gingival diseases and often presents distinctive clinical features. (Holmstrup, 1999a).
The causes of non-plaque-induced gingival diseases include bacterial, viral, and fungal infections, genetic disorders and mucocutaneous diseases (e.g., lichen planus). Traumatic tooth brushing and allergic reactions to drugs are other possible causes.

2.1 Gingival lesions associated with specific bacterial infections

Bacterial infections can affect patients with and without immunodeficiency. Neisseria gonorrhoeae, Treponema pallidum, streptococci, Mycobacterium chelonae, are the most common bacterial infections that give rise to gingival lesions. They can manifest as fiery red, edematous, and painful ulcerations, asymptomatic chancres, mucous patches or atypical non-ulcerated, highly inflamed gingiva (Holmstrup, 1999b). These lesions may be associated with lesions on other parts of the body.

2.2 Viral infections

The most common viral infections are herpes simplex virus type1(HSV-1) and 2 (HSV-2) and varicella-zoster virus.
HSV is the most common viral infection of the oral/facial area. It has two subtypes: type 1, which affects the oral cavity; and type 2, which affects the genitals. Primary herpetic gingivo-stomatitis is most commonly observed in children from 7 months to 4 years of age but can also be found in adolescents or young adults. Children are often infected with HSV by their own parents if these have recurrent herpes lesions. The primary infection may be asymptomatic but can manifest as severe gingivostomatitis, in which the gingiva are

painful, inflamed and ulcerated. Fever and lymphadenopathy are classic features and affected individuals experience difficulty in chewing.

The incubation period of the virus is 1 week, and healing occurs after approximately 10 to 14 days. Following infection and local replication at mucosal surfaces, HSV 1 enters sensory nerve endings and is transported by retrograde axonal transport to neuronal cell bodies, where a more restricted replication cycle takes place, usually culminating in the latent infection of these neurons. Latency allows maintenance of the viral genome in non-pathogenic and non-replicate form, serving as a reservoir for a later viral attack on the host. Reactivation of the virus in sensory ganglia causes cutaneous and mucocutaneous infection of the face, usually on the lips (Tovaru et al 2010). Reactivation of the virus is normally triggered by trauma, sun exposure, or menstrual periods, among other factors. These lesions are present in around 50% of the population and around 80% incubate the virus in latent form. Recurrent herpes infections can be found intra- and extra-orally. Intraoral herpes infection manifests as a group of painful ulcerations involving the gingiva and hard palate. The infection can be clinically diagnosed and confirmed by isolating the virus. HIV-positive individuals are more susceptible to viral infections and recurrence of herpetic lesions can be severe and potentially fatal in these patients.

Varicella-zoster virus causes chickenpox, primarily in children, and later reactivation of the virus in adults causes herpes zoster (shingles). Both can involve the gingiva, presenting as vesicle lesions that burst leaving fibrin-covered lesions.

This infection is readily diagnosed from the intense associated pain and unilateral lesions, which generally heal after 1-2 weeks. (Figure 1)

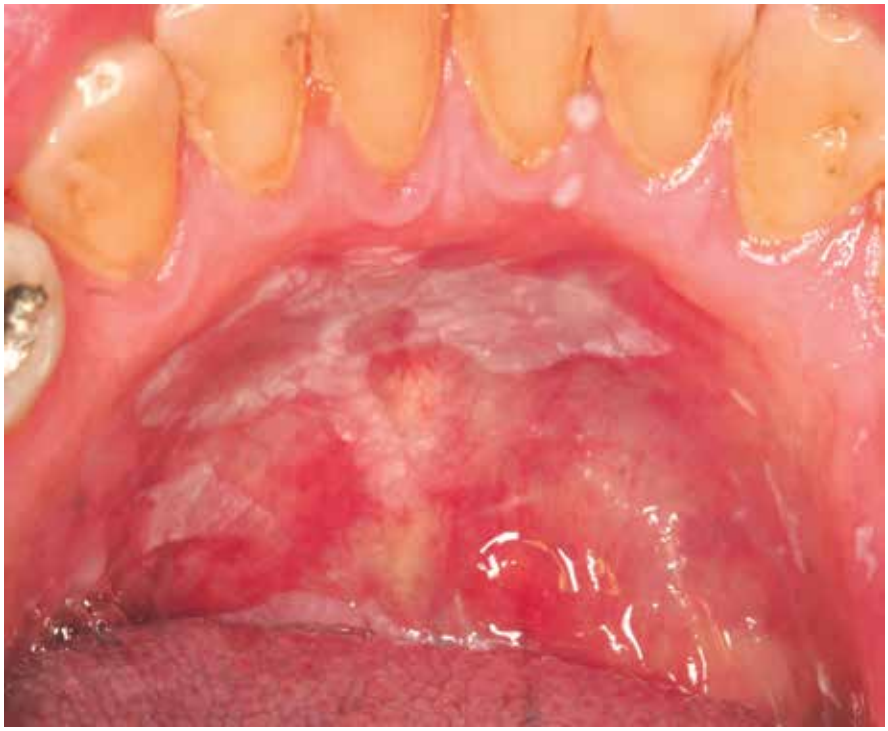

Fig. 1. HIV infection

2.3 Gingival lesions associated with fungal infections

Gingival inflammation can also be caused by fungal infections such as candidosis, linear gingival erythema, and histoplasmosis.

2.3.1 Candidosis

Candida albicans is a diploid fungus (a form of yeast) that causes opportunistic oral and genital infections in humans. This commensal species populates the gastrointestinal tract. It affects 80% of the human population with no harmful effects but its overgrowth results in candidosis.

Infection by candida albicans is the most common mycotic infection of the oral mucosa and is commonly associated with impaired host immune responses such as:immunodeficiency, reduced salivary flow rate, smoking, corticosteroid treatment, or the use of antibiotiotics Candidosis is not usually observed in the gingival tissue of healthy individuals but, when affected, the most frequent clinical feature is gingival redness associated with a granular surface. This infection appears in four main forms: acute pseudomembranous candidosis, acute atrophic candidosis, chronic hyperplastic candidosis, and chronic atrophic candidosis.

The diagnostic criteria for oral candidosis are: white plaques or diffuse erythematous areas, culture of C.albicans from saliva, presence of mycelium on direct examination of a smear from the lesion, biopsy showing hyphae in epithelium and characteristic histological changes, serum fluorescent antibody titer against Candida albicans > 1:16, and a positive antibody test with neat saliva. A further characteristic of primary oral candidosis is the alteration or disappearance of the lesion after treatment with antifungal agents (Holmstrup & Axéll, 1990).

2.3.2 Linear gingival erythema

Linear gingival erythema is a non-plaque-induced gingival lesion exhibiting a distinct erythematous band of the marginal gingiva, with either diffuse or punctate erythema of the attached gingiva. It is characterized by intense gingival inflammation which does not respond to treatment with scaling and root planing or hygiene control. According to recent studies, the prevalence of linear gingival erythema ranges from 2 to 25% (Umadevi et al 2006).

2.3.3 Histoplasmosis

Histoplasmosis is caused by the fungus Histoplasma capsulatum, a soil saprophyte mainly found in the feces of cats and birds. H. capsulatum exists in mycelial form at room temperature and in yeast form at body temperature. Infection by H. capsulatum usually occurs by inhalation of the spores and their deposition in the lungs. Most infections in the normal host are subclinical and asymptomatic.

There are three forms of histoplasmosis: a primary acute pulmonary form that is usually asymptomatic but may cause flu-like symptoms; a chronic pulmonary form, observed in a small percentage of patients, which is usually associated with underlying pulmonary disease; and a severe disseminated form, which is rare and observed in immunocompromised patients at the extremes of age or in otherwise debilitated patients. In recent years, histoplasmosis has joined other mycoses as a serious opportunistic infection associated with AIDS.

Histoplasmosis in the head and neck is mainly seen in patients with disseminated disease and may be their only symptom. Oral lesions have been reported in 30% of patients with pulmonary histoplasmosis and 66% of patients with disseminated disease. They have been reported throughout the oral cavity, including gingiva, tongue, palate, buccal mucosa, oropharynx, retromolar area, lip, and floor of mouth. The lesions have been described as flat, plaque-like, non-tender elevations, either papillary or nodular, which subsequently ulcerate and become painful. Other descriptions include an erythematous, granulomatous appearance, a pattern of scattered red and white granulomatous lesions, and ulcerations as large as 2.5 cm in diameter. The definitive diagnosis is based on smear or culture and histology. (Stanford & Rivera Hidalgo, 1999)

2.4 Gingival lesions associated with genetic disorders

Hereditary gingival fibromatosis is a very rare condition. It develops as an isolated disorder or as one feature of a syndrome; the most frequent characteristic is hypertrichosis;. Occasionally it is associated with mental retardation and epilepsy. The hyperplasic gingiva has a normal color and firm consistency with abundant stippling in adjacent gingiva. The buccal and lingual tissue of both mandible and maxilla may be involved, with inter-individual variation in the degree of hyperplasia. Gingival fibromatosis can also be inherited as an autosomal dominant or recessive condition. The gingival enlargement usually begins with the eruption of permanent dentition. Gingival fibromatosis cannot be cured and usually involves the removal of large amounts of gingival tissue by conventional external bevel gingivectomy. (Ramer et al 1996)

2.5 Gingival lesions associated with systemic conditions

Systemic conditions that are associated with gingival inflammation include lichen planus, pemphigoid, pemphigoid vulgaris, multiform erythema, lupus erythema, drug-induced mucocutaneous diseases, and allergic reactions. Dermatologic diseases include not only numerous primary skin diseases but also common cutaneous manifestations of visceral or systemic diseases that may involve the oral mucosa. Dermatology is currently of major scientific and odontological interest, since oral lesions can be very early or even the only signs of various diseases (Gonçalves et al 2010). One of the main gingival disorders unrelated to plaque accumulation is desquamative gingivitis, which is characterized by epithelial desquamation, erythema, ulceration and/or vesiculobullous lesions in the gingiva and other epithelial tissues.

2.5.1 Lichen planus

Lichen planus is the most common mucocutaneous disease involving the gingiva, with a prevalence of 0.5-2.5%. Some individuals present with lesions at other sites, such as the skin, while others are only affected in the oral cavity. The control and treatment of lichen planus is important, since it has premalignant potential. Its etiology is unknown. It manifests clinically in reticular or atrophic-erosive form, and the lesions show white papulae and striae, usually bilateral. Atrophic-erosive ulcerated lesions can sometimes be painful, whereas reticular forms have no significant symptoms. Lichen planus can affect any area of the oral mucosa, and its clinical appearance and extent can vary over the years, requiring differential diagnosis with other diseases such as leukoplakia. Subepithelial inflammatory reactions produced by lichen planus lesions are due to a specific unknown antigen, which is found at the point of attachment between the connective and epithelial tissues of the gum. (Holmstrup & Dabelsteen, 1979). (Figure 2,3)

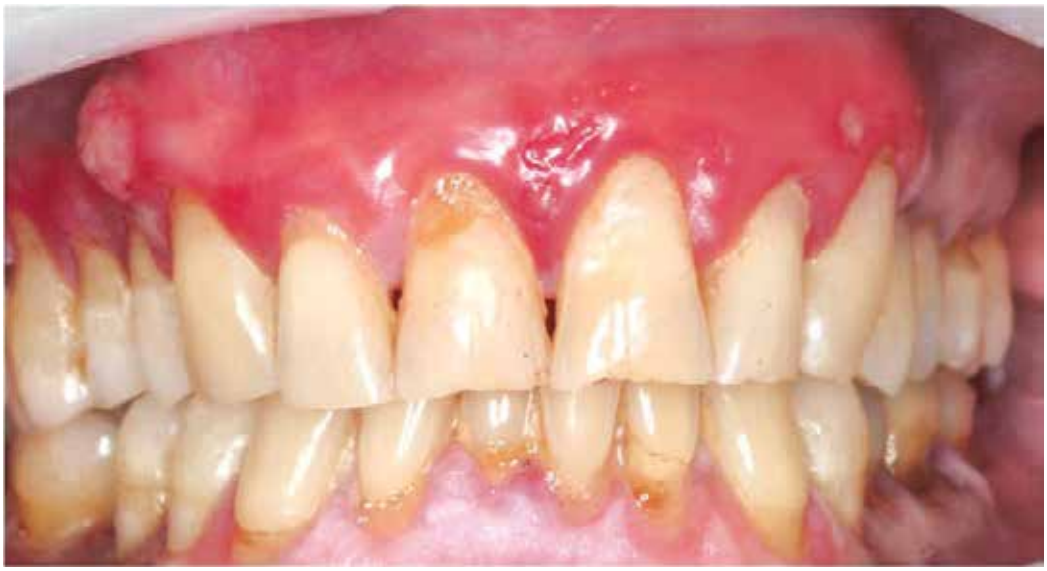

Fig. 2. Erosive formo of lichen planus

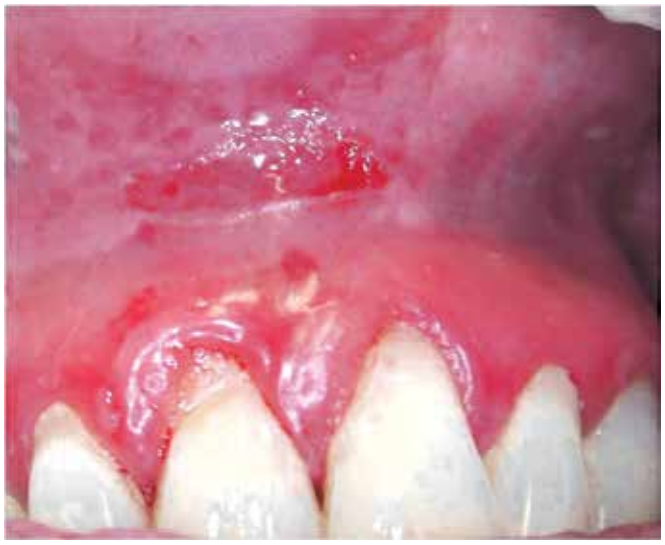

Fig. 3. Atrophic-erosive Lichen planus

Oral lichen planus is usually diagnosed by clinical and histological examinations, although the clinical appearance alone can suffice in classical lesions (bilateral white striae in cheek mucosa). The differential diagnosis includes lichenoid reactions to drugs or dental materials, leukoplakia, lupus erythematosus, and graft vs. host disease in bone marrow transplantation patients. Desquamative gingivitis may also be mistaken for pemphigus, pemphigoid, dermatitis herpetiformis, or linear IgA disease. Therefore, complementary examinations are essential in all cases to diagnose or rule out malignancy (Canto et al 2010).(Figure 4,5)

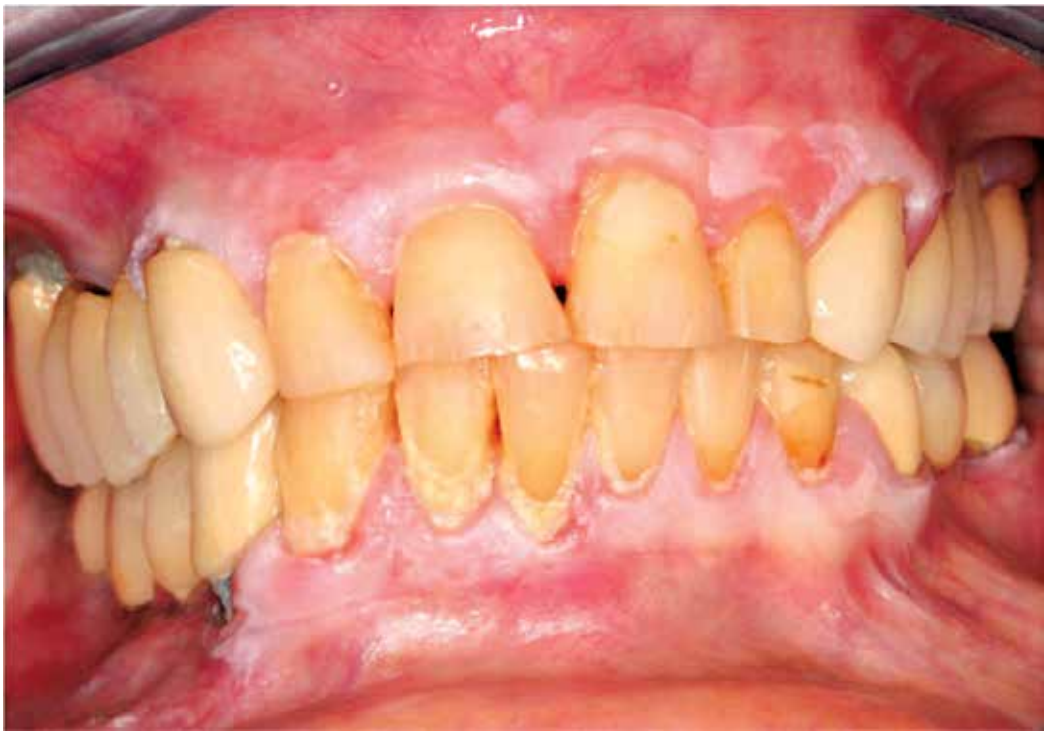

Fig. 4. Leukoplakia

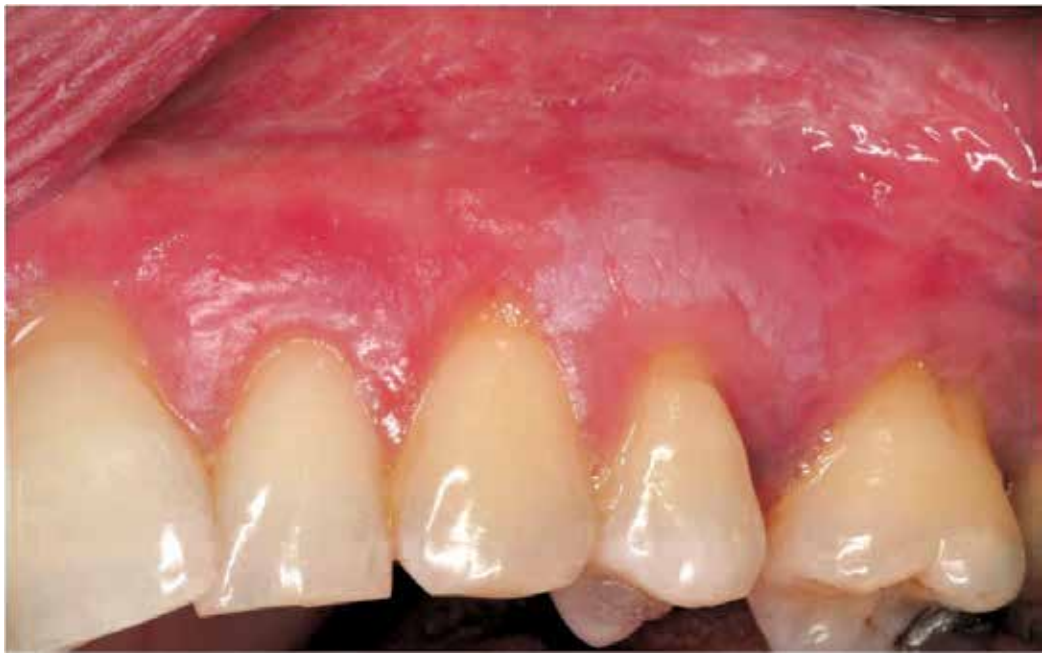

Fig. 5. Leukoplakia

2.5.2 Pemphigoid

Pemphigoid is a group of disorders in which antibodies against the basal membrane components cause the epithelium to detach from the adjacent connective tissue. This condition normally appears in women over 50 years of age. It mainly affects the skin but can also affect the oral mucosa. Oral manifestations are almost inevitable, and the mouth is frequently the first affected site. The course of the disease is slow and progressive. Oral lesions (blisters) can be found in gingival tissue, buccal mucosa, and palate. These remain intact for the first 24-48 hours but finally burst, producing pain and stinging. (*Nickolsky positive*) Extraoral lesions on other sites are less common, although symblepharons are found on ocular mucosa and can lead to blindness if left untreated. (Figure 6)

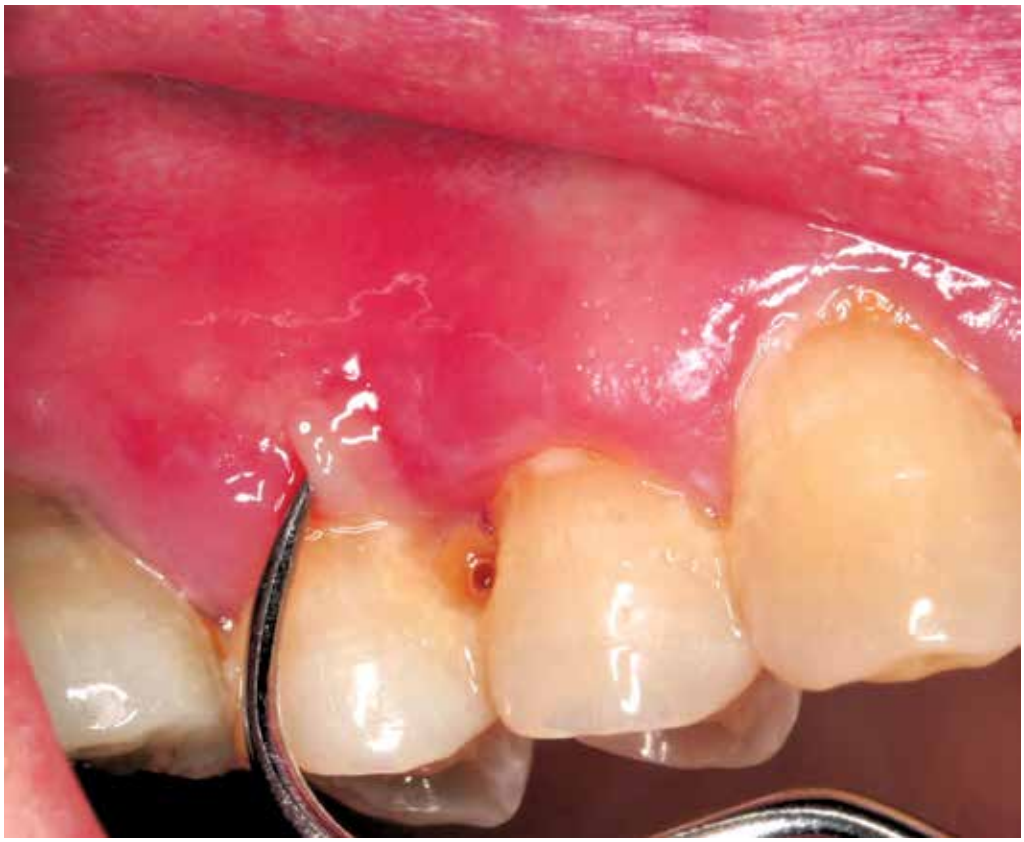

Fig. 6. Pemphigoid

2.5.3 Pemphigus

Pemphigus is group of autoimmune diseases characterized by the formation of blisters, due to loss of cohesion between keratinocytes in the epidermis caused by antibodies against desmoglein 1 and desmoglein 3. The mechanism by which the formation of antibodies is triggered remains unknown. Pemphigus vulgaris is the most common and severe form and is most frequent in the 6th and 7th decades of life. Its etiology appears to be genetic, while it is more frequent in individuals exposed to certain predisposing factors, including UV light and certain drugs. The blisters cover large areas of the skin and can be fatal if untreated.

Individuals suspected of this condition must be referred immediately to a dermatologist. (Figure 7,8)

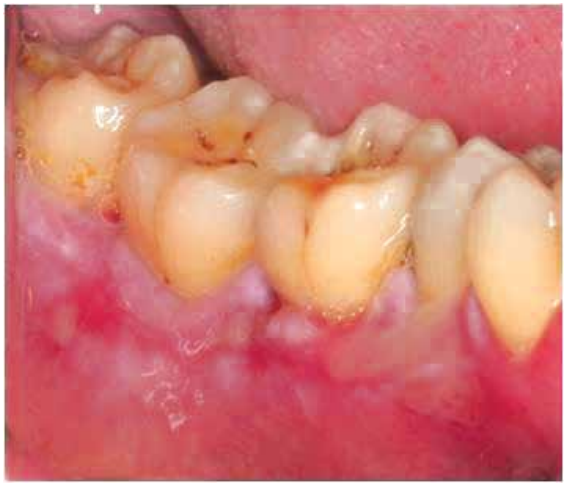

Fig. 7. Pemphigus

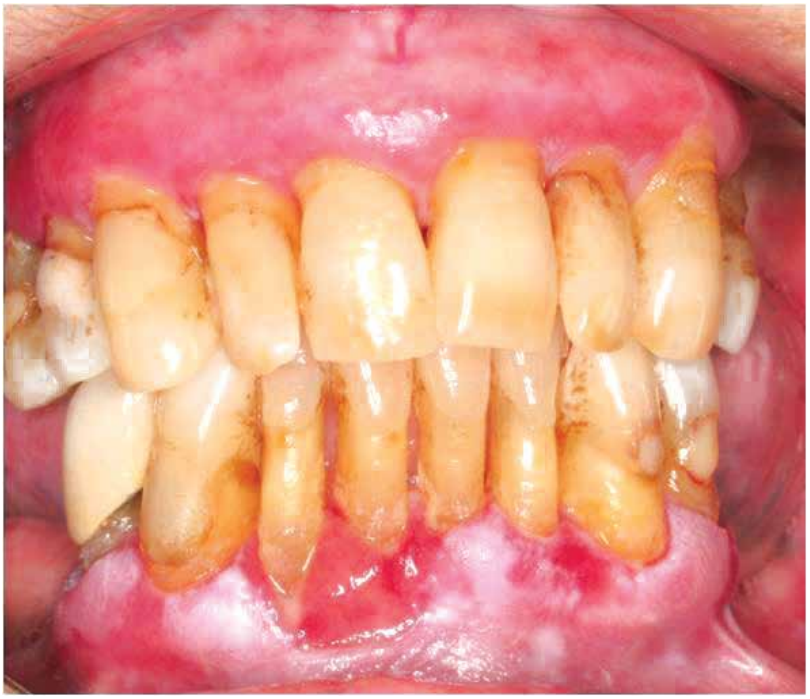

Fig. 8. Pemphigus

In the mouth, the lesions are localized in the gingival tissue and other sites. The blisters are small, fragile and asymptomatic until they burst, when they leave extremely painful and slightly bleeding erosions. The lesions contain non-adherent epithelial cells known as

Tzanck cells. The treatment for pemphigus was decidedly unsatisfactory before the appearance of glucocorticosteroids in the 1950s, with mortality rates of 60-90%. However, the high doses of glucocorticosteroids required to control pemphigus has led to a search for other agents with a higher therapeutic index. (Korman, 1988). The therapy currently consists of systemic corticosteroids and rigorous hygiene control.

2.5.4 Erythema multiforme

Erythema multiforme is a skin condition of unknown origin, possibly mediated by the deposition of immune complexes (mostly IgM) in the superficial microvasculature of the skin and oral mucous membrane. It usually follows infection or drug exposure. It is a common disorder, with peak incidence in the 2nd and 3rd decades of life. Onset of this disease is usually preceded by general malaise.

The disease spectrum includes a mild and self-limited skin variant with exanthematic characteristics and minimal oral involvement and a severe, progressive variant that leads to extensive mucocutaneous epithelial necrosis, also known as Stevens-Johnson disease. In the milder form, oral lesions in the labial and buccal mucosa, tongue, palate, and gums change from papulae to blisters. Recurrence is very frequent, and the healing of lesions can take several weeks. Erythema multiforme has always posed a diagnostic challenge due to its extremely varied features, which can mimic other diseases. Nevertheless, the natural history and clinical and histological findings usually allow other conditions to be ruled out and a definitive diagnosis to be reached (Lozada & Silverman, 1978). Treatment calls for corticosteroids and plaque control.(Figure 9).

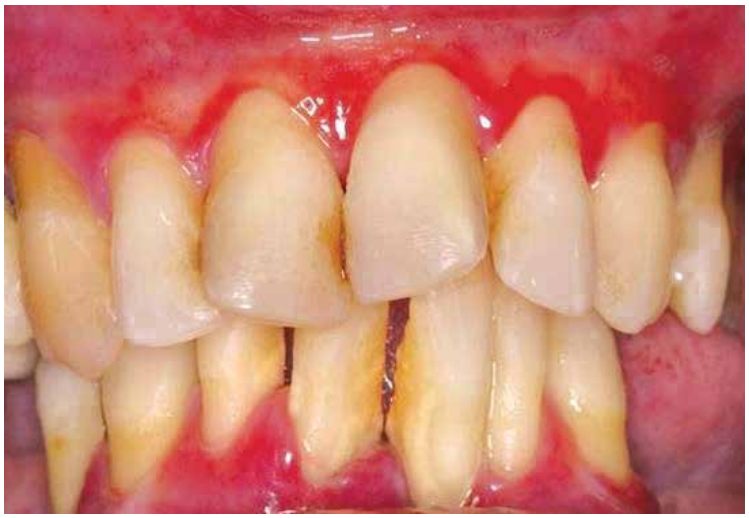

Fig. 9. Chronic desquamative gingivitis

2.5.5 Lupus erythematosus

Lupus erythematosus is a group of diseases in which anti-self antibodies are formed against various cell components, including the nucleus and cytoplasmic membrane. It more frequently affects females but shows no predilection for any anatomic site. The disease has two forms. One is systemic lupus erythematosus, which can affect mucosa, skin and other organs, including the kidneys and heart; the patients suffer from fever, weight loss, arthritis,

and a typical butterfly-wing erythema on the cheekbone. The other, localized form is discoid lupus erythematosus, which only involves skin and mucosa. Orally, discoid lupus presents round erythematous lesions with a depressed central region surrounded by white striae, as well as less typical lesions, similar to Wickham striae, in the buccal mucosa, gingiva, and tongue. Treatment of the disorder includes topical corticosteroids alone or combined with immunosuppressants, and systemic corticosteroids in more severe cases.

2.6 Drug induced gingival lesions

Drug-induced mucocutaneous disorders can produce gingival hyperplasia, also known as gingival enlargement. Drug-induced gingival overgrowth is a side effect of three types of drugs: anticonvulsants (eg.Epanutin) , immunosuppressive agents (eg. Cyclosporine), and various calcium channel blockers (e.g., Nifedipine) used for cardiovascular diseases. The overgrowth is characterized by the accumulation of extracellular matrix in gingival connective tissues. Recent studies suggested that these disorders may be induced by disruption of collagen synthesis homeostasis and gingival tissue degradation (Kataoka et al 2005a)

2.7 Allergic reactions

Oral manifestations of allergic reactions are uncommon. Reactions are mainly type I (immediate, mediated by IgE) or type IV (deferred, mediated by T cells). There are various possible causal agents, including materials used in dental procedures, oral hygiene products, chewing gum and foods. Materials such as mercury, gold, and acrylics can trigger type IV reactions, followed by the onset of white or erythematous lesions in the gingiva after 24-48 hours. Removal of the allergenic material is sufficient to stop the reaction. Dentifrices and mouthwashes can can cause edematous and red gingiva and affect the tongue. Foods that can potentially cause allergic reactions type I and IV include peanuts, kiwi fruit, and peaches.

2.8 Other gingival manifestations of systemic diseases

Other systemic diseases with gingival manifestations include gastrointestinal diseases (e.g., Crohn´s disease), leukemia, and diabetes mellitus.

2.8.1 Crohn´s disease

Crohn's disease, also known as regional enteritis, is an inflammatory disease of the intestines that can involve any part of the gastrointestinal tract, causing a wide variety of symptoms. It primarily causes abdominal pain, diarrhea (which may be bloody if the inflammation is severe), vomiting, or weight loss, but may also cause complications outside the gastrointestinal tract, such as rashes, arthritis, eye inflammation, tiredness, and lack of concentration. The oral lesions in Crohn´s disease are similar to those of the gastrointestinal tract, including large ulcerations. The oral lesions are sometimes the first signs of the disease. Typical oral manifestations are folds in the labial or buccal sides of the sulcus.

2.8.2 Leukemia

Leukemia is a malignant haematologcal disorder characterized by an abnormal increase in white blood cells.. Leukemia is classified according to the course of the disease (acute or chronic) and the cell type involved. The main types are acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, and chronic myelogenous

leukemia. Oral manifestations have been reported in patients with acute monocytic leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, and chronic lymphocytic leukemia. Gingival infiltration is the initial presenting complication in 5% of acute monocytic leukemia cases. Gingival infiltration of leukemic cells is most commonly seen in acute monocytic leukemia (M5) and acute myelomonocytic leukemia (Demirer et al 2007). Manifestations of leukemia include extended edema, ulceration, petechiae, and erythema. These are much more common in the acute than in the chronic form of the disease. Gingival edema in leukemic patients is mainly caused by plaque-induced inflammation, and plaque control is important. Gingival bleeding is also frequent in these patients due to secondary thrombocytopenia. Dental treatment is based on plaque control and, sometimes, on oral antibiotics administered one day before and one day after mechanical debridement.

2.8.3 Diabetes mellitus

Diabetes mellitus is characterized by disorders in insulin production, the metabolism of carbohydrates, fats, and proteins and in the functioning and structure of blood vessels. Patients with type I diabetes are at greater risk of developing gingivitis. Both children and adults with poor metabolic control show a greater tendency towards more severe gingivitis. The prevalence of gingivitis in children and adolescents with diabetes is nearly twice that observed in children and adolescents without this disease. The association between diabetes and gingivitis in children and adolescents is so widely accepted that diabetes mellitus–associated gingivitis appears as a specific entity in the most recent classification of periodontal diseases. Adults with type II diabetes may show higher rates of gingival inflammation *versus* adults without diabetes. Almost 64% of diabetics are estimated to have gingival inflammation, in comparison to 50% of non-diabetics. (Figure 10).

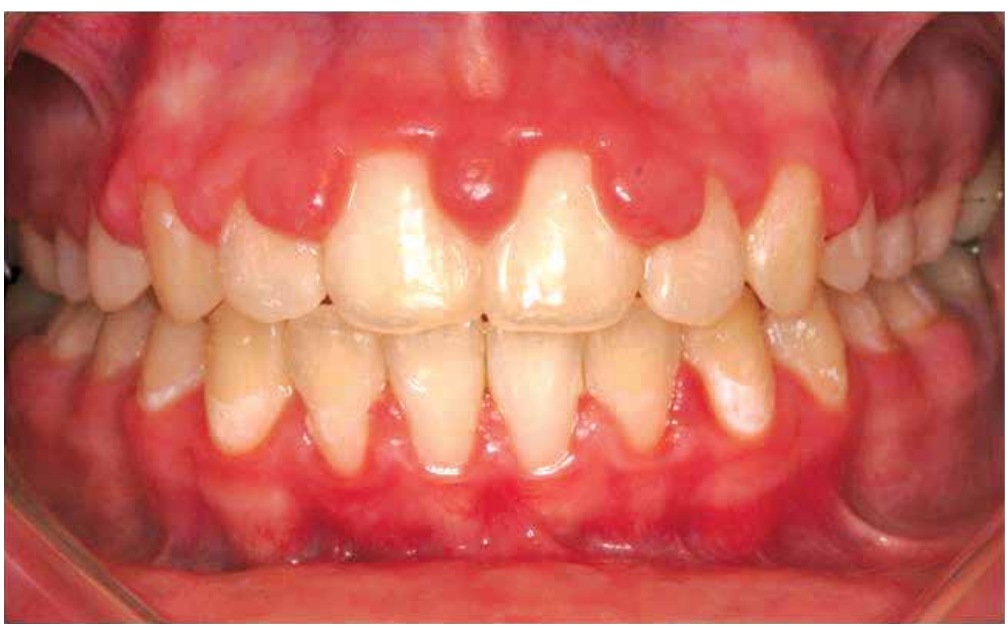

Fig. 10. Diabetes

The degree of metabolic control in diabetes appears to be an important factor in the development and progression of gingivitis. Serum fructosamine levels, reflecting the patient's glycemic control over the preceding 2-3 weeks, are positively correlated with the degree of gingival bleeding and the severity of gingival inflammation in adults with type II and children with type I diabetes, respectively. The correlation between serum fructosamine levels and gingival inflammation in children was recently reported to persist into adolescence. Glycosylated hemoglobin (HbA_{1c}) values (reflecting blood glucose concentrations averaged over the preceding 6-8 weeks) above 10% (normal values = 4–6%) appear to especially predispose children and adolescents to gingivitis. Notably, the presence of gingivitis in diabetic patients is not related to higher levels of plaque accumulation. The normalization of glycemic levels may significantly reduce the severity and extent of gingivitis in diabetic patients (Ryan et al 2003).

2.9 Gingival lesions associated with trauma

Injuries to oral soft-tissues can be produced by accidental, iatrogenic, and factitious traumas. Traumatic lesions, whether chemical, physical, or thermal, are relatively common in the mouth. Physical injury can also be self-inflicted (gingivitis artefacta), i.e., resulting from accidental trauma, premeditated infliction, or chronic habits, e.g., fingernail biting, digit sucking, or sucking on objects such as pens, pencils, or pacifiers (Dilsiz & Aydin, 2009).

Physical trauma (e.g., from aggressive tooth brushing) can cause gingival lesions. Hyperkeratosis is the gingival response when the trauma is limited, whereas gingival laceration of the surface and tissue loss (gingival recession) can result from more violent traumas. Horizontal movement of the brush, abrasive dentifrices, and dental floss can also produce physical trauma of the gingiva. It is difficult to diagnosis these lesions by clinical evaluation, and the etiology cannot be identified in some cases.

Chemical injuries, such as those caused by chlorhexidine, are reversible and resolved by removal of the toxic substance.

Thermal injuries to the oral mucosa are commonly caused by hot drinks or food and most frequently affect the palate and labial mucosa. These lesions are painful, with an erythematous appearance, and may present vesicles, ulcerations or erosions of the mucosa.

Foreign bodies can also cause lesions in the oral cavity through the entrapment of materials, e.g., dental amalgam, in the gingival connective tissue. Amalgam pigmentation, generally called amalgam tattoo, is a relatively common finding in the oral mucosa. Tissue reaction to amalgam can vary considerably. It can arise as a macrophage or chronic inflammatory response, usually in the form of a foreign body reaction, or there can be no reaction (Santos Parizi & Nai, 2010)

3. Plaque-induced gingival diseases

This group of gingival diseases are very prevalent and are initiated by dental plaque. The clinical features reflect the hosts's inflammatory and immune responses to the plaque bacteria. The clinical features of this condition include redness, swelling, and bleeding on probing. Other factors such as systemic disease, hormones, genetics, drugs, and malnutrition may influence the signs and symptoms of the disease.

The classification of plaque-induced gingival diseases is based on the presence of dental plaque and the local or systemic factors that influence the level of gingival inflammation.

3.1 Local modifying factors

3.1.1 Tooth anatomy

3.1.1.1 Tooth position

The position or inclination of teeth can predispose the periodontium to plaque accumulation and subsequent inflammation. While studies show that areas of the periodontium adjacent to malaligned teeth can be maintained in a good state of health, periodontal disease can occur if meticulous oral hygiene is not practiced. In children, plaque and gingival inflammation scores have been correlated with malalignment.

3.1.1.2 Root proximity

Root proximity may present an impediment to self-performed or professionally applied plaque removal, increasing the risk of gingival inflammation.

3.1.1.3 Open contacts

Food impaction is likely if there are open contacts between the teeth. A significant relationship has been observed between food impaction and probing depth in a group of 40 healthy young male naval recruits, and the researchers concluded that food impaction contributes to periodontal disease.

3.1.1.4 Root abnormalities

Palato-gingival grooves are developmental abnormalities mainly observed in maxillary incisors. The presence of a groove from the crown that extends apically at the gingival margin can impede the removal of plaque and allow access of plaque microorganisms to the subgingival area. Proximal root groves can also be found on incisor teeth and maxillary premolars. These grooves have been associated with poor periodontal health, including attachment and bone loss, and they can appear on any tooth surface.

3.1.1.5 Tooth restorations

Poor restorations with ill fitting margins and orthodontic appliances can negatively influence the health of adjacent gingival tissues. It has been hypothesized that restorations violating the so-called ¨biological width¨ can produce an inflammatory response that may result in the loss of bone and connective tissue attachment and the migration of epithelial attachment. In most cases, the amount of damage to the periodontium is influenced by the severity and duration of the marginal discrepancy and the ability of patients to maintain the areas free of plaque.

3.1.1.6 Effects of restorative materials

Allergies to metals and acrylics commonly used in dental restorations have been reported. Damage to the periodontium can result from allergy to one or more of these materials (Blieden, 1999).

3.2 Systemic modifying factors

3.2.1 Endogenous hormones

Periodontal tissues are modified by androgens, estrogens, and progestins. The homeostasis of periodontal tissues is a complex, multifactorial relationship that involves, at least in part,

estrogen hormones. The intricate relationship between estrogen hormones and periodontal health has largely been studied in the gingiva. Clinical observations confirmed an increase in the prevalence of gingival disease with fluctuating plasma estrogen levels even when the oral hygiene remained unchanged. The etiology of estrogen-associated gingival diseases remains an enigma. Various authors have suggested that estrogens may modulate putative periodontal pathogens, blood vessels, and the immune system in the gingiva, but the influence of estrogen on these theoretical factors remains to be defined (Mariotti, 2005).

3.2.1.1 Gingivitis associated with puberty

The marked increase in steroid hormones in both sexes during puberty has a temporary effect on the inflammatory status of the gingiva. The signs of gingivitis in these cases are similar to those of classic plaque-induced gingivitis, although gingival inflammation can be found in adolescents with only a small amount of dental plaque accumulation.

3.2.1.2 Gingivitis associated with the menstrual cycle

Gingival tissues contain receptors for androgens, estrogens, and progesterone, which exert effects on the oral mucosa and periodontium. Changes in the circulating levels of female sex hormones also affect the host response against dental plaque (Becerik et al 2010). Women with gingivitis experience greater inflammation during ovulation with an associated increase in crevicular fluid exudate. Changes in gingival tissue during menstrual phases may be related to changes in inflammatory markers in the gingival crevicular fluid.

3.2.1.3 Gingivitis associated with pregnancy

The rise in hormone levels during pregnancy increases the risk of gingivitis, regardless of plaque levels. Various studies have found more gingival inflammation in pregnant than in postpartum women with the same amount of plaque. (Löe et al 1963). Pyogenic granuloma is an inflammatory hyperplasia that can be caused by hormonal factors; it appears on the gingiva as a smooth or lobulated exophytic lesion with small red erythematous papules on a pedunculated or sometimes sessile base, which is usually hemorrhagic and compressible. (Jafarzadeh et al 2006). This lesion is more frequent during the first trimester of pregnancy and normally disappears after giving birth.

3.2.2 Gingivitis associated with drugs

Gingival enlargement is a frequent side effect of certain drugs, including anticonvulsants (phenytoin, sodium valproate), immunosuppressive agents (cyclosporine A), and calcium channel blockers (nifedipine).

Phenytoin-induced lesion has aroused the curiosity of dental practitioners ever since its first description by Kimball in 1939, and interest has developed among cell biologists, connective tissue biochemists and geneticists over the past decade. However, intense research efforts have not elucidated the precise mechanism by which this simple molecule simultaneously prevents seizure activity in the brain while eliciting an adverse connective tissue reaction in the gums. For almost half a century, phenytoin was the only chemical compound regularly associated with a connective tissue response limited almost exclusively to the gingiva. However, phenytoin has been joined over the years by other systemic medications that have gingival overgrowth as an adverse effect. Although these new medicines are not structurally related in any way to phenytoin, they elicit gingival manifestations with an uncanny clinical and microscopic resemblance to the gingival enlargement associated with phenytoin (Hassel & Hefti 1991).

Reported prevalence rates vary widely from 10 to 50% for phenytoin, 8 to 70% for cyclosporine A, and 0.5 to 83% for nifedipine. The presence of various degrees of gingival inflammation hampers the accurate assessment of drug-induced gingival overgrowth per se, which is exacerbated by the inflammation (Kataoka et al 2005b).

3.2.2.1 Anticonvulsants (Phenytoin)

Phenytoin is an anticonvulsant used in the treatment of epilepsy. Approximately 40-50% of all individuals treated with phenytoin develop esthetically disfiguring enlargement of the gingiva. The lesion is histologically characterized by an accumulation of the connective tissue matrix within the gingiva propria. Although the pathogenesis is multifactorial, there is a consensus that direct action of phenytoin upon resident gingival fibroblasts is the primary causative factor (Hassel, 1983).

3.2.2.2 Immunosuppressive agents (Cyclosporine A)

This drug prevents the rejection of transplanted solid organs and bone marrow. Despite its unequivocal success as a truly selective immunosuppressive drug, cyclosporine A is also associated with gingival enlargement.(Hassel & Hefti, 1991), with a prevalence ranging from 6 to 80%. It appears as a progressive gingival growth that starts with a more intense enlargement of interdental papillae in the anterior area of the mouth. The presence of dental plaque plays an important role in worsening the clinical picture.

3.2.2.3 Calcium channel blockers (Nifedipine)

In clinical use since 1977 for the treatment and prophylaxis of acute and chronic coronary insufficiency, nifedipine was first reported in 1984 to be causatively associated with gingival enlargement (Hassel & Hefti, 1991). The prevalence of gingival overgrowth in treated patients ranges from 15 to 25%. These drugs selectively block the entrance of calcium channels at cell level, hence increasing the extracellular calcium count. The enlarged gingiva is firm in these cases, with no tendency to bleed, and is normally more prominent in the anterior vestibular area.

3.2.2.4 Gingivitis associated with oral contraceptives

Oral contraceptives were first approved in the USA in 1960 and are currently used by almost 12 million women in the USA and more than 100 million women worldwide. Usage varies widely by country, age, education and marital status. It has been suggested that hormonal contraceptive use by women of childbearing age increases their risk of periodontal disease. Early small-scale clinical studies found that combined oral contraceptives containing high doses of estrogen increased the risk of gingival disease, suggesting an adverse effect on the underlying supporting periodontal tissues. Other studies reported that gingival inflammation increased in direct relationship with the duration of combined oral contraceptive use. Limited animal studies also have lent support to these notions, indicating that oral contraceptives have marked effects on the gingival microvasculature, altering capillary permeability and producing the cell immune response seen in gingival diseases. As a result of these data, the dental community has often attributed poor gingival health to the use of oral contraceptives, despite these studies being more than 25 years old.

A recent small-scale clinical study found no association between the use of low-dose oral contraceptives and gingivitis, but the relationship between OCs and periodontal diseases has yet to be investigated in a large representative population-based sample. A cross-sectional study in premenopausal women found no association between previous high-dose

or current low-dose oral contraceptive intake and increased levels of gingivitis or periodontitis, emphasizing the need for a reexamination of the perceived association between these drugs and periodontal diseases (Taichman & Eklund, 2005).

3.2.3 Gingivitis associated with malnutrition

Severe periodontal disease, accompanied by gingival hemorrhage, tooth mobility and attachment loss, was traditionally considered a clinical feature of ascorbic acid deficiency. However, it has been suggested that various forms of gingivitis and periodontitis mainly result from the activities of oral microorganisms that colonize the teeth and adjacent periodontal tissues, assigning a secondary role to ascorbic acid deficiency; in fact, most of the epidemiological and experimental evidence accumulated over recent decades has failed to demonstrate any significant etiological relationship between ascorbic acid deficiency and periodontal disease (Leggot et al 1991).

3.2.4 Gingivitis associated with ulcerated lesions

Necrotizing gingivitis (NG) or necrotizing ulcerative gingivitis (NUG) is an acute opportunistic gingival infection caused by bacterial plaque. It appears more frequently in undernourished children and young adults and in immunodeficient individuals. The disease is characterized by pain, bleeding, and papillary necrosis and has a tendency to relapse (Bermejo Fenoll & Sanchez Pérez 2004). Its prevalence is fairly low (<0.5% in industrialized countries), although an increase has recently been observed among young adults in relation to smoking, stress and other factors. HIV-positive individuals are also more susceptible to necrotizing periodontal disease, with a reported prevalence ranging from 0% to 11%.

4. Conclusions

Gingivitis can have multiple origins and can be the manifestation of a wide range of systemic diseases. Gingival tissue inflammation is one of the most common lesions encountered in the clinical setting and may be the first symptom in many types of disease. Gingivitis may therefore have important diagnostic relevance, and it is vital for clinicians to be aware its different possible causes to ensure a correct diagnosis and treatment.

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5

Components of Host Response to Pathogenic Bacteria in Gingivitis

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1. Introduction

A classification based on infection as the principal etiology of periodontal diseases divides categories based on gingival inflammation and periodontal attachment loss and recognized health, gingivitis and periodontitis as separate entities (Armitage 1999). Separation of gingivitis from periodontitis suggests that there are differences in these conditions that might include type or severity of infection, and/or adequacy of host response. Data shows that gingivitis in adults can remain stable throughout many years and not endanger the life of the dentition, whereas periodontitis, despite extensive, continues to break down the surrounding hard and soft tissue, leading ultimately to tooth loss (Seymour 1987).

Bacterial biofilms have been shown to be the primary etiological factor in the initiation of gingival inflammation and subsequent destruction of periodontal tissues (Haffaje & Socransky 1994). Although chronic bacterial and endotoxin exposure is a prerequisite for gingival inflammation and periodontal tissue destruction to occur, its presence alone accounts for a relatively small proportion (i.e. 20%) of the variance in disease expression and is not enough to explain the (Grossi et al. 1994).

2. Bacterial specificities

An attractive hypothesis concerning the role played by Toll Like Receptors (TLRs) in the activation of adaptive immunity holds that the precise combination of TLRs activated by a given microbial infection leads to "tailoring" the adaptive immune response so that it can deal with that specific infection (Hoebe & Ulevitch 2003). Hence, dendritic cells-stimulated, stimulated by LPS, might direct the development of an adaptive response that is better suited for dealing with Gram-negative organisms than Gram-positive organisms. When the

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ability of various oral bacteria to induce innate immune responses from gingival epithelial cells is investigated, the results show that non-periodontopathic and orange-complex bacteria induce weak and strong responses, respectively, but that red-complex bacteria suppress immune responses, live and lysed bacteria showed a difference in their ability to induce immune responses from epithelial cells (Ji et al. 2007). The down-regulation of interleukin-8 by *Porphyromonas gingivalis* in both mRNA and protein levels, the so-called "chemokine paralysis", has been well characterized (Darveau et al. 1998).

It has been controversial whether oral epithelial cells express TLRs (Asai et al. 2001), in relation to their apparent unresponsiveness to various microbial products in terms of proinflammatory cytokines products. Recently, the authors demonstrated, by immunohistochemical analysis the clear expression of NOD1 and NOD2 in normal oral epithelial tissues, and also showed, using PCR, flow cytometry, and immunostaining, that primary oral epithelial cells in culture expressed these molecules (Sugawara et al. 2006). On the other hand, gingival fibroblasts constitutively expressed proinflammatory cytokines. Upon stimulation, with chemically synthesized ligands mimicking microbial products for the receptors in gingival fibroblast, the production of pro-inflammatory cytokines, such IL-1, IL-6, IL-8 and MCP-1 was markedly up regulated (Uehara et al. 2007). In other study, viable *P. gingivalis* induced a strong *in vitro* inflammatory response in both gingival and periodontal ligament fibroblasts incremented gene expression of interleukin (IL)-1β, IL-6, IL-8, TNF-α and secreted RANTES (Scheres et al. 2010). These findings indicate that these innate immunity-related molecules in gingival fibroblasts are functional receptors involved in inflammatory reactions in periodontal tissue, which might be responsible for periodontal pathogenesis.

P. gingivalis, a Gram-negative, anaerobic oral black-pigmented rod, is suspected to be a periodontopathic bacterium and has been frequently isolated from the periodontal pockets of patients with periodontal diseases (Slots & Listgarten 1988), and the virulence of *P. gingivalis* has been attributed to a variety of factors, including hemagglutinins, cysteine proteinases, and fimbriae (Lamont & Jenkinson 1998). Although fimbriae function as a colonization factor (Lamont & Jenkinson 1998), evidence suggests that their virulence role may extend to immunomodulation of the macrophage response to *P. gingivalis*, through coordinated interaction with pattern-recognition receptors (Hajishengallis et al. 2006). Recently, a study demonstrated that *P. gingivalis* expressing wild-type fimbriae may exploit the TLR2/complement receptor 3 pathway for intracellular entry, and persisting in macrophages and by reducing the production of IL-12p70, which may facilitate the survival of other microbes that cohabit its niche in the oral biofilm (Wang et al. 2007). These results support the concept that pathogens evolved to manipulate innate immunity for promoting their adaptive fitness and, consequently, their capacity to cause disease (Finlay & McFadden 2006). The chemical and biological properties of *P. gingivalis* LPS and its lipid A are different from those of enterobacterial LPS and their lipid A (Ogawa 1994), and different forms of *P. gingivalis* lipid functionally interact with only TLR4 (Sawada et al. 2007). Milward et al. (Milward et al. 2007) demonstrated that *Fusobacterium nucleatum* and *Porphyromonas gingivalis* have differential effects at the molecular level on oral epithelial cells and that their differences in activating NF-κB nuclear translocation in oral epithelial cells may at least in part be responsible for the change in dynamics and kinetics of downstream gene expression. Genes encoding for structural components of the cytoskeleton, namely cytokeratins, were

detected as being differentially expressed in oral epithelial cells in response to challenge by *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. It is therefore conceivable that bacteria may stimulate alterations in cytokeratin expression to gain entry into the underlying gingival tissues.

Challenge of human gingival epithelial cells with *Aggregatibacter (Actinobacillus) actinomycetemcomitans* has been shown to induce the secretion of several pro-inflammatory cytokines (Dongari-Bagtzoglou & Ebersole). A previous focus in research on *A. actinomycetemcomitans* leukotoxin has been on its ability to kill neutrophils, the primary defense line against bacteria in the periodontal pocket (Johansson et al. 2000a). Other lines of research have associated the presence of *A. actinomycetemcomitans* with concentrations of cytokines. *Aggregatibacter (Actinobacillus) actinomycetemcomitans* is a facultative anaerobic Gram-negative bacterium associated with aggressive periodontitis (Slots and Ting 1999). *A. Actinomycetemcomitans* possesses several different well-studied virulence factors, among which the leukotoxin us suggested to play an important role in the pathogenicity (Feng & Weinberg). Leukotoxin is assumed to contribute to the severity of the periodontal disease by disrupting the local defense mechanisms (Guthmiller et al. 2001). Macrophages are one of the cell types involved in the local immune response and various homeostatic, immunological and inflammatory processes and contribute to specific immunity via antigen presentation and release of IL-1; they are the main source of IL-1 in inflamed tissues (Dinarello 1996). Recently a study showed that purified leukotoxin from *A. actinomycetemcomitans* activates caspase-1 in human monocytes/macrophages *in vitro* and consequently leads to secretion of bioactive IL-1β (Kelk et al. 2005). Recently, a study showed that enhanced concentration IL-1β correlated with a high proportion of *A. actinomycetemcomitans* in samples from diseased sites of a localized aggressive periodontitis patient infected with a minimally leukotoxic strain of *A. actinomycetemcomitans*. This leukotoxin is the main cause of IL-1β secretion from human macrophages exposed to highly as well as minimally leukotoxic strains of *A. actinomycetemcomitans*, may indicate that leukotoxin has the potential to trigger the inflammatory response (Kelk et al. 2007). In a site of inflammation, monocytes are recruited from the peripheral circulation and need to pass through the blood vessel wall to enter the infected tissues. During this migration and later in the tissue they differentiate into macrophages and are primed by inflammatory components from the host (Perregaux et al. 2002) and microbial components from the infection (Loesche 1993). If the macrophages are exposed to a secondary factor (leukotoxin) that activates caspase-1, the level of IL1β secretion from these primed macrophages can be substantial and devastating for the tissue (Kelk et al. 2007).

3. Immune response

The transition process from gingival health to early inflammatory changes is characterized by a local increase in vascular permeability, redness, swelling and by the recruitment and activation of polymorphonuclear nutrophils (PMNs) (Delina & Van Dyke 2000). In the course of this acute phase, several products modulate vasodilatation (e.g. bradykinin and prostaglandins), vascular permeability (e.g. histamine and leukotriene) and additional recruitment of inflammatory cells through chemotaxis (e.g. complements products and chemokines) (Offenbacher 1996). The subsequent immune response starts

when antigen-presenting cells become involved, presenting the foreign microorganisms or antigens to immunocompetent cells such as T lymphocytes. This leads to the expansion of antibody-secreting plasma cells and the development of a chronic lesion (Gemmel & Seymour 1994).

Based on clinical and immunological findings, the immune response in the stable gingivitis lesion is dependent on proinflammatory reactivity in progressive periodontitis lesion. For inflammatory mediators, IL-1β, interferon (IFN)-γ and receptor activator of nuclear factor (NF)-κB ligand tended to be higher in periodontitis, whereas tumor necrosis factor (TNF)-α and IL-12 p40 showed no difference (Honda et al. 2006). These findings suggest that perhaps subtle differences in the balance of cytokines may result in different disease expression.

Anti-microbial peptides produced from mucosal epithelium appear to play pivotal roles in the host innate immune defense system in the oral cavity. In particular, human beta-defensins (hBDs) a cathelicidin-type antimicrobial peptide, LL-37, were reported to kill periodontal disease-associated bacteria (Bals 2000). Recent studies using immunohistochemistry have demonstrated the expression of hBD-1 and hBD-2 in the gingival epithelium (Dale 2002). A variety of bacterial components are reported to induce hBD-2 mRNA expression by primary gingival epithelial cell (GEC) cultures (Weinberg et al. 1998). Recently data revealed that neutrophils expressed only LL-37, but not hBD-2 or hBD-3, and that such expression was prominent in the inflammatory lesions when compared to healthy gingival which showed very few or no LL-37 expressing neutrophils. GEC, however, expressed al three examined anti-microbial peptides, irrespective of the presence or absence of inflammation. Moreover, as determined by ELISA, the concentration of LL-37 in the gingival tissue homogenates correlated positively with the depth of the gingival crevice (Hosokawa et al. 2006).

3.1 Polymorphonuclear neutrophils

PMN play an important role in protection of host against invading bacteria during gingivitis. In fact, histopathology features of the gingivitis lesions show that PMN are prominent within the gingival tissues. Abundant neutrophils have been found within the connective tissue, junctional epithelium, and gingival crevice (Schroeder 1970; Attström 1971). On the other hand, absence or severe impairment of their functions results in an increased susceptibility to bacterial infection and some patients with severe peripheral blood PMN defects suffer from unusual gingivitis and destructive periodontal diseases (Ryder 2010).

PMN perform a complex series of functions during inflammatory response against bacteria: (1) adherence, required for subsequent functions performed by PMN at the inflammatory site, (2) directed migration towards chemotactic gradients, to reach the inflammatory site, (3) expression of receptors for both IgG and C3b on the cell surface, for the ingestion of opsonized bacteria, (4) phagocytosis, which is necessary for the clearance of pathogens, (5) release of the contents of specific granules, which is important in the regulation of themselves and other immune cells, and (5) production of antimicrobial substances.

Delivery of antimicrobial substances by PMNN is essential to control the bacterial targets and it is performed using different mechanisms: (1) Synthesis of oxygen metabolites: When PMNN contact certain stimuli (such as opsonized bacteria or chemoattractants) consume

dioxygen through a process called respiratory burst and, by transferring 1 or 2 electrons from cytosolic NADPH to the extracellular dioxygen via the NADPH oxidase system, form toxic superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), respectively (Shapira et al. 1991; Ryder 2010). (2) Discharge of lysosomes and secretion: Antimicrobial product secretion by movilization of cytoplasmic granules called lysosomes, fusion between the lysosomes and phagosomal or plasma membrane, and discharge of granule contents into the phagosome or the external environment. (3) Phagocytosis: Implied the engulfment of particles or bacteria and their components within a phagosome. Fusion between lysosomes and phagosome form the phagolysosome, which bacteria destruction by delivered antibacterial substances at a very high concentration by intraphagolysosomal secretion and respiratory burst activity. (4) Death: Cytosolic and granule component can be delivered by apoptotic or cytolytic mechanisms.

3.2 Cytokines

The immune system is alerted to the presence of a pathogen through the activation of the innate immune system. The message is transmitted to the cells of the adaptive immunity through activated antigen-presenting cells. The development of specific immunity capable of eliminating the pathogen is orchestrated by cytokines and chemokines produced by the innate system. Cytokines mediate a variety of biological functions, and the network in which they act is essential (Bendtzen 1994). They act in the initiation of the effectors phase and regulate the length and extent of the response. Initially, cytokines were believed to be produced exclusively by CD4 T cells (Abbas et al. 1996), but now is clear that the arrangements of cytokines produced by cells is a result of their function and not of their phenotype (O'Garra 1998). Furthermore, cytokine production is related to the cells differentiation stage and to transitory phases of their response (O'Garra 1998). Considerable effort has been made to study the cytokine released by different host cells when exposed to components of periodontopathogenic bacteria (Offenbacher 1996). These studies have demonstrated that a number of pro-inflammatory cytokines are synthesized in response to periodontopathogenic bacteria and their products, hence inducing and maintaining an inflammatory response in the periodontium (Page 1991).

IL-1β is a multifunctional inflammatory mediator able to modulate bone resorption by the activation of osteoclasts (Dewhirst et al. 1985) and by stimulating prostaglandin E2 synthesis (Tatakis et al. 1988). Although this cytokine was originally considered to be a product of mononuclear phagocytes, evidence suggested that both keratinocytes and gingival fibroblasts could also produce it, in response to stimulation by bacterial products (Dinarello 1998). Data indicate that the local production of IL-1β, in the gingival crevicular fluid, increases with increasing inflammation (Orozco et al. 2006). The levels of IL-1β are increased significantly in sites belonging to the adults periodontitis and early onset periodontitis (EOP) patients, compared to those belonging to the healthy and gingivitis groups (Giannopoulou et al. 2003; Girolomoni et al. 2002). Marked differences of IL-1β were observed in the different disease categories groups, as compared to the healthy group; a 3-fold increase was noticed in the gingivitis patients, a 6-fold increase in the adults periodontitis patients and an almost 9-fold increase in the EOP group. The IL-1 family consists of two proteins with similar biologic activities, IL-1α and IL-1β, as well as the IL-1 receptor antagonist (IL-1Ra), a non-signaling ligand. These ligands bind of two distinct and

separate receptors, the IL-1 receptor type I (IL-RI) and IL-1RII, respectively, which are expressed on a variety of cells and the binding of IL-1α and IL-1β to IL-1RI leads to cellular signaling and biologic effects but the binding of IL-1α and IL-1β to IL-1RII does not lead to cellular signaling (Dinarello 1998).
IL-6 is a pleiotropic cytokine that stimulates immunoglobulin secretion by human B-lymphocytes, activates T cells, stimulates the synthesis and secretion of acute phase proteins by hepatocytes, and activates the complement cascade (Revel 1999). The results showed that IL-6 is augmented in adult's periodontitis and EOP when is compared with to the healthy and gingivitis groups; the levels IL-6 in the healthy and gingivitis groups was extremely low, but showed a 2-fold and 7-fold increase in the adult and EOP groups (Giannopoulou et al. 2003).

3.3 T and B cells

Studies in chronic periodontitis using and immunofluorescence support the concept that T lymphocytes dominate the cellular infiltrate in healthy/gingivitis lesions, while periodontitis lesions are associated with high numbers of B cells in the inflammatory infiltrate (Seymour & Greenspan 1979; Berglund et al. 2002). Several studies revealed no statistically significant difference between the aggressive periodontitis or early onset periodontitis and chronic or adults periodontitis groups and healthy controls with regard to the relative counts of B-cells, T-cells, T-helper, T-cytotoxic/suppressor, activated T-cells and NK cells (Budunelli et al. 2001; Emingil et al. 2001; Takahashi et al. 1995). In aggressive periodontitis, existed a low number of T cells (CD3+) compared to healthy/gingivitis biopsies and this reduction of CD3+ could indicate that other lymphocytic cells like B lymphocytes may play an important role in the immune response that mediates damage in aggressive periodontitis (Suarez et al. 2004).
T cells are present in the inflammatory infiltrates of periodontal disease lesions and require antigen presentation by antigen-presenting cells (APCs). The presence of numerous CD1a+ Langerhans cells was noted in the epithelium with no difference between the healthy/gingivitis and periodontitis group (Gemmell et al. 2003). Five times as many CD1a+ Langerhans cells have been shown in clinically inflamed gingival epithelium compared with numbers in the same patients following periodontal treatment (DiFranco 1985).
While T cells exhibit immunoregulatory features via T-helper 1 and T-helper 2 subsets, B cells upon activation transform into antibody producing plasma cells. Polyclonal B-cell activation is believed to be of major significance in the development of B-cell lesions (Tew et al. 1989). Yamazaki et al. showed that the frequency of B-cells and activated B-cells in the periodontitis was much higher than that of gingivitis lesions, suggest that the periodontal lesions is also dominated by B cells in the tissue (Yamazaki et al. 1993,28). This was substantiated by the results of Afar et al., who demonstrated a marked increase in a B-cell subpopulation –namely the autoantibody-producing CD20+ CD5+ cells- of patients with periodontitis (Afar et al. 1992). The results of CD40L expression, in the gingival tissue of periodontitis patients, suggest that , CD40L-expressing activated T cells stimulate B cells by cross-linking CD40 molecules to proliferate and differentiate to plasma cells and subsequent immunoglobulin production (Orima et al. 1999).

3.4 Matrix metalloproteinases (MMPs) in gingivitis

Extracellular proteolysis and remodeling of the extracellular matrix from periodontal tissues is an integral feature of periodontal homeostasis involving a tight balance among protease

activities and inhibition. Matrix metalloproteinase's (MMPs) are genetically distinct but structurally related zinc-dependent metalloendopeptidases, described almost fifty years ago (Gross and Lapiere, 1962). MMPs can synergistically degrade almost all extracellular matrix and basement membrane components and regulate several cellular processes including inflammatory responses, representing the most prominent and widely studied family of proteinases associated with periodontal diseases (Folgueras et al., 2004; Overall, 2002; Sorsa et al., 2006).

Type I collagen is the major extracellular matrix component of connective periodontal tissues setting collagenolytic MMPs among the most prominent mediators and biomarker candidates for periodontal tissue breakdown (Golub et al., 1997; Kiili et al., 2002; Rodan and Martin, 2000). Since their discovery, collagenolytic MMPs were promptly identified in gingival (Geiger and Harper, 1980; Gibson and Fullmer, 1966) and gingival crevicular fluid (GCF) (Golub et al., 1976) in association with the degree of gingival inflammation (Overall and Sodek, 1987). So far, a burst of evidence supports that collagenases along with other MMPs play a pivotal role in periodontal tissue destruction (Sorsa et al., 2006).

The major collagenolytic MMPs associated with severity of periodontal inflammation and disease are MMP-8 and MMP-13. MMP-2 and MMP-9 belong to the gelatinase family of MMPs that further degrade denatured collagen basement membranes, among other substrates. Whereas MMP-2 expression is constitutive, MMP-9 is strongly induced during inflammatory conditions (Kiili et al., 2002; Sorsa et al., 1988; Sorsa et al., 2006; Tervahartiala et al., 2000; Uitto et al., 2003).

MMP-8 and MMP-9 are by far the predominant MMPs in GCF and their major source are regarded to be neutrophils, monocytes and macrophages, although resident periodontal cells can also produce these MMPs. MMP-9 has also been detected in junctional sulcular and pocket gingival epithelial cells and thus it might be involved in epithelial response during periodontitis (Smith et al., 2004). Increasing of MMP-8 and MMP-9 are substantially involved in chronic periodontitis (Hernandez et al., 2010; Hernandez Rios et al., 2009; Pozo et al., 2005) and represent the most promising biomarkers for periodontal inflammation and disease severity (Sorsa et al., 2009). Active forms of MMP-8 tend to increase during disease progression, whereas neutrophil MMP-8 is the predominant isotype. Nevertheless, physiological MMP-8 levels might be required for normal tissue turnover and are thought to be rather protective against periodontal destruction (Hernandez et al., 2011; Kuula et al., 2009).

MMP-13 on the other hand, is much less abundant in GCF. MMP-13 is expressed by sulcular epithelial cells, macrophage-like cells, fibroblasts, plasma cells and osteoblasts (Hernandez et al., 2006; Rydziel et al., 2000). MMP-13 levels are shown to increase in chronic periodontitis in comparison to healthy periodontium, whereas MMP-13 increased activity has mainly been related to the occurrence of the loss of periodontal supporting tissue and alveolar bone resorption. Additionally, MMP-13 can activate proMMP-9 in diseased gingival tissue (Hernandez Rios et al., 2009).

Although a strong body of evidence supports that MMP-8, MMP-9 and MMP-13 play an important role in periodontal inflammation and/or supporting tissue loss, few studies have focused on the role of these proteinases in gingivitis and reports are somewhat conflicting. Previous determinations of MMP -1, -2, -9 and -13 expression through real time-PCR showed no differences among gingival samples from healthy, gingivitis and periodontitis

subjects, but a tendency for both gelatinases to increase in inflamed tissues (Goncalves et al., 2008). Conversely, MMP-8 and MMP-9 protein levels, as well as total collagenase activity have been found to increase progressively from healthy periodontal tissues and gingivitis to periodontitis in GCF and/or saliva samples (Mantyla et al., 2003; Rai et al., 2008; Xu et al., 2008). Nonetheless, a recent study reported significant increases in GCF IL -1β levels along with an overall decrease of MMPs in experimental gingivitis model, except for MMP-8. The authors also described different subsets of inflammatory phenotypes based on cluster analysis of changes in GCF mediators that resulted in a similar clinical phenotype. One of the inflammatory phenotypes appeared to be similar to periodontitis, consisting of increases in IL-1α and IL-1β, IL-6, MMP-8 and MMP-9, along with a significant decrease in macrophage inflammatory protein (MIP)-1β. This finding suggests that host response during gingivitis might be heterogeneous and some of the inflammatory phenotypes could be more susceptible to periodontitits development (Offenbacher et al., 2010). Accordingly, elevation of active MMP-8 has previously been associated with the conversion of gingivitis to periodontitis and established periodontitis (Mantyla et al., 2006).

Despite MMP-13 has been more related to chronic periodontitis progression than severity of inflammation, it has previously been reported that MMP-13 also increase progressively from healthy subjects to periodontitis in GCF. Higher levels of active forms and total MMP-13 have been described in gingivitis versus healthy sites; whereas MMP-13 levels and active forms were significantly higher in chronic periodontitis, compared to both, healthy and gingivitis subjects (Ilgenli et al., 2006). Furthermore, chronic periodontitis subjects show higher MMP-13 activity in active sites compared to inactive sites and healthy controls.

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6

Innate Immunite and Inflammation

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1. Introduction

A bacterium can find in the human oral cavity a variety of environmental niches that provide the warmth, moisture and food necessary for growth. Bacteria are able to invade this environment, adhere and colonize, gain access to food sources, and escape clearance by host nonimmune and immune responses thanks to genetic traits they have acquired. Unfortunately, diseases can be caused by many of the mechanisms the bacteria use to maintain their niches. This can occur either by destroying the tissue directly or indirectly, since the surface structures of the bacteria stimulate strong inflammatory host responses which can/may be protective but are often the main causes of the disease symptoms.

The human body is colonized with numerous microbes as normal flora, many of which serve important functions for their hosts, such as protecting the host from colonization with pathogenic microbes. Therefore, not all bacteria cause disease. Almost 1000 microbial species have been identified in dental biofilm intimately integrated forming a consortia in which an interchange of nutrients and metabolic factors occurs. Genetic characteristics expressed by the biofilm let the microbial consortia to compete, cooperate, and survive a changing environment, resist antibiotics and acquire virulence factors to the detrimental of oral health.

A "*septic*" gingival inflammation occurs when microbes and their virulence factors compromise the junctional epithelium health. Some of the natural defense mechanisms and physical and chemical barriers are salivary pH, mucus, secretions containing antibacterial substances such as lysozyme and collectins, phagocytic cells as neutrophils, macrophages and natural killer cells, blood proteins, including the complement system and other inflammatory mediators such as cytokines that regulate and coordinate the innate immune response.. The role of these factors is to make it difficult for the bacteria to enter the body. However, bacteria often have the means to compromise the epidermal barrier and invade the body. Initially gram-positive bacteria infects the mouth, while peptidoglycan and its breakdown products, teichoic and lipoteichoic acids, are released, which induce a pyrogenic acute-phase responses. New Gram-negative species, such as *Porphyromonas gingivalis, Campylobacter rectus, Eikenella corrodens, Actinobacillus actinomycetemcomitans,* and oral *spirochetes (Treponema* species) may be found present in the biofilm in the first four days following the beginning of plaque accumulation. While the dental plaque formation continues, Gram-negative species become dominant over the Gram-positive species. The overgrowth of Gram-negative anaerobic bacteria is considered one of the main causative factors that induce a complex sequence of events known as the inflammatory response of gingivitis. The lipopolysaccharide (LPS) produced by gram-negative bacteria is an even

more powerful activator of acute-phase and inflammatory reactions and its lipid A portion is responsible for its endotoxin activity. Most bacterial components and structures binds to specific receptors on macrophages and neutrophils, the sentinel cells of the human body and crucial mediators of the inflammatory response. These cells can sense the non-self or pathogen-associated molecular patterns (PAMPs) through multiple membrane receptors such as Toll Like Receptors (TLR). The recognition through most TLR induces the activations and up regulated of the MyD88 dependent pathway that culminate with the phosphorylation of NF-kB and induction of the expression of the pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 and prostaglandins. However, other pathways, which are independent of MyD88, may also be activated, using mainly TRIF as an adaptor molecule, with the purpose of regulating the first via secretion of anti-inflammatory cytokines, avoiding the progression of inflammation. There are several other classes of phagocyte receptors that bind microbes and mediate their internalization, such as mannose receptors that bind terminal mannose and fucose residues of glycoproteins and glycolipids. Scavenger receptors bind and mediate endocytosis of oxidized or acetylated low-density lipoprotein (LDL). Macrophage integrins, notably Mac-1, may also bind microbes for phagocytosis. G protein-coupled receptors, Fc and C3 receptors, and receptors for cytokines, mainly IFN-γ, function cooperatively to activate phagocytes to kill ingested bacteria. Phagolysosomes are formed by the fusion of phagocyte vacuoles with lysosomes, in which the microbicidal mechanisms, such as reactive oxygen intermediates (ROIs) (highly reactive oxidizing agents that destroy bacteria) are concentrated in bacteria. Besides ROIs, macrophages produce reactive nitrogen intermediates, mainly nitric oxide, by the action of an enzyme called inducible nitric oxide synthase (iNOS). Activated neutrophils and macrophages also produce several proteolytic enzymes in the phagolysosomes, which function is to destroy bacteria. One of the most important enzymes in neutrophils is elastase, a broad-spectrum serine protease known for being essencial to kill many types of bacteria. Endotoxin also stimulates the growth of B cells, and induces the expression of class II MHC molecules permits these cells to function as an antigen-presenting cell (APC). An example is a dendritic cell that acquires the antigen by phagocytosis or endocytosis and after the antigen is processed, the mature dendritic cells present it to TH cells. On the other hands, "*aseptic*" gingival inflammation can occur. Many factors can contribute for the development of this inflammation: drugs, hormones, autoimmunity diseases, and GVHD among other disorders.

2. Innate immunity and inflammation

Innate immunity can be seen to comprise four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory.

2.1 The oral mucosal surfaces provide protective barriers against Infection

The physical and anatomic barriers that usually prevent the entry of pathogens are of distinct layers. The outer epithelial layer and underlying layer of connective tissue are the first line of defense against infection. IThese protect the deeper tissues (fat, muscle, nerve and blood supplies) from mechanical insults, such as trauma during chewing, and also prevents the entry of bacteria and some toxic substances into the body. The hard surface of some mucosa (such as the hard palate, the gingivae, and some areas on the dorsum of the tongue) is inflexible but resistant to abrasion, and is tightly bound to the underlying tissue.

The acquisition of organisms and the subsequent course of either stable colonization or invasion of the host involves complex host-parasite interactions. From one perspective, host factors are operative, appearing to select against certain species while being permissive to others. From another perspective, microbial species that are successful at colonization must overcome certain host factors to maintain a selective advantage and flourish within a particular body habitat. It is interesting to observe that while host mucosal defenses play a significant role contributing to the selection of the resident flora, it is this established flora that provides the host with what might be its most important local defense system. More than 1000 bacterial species or phylatypes have been detected in the oral cavity. These organisms are rarely involved in infection unless there is some breach of the mucosal surface or some upset in the balance of the normal flora. When this occurs, the host is susceptible to infection from both recently acquired organisms and those present before that may now become invasive resulting in the formation of microbial biofilms such as supre and subgingival dental plaque, and tongue surface debris, leading to dental caries, gingivitis, periodontal disease and oral malodor. The biofilm bacteria and their toxins perturb gingival epithelial cells as the first stage in a cascade of inflammatory and immune processes that lead to the destruction of gingival tissues and ultimately, in susceptible patients, alveolar bone loss and tooth loss as a result of periodontal disease.

2.2 Physiologic barriers to infection include general conditions and specific molecules

There are physiologic barriers that contribute to innate immunity such as pH, temperature, saliva containing antibacterial substances like lysozyme, collectins, complement system and proteins called cytokines, that regulate and coordinate many activities of the cells of innate immunity.

2.2.1 Saliva is the fluid present in the oral cavity that is produced by different salivary glands and its function is to protect the epithelial tissue against external harmful effects. The protection is achieved by the physical movement of exocrine glandular secretions that mechanically entrap and effectively remove many potentially harmful microorganisms and compounds. Its components, like lysozyme, collectins, blood proteins, complement system, soluble factors and secretory Immunoglobulin A can also bind to microorganisms inhibiting their adhesion to the mucosal surface. In addition, antibacterial mechanisms can influence the metabolism of bacteria by bacteriolysis, membrane damage, inhibition of growth, and cell killing. It is likely that besides the sharing of these general needs, the mucosal secretions also share a variety of protective components (Table 1).

2.2.2 A variety of **soluble factors /proteins** that are present in many different body fluids, including saliva, that are necessary to protect the oral epithelia from the large number of possible invading microbes and maintain the oral homeostasis of commensal and pathogenic bacteria. Moreover, the expression of anti-microbial proteins is differentially regulated by different periodontal pathogens (Handfield et al.2005) (Table 2), suggesting that a specific antimicrobial ''cocktail'' constitutes the physiological response to individual pathogens. This mix may also play a role in maintaining an appropriate balance between oral pathogens and commensals. **Histatins** are a family of several low molecular-weight histidine-rich peptides that are secreted mainly in parotid saliva and, to a lesser extent, in submandibular saliva. Histatins possess antimicrobial properties against a few strains of *Streptococcus mutans* and inhibit hemagglutination of the periopathogen *Porphyromonas*

Protective Funcions
Tissue coating (mucosal and tooth pellicle) Lubrification Humidification Remineralization of the teeth
Host Defense Functions
Immunological activity Anti-bacterial activity Anti-viral activity Anti-fungal activity
Digestion
Digestive enzymes Bolus formation Taste

Table 1. Functions of Human Salivary Secretions

gingivalis. In addition, histatins neutralize the endotoxic lipopolysaccharides located in the outer membranes of Gram-negative bacteria, which may be an important part of the host's defense system. Histatins are involved in functions that are specific of the oral cavity. Histatins 1, 3, and 5 bind to hydroxyapatite and may therefore be precursors of components of the acquired enamel pellicle. The formation of pellicle is the first step in dental plaqueformation. and an important participant in the mineralization dynamics of oral fluids. **Proline-rich proteins (PRPs)** are a heterogeneous group of proteins that comprise about 70% of the parotid proteins. PRPs are classified into three groups: acidic, basic and glycosylated. Hence, the acidic PRPs are involved in typical oral processes like mineral homeostasis and neutralization of toxic substances in the diet. Glycosylated basic PRPs function as masticatory lubricants and have also been shown to interact with several types of microorganisms such as *Fusobacterium nucleatum*. Basic PRPs have a more general protective function among all these proline-rich proteins. **Mucins** are proteins that give the typical visco-elastic character to all the mucosal secretions. In general, the physiological functions of the mucins include, among others, cytoprotection, lubrification, protection against dehydration, and maintenance of visco-elasticity in secretions. Human saliva contains two saliva-specific types of mucins, low and high-molecular-weight mucin glycoproteins, MG2 (150-200 kDa) and MG1 (>1000 kDa). MG1 adheres to the tooth surface, forming a barrier against acidic attacks, while MG2 binds to a large number of different microorganism, including *Candida albicans* and *Actinobacillus actinomycetemcomitans.* **Cystatins** belong to the class of cysteine proteinase inhibitors and their role is to regulate the activity of cathepsins, liberated during inflammatory reactions, e.g., in gingivitis and periodontitis. They are very important in the inhibition of several viruses presumably by blocking necessary cysteine proteinases, and may control the proliferation and invasion of tumor cells. **Secretory Immunoglobulin A (sig A)** is a member of the adaptive immune response and is the predominant immunoglobulin of the mucosal immune system.

Secretions of glands that are anatomically remote from the site of immunization, such as salivary glands, can contain sigA antibodies to antigens encountered through the oral cavity. In the same way, IgA-producing cells are induced by the common mucosal immune response, consisting of lymphoid tissues concentrated in special structures, such as Waldeyer's ring or pharyngeal lymphoid ring, which are anatomical terms describing the lymphoid tissue ring located in the pharynx and to the back of the oral cavity. It was named after the nineteenth century German anatomist Heinrich Wilhelm Gottfried von Waldeyer-Hartz. The ring consists of (from superior to inferior): Pharyngeal tonsil (also known as 'adenoids' when infected), tubal tonsil (where Eustachian tube opens in the nasopharynx), palatine tonsils (commonly called "the tonsils" in the vernacular, less commonly termed "faucial tonsils") and lingual tonsils. The protective role of sigA is via neutralizing antigens from viruses, toxins and enzymes, interact together with the innate immune factors (e.g., lysozyme, lactoperoxidase, lactoferrin). **Lysozyme**, a hydrolytic enzyme, is able to cleave the peptidoglycan layer and induce killing and lysis of the bacterial cell. **Kallikreins**, are a group of serine proteases that are found in glandular cells, neutrophils, and biological fluids, including saliva, and have a role in blood coagulation, via the activation of the Hageman factor. However, in saliva some hydrolysis of proline-rich proteins occurs by kallikrein. It has been implicated in the regulation of local blood flow in salivary glands, the processing of polypeptide hormones such as epidermal growth factor, in ion transport in epithelial cells, and neutrophil chemotaxis. **Collectins** are surfactant proteins that may kill certain bacteria directly by disrupting their lipid membranes or, alternatively, by aggregating the bacteria to raise their susceptibility to phagocytosis. **Extra-Parotid GlycoProtein (EP-GP)** is an acidic salivary glycoprotein of low molecular weight, 18-20 kDa, that can be localized only in the submandibular glands (in the serous acinar cells) and is absent from the parotid gland. It has been shown to have a strong affinity to hydroxyapatite and has a specific function in the oral cavity, e.g., as a component of the dental pellicle and modulation of oral microflora. **Albumin** is the most abundant protein present in serum plasma, constituting from 55 to 62% of the total serum proteins. In saliva of orally healthy individuals, albumin can be detected in only very small amounts. Salivary albumin concentrations are significantly increased in individuals with gingivitis or periodontitis Albumin is also found in a complexed form with proline-rich glycoprotein (PRG), and this complex, as a pellicle constituent, appears to play an effective role in lubrificating the oral tissue surfaces. **Calprotectin** is a protein that inhibits bacterial growth by acting as divalent cation scavengers. **Secretory leucocyte protease inhibitor and elastase-specific inhibitor** present in the human submandibular gland and saliva exhibit anti-elastase activity and can kill both Gram-negative and Gram-positive bacteria. **Beta-2-microglobulin** causes agglutination of *S. mutans* in the presence of calcium. **Fibronectin** is a glycoprotein that is expressed in hepatocytes, epithelial cells and other cells, and is present in saliva. The protein induces bacterial agglutination and plays a role in reducing bacterial adhesion to oral surfaces. Fibronectin also binds directly to fimbrillin from *P. gingivalis* inhibiting the fimbrillin induced expression of inflammatory cytokines in macrophages. **Lactoperoxidase and myeloperoxidase** form the principal components of the peroxidase system of saliva. The enzymes catalyse the oxidation of thiocyanate ions (SCN) by hydrogen peroxide, and the reaction product hypothiocyanite (OSCN) is bactericidal. Hydrogen peroxide-mediated oxidation of chloride and iodide produces further bactericidal reaction products.

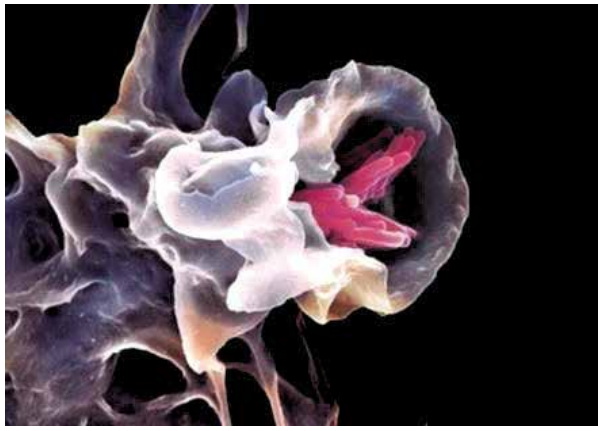
2.2.3 The **antimicrobial peptides** are necessary to protect the oral epithelia from the large number of possible invading microbes and maintain the oral homeostasis of commensal and pathogenic bacteria. Moreover, the expression of anti-microbial proteins is differentially regulated by different periodontal pathogens (Handfield et al. 2005), suggesting that a specific antimicrobial ''cocktail'' constitutes the physiological response to individual pathogens. This mix may also play a role in maintaining an appropriate balance between oral pathogens and commensals. Antimicrobial peptides exhibit striking variation in their ability to kill different species of oral bacteria (Gram-negative and Gram-positive) or different strains of the same species, as well as against yeast and viruses. In humans these antimicrobial peptides include defensins, adrenomedullin, cathelicidin (family member LL-37 in skin and oral mucosa and other epithelia), statherin and azurocidin. **Defensins** exhibit broad antibacterial activity to both gram-positive and gram-negative bacteria. The peptides bind to or are inserted into the bacterial cell membrane, causing membrane permeabilization and cell lysis. Defensins are most active against negatively charged phospholipids and increased salt concentrations inhibit their activity, suggesting that ionic interactions between membrane lipids and the cationic peptide are involved in their activity. The human defensins include the α-defensins. Alpha-defensins are expressed in neutrophils and have been identified in the gingival crevicular fluid of both healthy and diseased sites. Beta-defensins are found in gingival epithelial cells and whose expression is correlated with cellular differentiation and is regulated by calcium and phospholipase D. **Adrenomedullin** is a cationic amphipathic peptide with one disulfide bond. It is found in gingival crevicular fluid and glandular and whole saliva. In gingival crevicular fluid, the amount of adrenomedullin is about twice as high in periodontal disease sites than in healthy sites. **Cathelicidin,** precursor of the antimicrobial peptides FALL-39 and LL-37. LL-37, is a cationic alpha-helical peptide, and is expressed by neutrophils, epithelial cells, and can be found in saliva and gingival crevicular fluid. In addition to antibacterial activity, LL-37 also binds to and neutralizes lipopolysaccharide from gram-negative bacteria. In Candida albicans, the peptide causes disintegration of the plasma membrane. **Statherin** is found in gingival crevicular fluid and saliva. The peptide is secreted by the parotid and submandibular glands and inhibits the crystallization of calcium phosphate but also inhibits growth of anaerobic bacteria isolated from the oral cavity. It is the C-terminal peptide of statherin that exhibits the antibacterial effect**. Azurocidin** is expressed in azurophil granules of neutrophils present in human saliva and is antibacterial to gram-negative bacteria, presumably because of a strong affinity for lipopolysacchaide.

2.2.4 The **Complement system consists of a** group of zimogen serum proteins that can be activated through a variety of specific and nonspecific immunologic mechanisms. These mechanisms change the inactive forms of complement proteins into an active state with the ability to damage the membranes of pathogenic organisms, either by lysis of the pathogens or facilitating their clearance. Complement may function as an effector system that is set off by binding of antibodies to certain cell surfaces, or it may be activated by reaction between complement molecules and certain components from microbial cell walls. Reactions between complement molecules or fragments of complement molecules and cellular receptors set off activation of cells of the innate or adaptive immune systems. When periodontal tissues become inflamed, serous exudates begin to appear that mix with the salivary secretions at the gingival margin. The serous exudates contain a functional complement system, and the saliva secretions appear to cooperate and indeed potentiate the

complement system. Salivary secretions containing complement-reactive substances have the potential to cooperate in potentiating the activation of the classic and alternative complement pathways with enhanced C4b and C3b deposition, such as secretory IgA and high-molecular-weight nonimmunoglobulin agglutinins (NIA). Secretory IgA, when aggregated, activates the alternative complement pathway, while the aggregation of NIA, activate C1, causing C4 conversion and C4b deposition. Since NIA interacts with C1 and with immune complexes whichnot only involving secretory IgA but also IgG and IgM immune complexes. For that reason, NIA may have a role in (1) mediating activation of the classic complement pathway by secretory IgA and (2) enhancing the effectiveness of complement activation and deposition once serum or serous exudates contact human saliva. Proteins of the complement are low levels of gingival crevicular fluid (GCF) are present in health, the flow rate of this complement containing fluid dramatically increases as a consequence of the inflammation of periodontal tissues. In summary, human salivary secretion tend to potentiate the serum complement system. Dental plaque, and microorganisms in dental plaque activate the classic and the alternative complement pathways. Enhancement of C1q, C4b and C3b deposition by saliva-complement interactions occurring at supragingival margin may aid in the elimination of oral microbes in that area. A reversal of these enhancing effects might occur if certain bacteria, which are strong protease producers, colonize the dental plaque and digest residual native complement components and the C4b and C3b deposited on the microbial surfaces.

3. Cells that ingest and destroy pathogens make up a phagocytic barrier to infection

Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages (MØ), which are present in large numbers at portals of entry from the outside environment, just as within the oral cavity, which are constantly exposed to foreign particles (e.g., amalgam), viruses, bacteria, and fungi. Epithelial oral MØ express high levels of PRR including Toll-like receptors (TLR) and families of cytosolic proteins (e.g., NODs, NALPs), scavenger receptors (SR), mannose receptors (M R), G protein-coupled receptors, Fc and C3 receptors. MØ also express receptors for cytokines, mainly IFN-γ, which function cooperatively to activate phagocytes to kill ingested bacteria. Natural ligands on gram-positive bacteria that initially infect the mouth include peptidoglycan and their breakdown products, teichoic and lipoteichoic acids, which are released and stimulate toxin-like pyrogenic acute-phase responses. The lipopolysaccharide is produced by gram-negative bacteria is an even more powerful activator of acute-phase and inflammatory reactions. The lipid A portion of lipopolysaccharide is responsible for endotoxin activity. For TLRs to transmit signals via adaptor molecules selectively they depend on other surface molecules such as CD14/MD2 and C-type lectin-like receptors for proximal ligand binding and recognition. Collaboration among different membrane receptor families, as well as with nonclassical opsonins and proteinase cascades such as complement, is likely to be an important mechanism to enhance affinity of binding and specificity. The surface receptors of the MØ regulate a scope of functions, such as differentiation, growth and survival, adhesion, migration, phagocytosis, activation, and cytotoxicity. Their ability to recognize a wide range of endogenous and exogenous ligands, and to respond appropriately, is central to MØ functions in homeostasis as well as host defense in innate and acquired immunity, autoimmunity, inflammation, and immunopathology.



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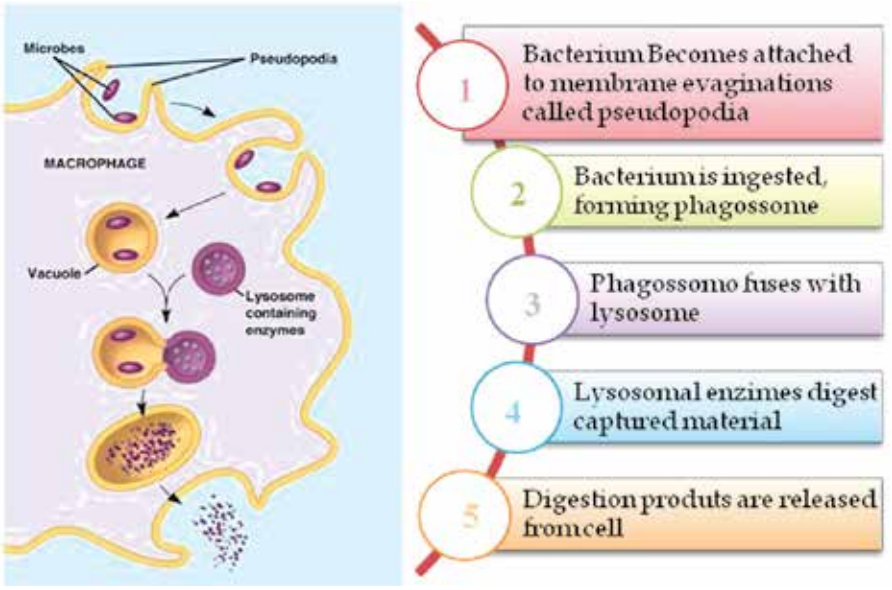


b)

Fig. 1. (a) Macrophage engulfing TB bacteria. Colored scanning electron micrograph (SEM) of a macrophage white blood cell (purple) engulfing a tuberculosis (Mycobacterium tuberculosis) bacterium (pink). This process is called phagocytosis. Macrophages are cells of the body's immune system. They phagocytose and destroy pathogens, dead cells and cellular debris. Credit: SCIENCE PHOTO LIBRARY (b) Schematic diagram of the steps in phagocytosis of a bacterium. [Part a, www. Nicerweb.com]

4. Inflammation represents a complex sequence of events that stimulates immune responses

4.1 Septic gingival inflammation

Tissue damage by microorganisms in gingival plaque induces a complex sequence of events collectively known as the inflammatory response. There is individual variation in this

response with some individuals taking longer to manifest disease compared to others. So, while it has been known for many years that plaque is the etiological agent, the factors contributing to patient susceptibility, for example, innate individual susceptibility, may involve both host-related genetics and the nature of the microbial challenge (the biofilm specific antigens involved in periodontal disease and of the immune response to them). Innate immunity is a consistent feature of both gingivitis and periodontitis. Strong innate immune responses, with high levels of IL-12, are associated with a Th1 response, while poor innate immune responses are suggested to favor a Th2 response. All individuals with dental plaque will advance to gingivitis, and not all individuals will progress to periodontitis. The advancement from health to gingivitis and then to periodontitis can be loosely divided into four stages: initial, early, established and advanced lesions.

4.1.1 The initial stage occurs almost immediately after toothbrushing. Some minutes after brushing your teeth, saliva derived glycoprotein deposits start to cover the tooth surface with what is referred to as "pellicle". The formation of pellicle is the first step in dental plaque formation. The pellicle is then colonized by Gram-positive bacteria such as *Streptococcus sanguis*, *Streptococcus mutans*, and *Actinomyces viscosus,* becoming what is known as dental plaque. Bacteria cells interact with pellicle components enabling plaque to firmly adhere to the tooth surface (Figure 2). Substances produced by the already accumulated bacteria enrich the plaque environment making it favorable for the growth of other species of bacteria. The presence of pathogenic bacteria is necessary but not sufficient for this process. The host immune and inflammatory response to the microbial challenge is a critical determinant of susceptibility to develop the destructive disease, and is under the influence of multiple behavioral, environmental, and genetic factors.

During the first four days of plaque accumulation, new Gram-negative species, such as *Porphyromonas gingivalis, Campylobacter rectus, Eikenella corrodens, Actinobacillus actinomycetemcomitans,* and oral *spirochetes* (*Treponema* species) can be found in the plaque biofilm. On the following days, Gram-negative species become dominant over the Gram-positive species. The overgrowth of Gram-negative anaerobic bacteria is considered one of the main causative factors that induce a complex sequence of events known as the inflammatory response of gingivitis. As plaque accumulates, bacterial enzymes and metabolic end products increase the permeability of the junctional epithelium, allowing both the ingress of further bacterial products and, at the same time, the outflow of gingival fluid.

This gingival fluid is essentially a serum product, which contains all the components of complement. Activation of complement via the so-called ''alternative pathway'' in the gingival sulcus results in production of the anaphylatoxins C3a and C5a, which in turn lead to the release of vasoactive amines from mast cells. These vasoactive substances lead to an increase in vascular permeability facilitates influx of fluid and polymorphonuclear leukocytes (PMNs) from the capillaries into the gingival sulcus and the formation of edema.

Macrophages are known to secrete TNF-α and IL-1β in response to serial stimulation with lipopolysaccharide (LPS). Caspase 1 activation by inflammasome complexes in response to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) induces the maturation and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β), IL-18 and IL-33. Both IL-1β and IL-18 are highly potent proinflammatory cytokines. IL-18 induces interferon γ expression and secretion from IL-12-primed naïve T cells to promote the differentiation of type 1 helper T cells. IL-33 has recently

been identified as the ligand of the IL-1 receptor family protein ST2 and promotes responses mediated by type 2 helper T cells. Upon cleavage of their proforms by caspase-1, these cytokines become active and are secreted. Thus, caspase-1 activity is critical for the inflammatory response. The release of DAMPs is a common event, as tissue damage and cell lysis are often associated with infections and lead to the release of host molecules. The importance of the recognition of these DAMPs by the immune system is that it not only allows the sensing of an ongoing infection and subsequent recruitment of more immune cells, but also can initiate the repair of the damaged tissue. It seems, then, that the innate immune pathway not only scans the cellular environment for signs of invading pathogens, but also recognizes the damage caused by them. Cytokines such as tumor necrosis factor and IL1 simulate IL8 synthesis which is also a potent chemoattractant for neutrophils.

The emigration of phagocytes is a multistep process that includes adherence of the cells to endothelial wall of the blood vessels (margination), followed by their emigration between the capillary-endothelial cells into the tissue (diapedesis or extravasation), and finally, their migration through the tissue to the site invasion (chemotaxis). This event is initiated by a variety of chemical mediators, some derived from bacteria, others generated by several plasma enzyme systems, and some others from products of various white blood cells. The mast cells release preformed tumor necrosis factor-a (TNF-a), which is largely responsible for the expression of adhesion molecules by endothelial cells (e.g., ELAM-1, ICAM-1). Combined with an increase in Interleukin-8 (IL-8) production by the epithelial cells, which helps establish a fast flow of PMNs through the junctional epithelium and the subsequent sticking and migration of PMNs. In addition, the presence of of neutrophil mediators, including leukotriene B4, platelet activating factor, thromboxane B2, elastase, and collagenase into the gingival sulcus contribute to the process. (Figure 3). Once in the gingival sulcus, however, the PMNs are unable to phagocytosis the bacteria.

Clinically, gingival inflammation is characterized by gingival redness, swelling and increased tendency of bleeding of the soft tissue.

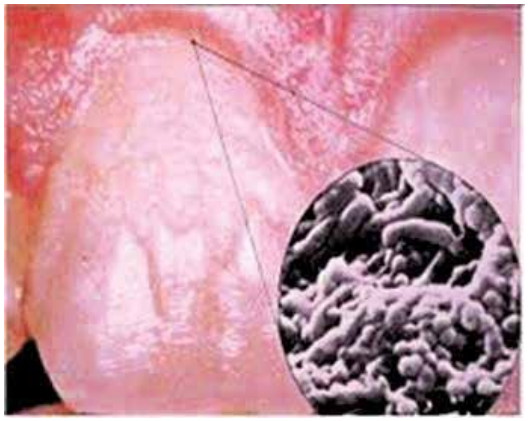
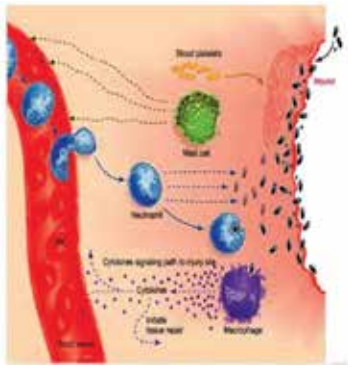


Fig. 2. Dental plaque formation, saliva derived glycoprotein deposits start to cover the tooth surface with what is referred to as "pellicle". The formation of pellicle is the first step in dental plaque formation. The pellicle is then colonized by Gram-positive and Gran-negative bacteria that interact with pellicle components enabling plaque to firmly adhere to the tooth surface.



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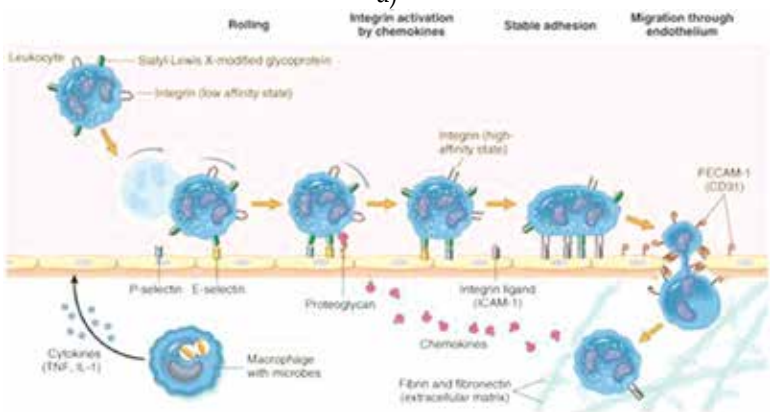

b)

Fig. 3. (a) Bacteria adhere the surface of gingiva. Mast cells secrete factors that mediate vasodilation and vascular constriction. Delivery of blood, plasma, and cells to injured area increases. Neutrophils secrete factors that kill and degrade pathogens. Macrophages secrete cytokines that attract immune system cell to the site and activate cells involved in tissue repair. (b) Rolling, margination and adhesion of neutrophils: interaction between selectins, integrins and cell adhesion molecules like VCAM. These process by diapedesis are madee for chemokines, bacterial peptides, C5a, LTB4 (Leukotreine), IL 8(interleukin). Actin myosin interactions in the leucocyte are responsible for, transmigration by diapedesis. (Figures of link: http://medicinembbs.blogspot.com/2011/02/inflammation.html)

4.1.2 The early lesion or stable lesion. At approximately 4–7 days of plaque accumulation, the nature of the developing lesion changes from one consisting primarily of PMNs to one with increased numbers of lymphocytes and macrophages. This is the early lesion in which vascular changes can be better observed, as illustrated by the activation of previously dormant capillary beds, and the development of perivascular inflammatory infiltrates below the junctional epithelium. The result is a net increase in the flow of fluid into the affected gingival tissues, and, after that, an increase in the flow of gingival crevicular fluid. Further concurrent widening of intercellular spaces between the epithelial cells of the junctional epithelium allows increased diffusion of bacterial products into the gingival tissues and escalation of the inflammatory response. The lesion begins as small perivascular infiltrates which progressively enlarge and conflate until they become clinically evident at around day 12 to 21. By day 21, lymphocytes make up 70 per cent of the infiltrate. As noted above,

gingivitis develops as perivascular lymphocyte/macrophage lesions. Increasing in size, they conflate and merge together, eventually becoming clinically evident**.** The lymphocytes are predominantly T cells with a CD4:CD8 ratio of around 2:1. Increased numbers of Langerhans cells are seen in the oral as well as oral sulcular epithelium. While interdigitating dendritic cells can be found in the perivascular spaces, the majority of macrophages in the developing lesion are acid phosphatase positive phagocytic cells. As soluble antigen enters the tissues, it is taken up by the resident Langerhans cells and carried to the regional lymph nodes where antigen specific T cells are sensitized. These sensitized cells then travel back to the site of original antigen challenge (i.e., the gingival tissues). Following further antigen presentation by dendritic cells, they are activated and control, together with the infiltrating phagocytic macrophages, the ingress of antigen and achieve a balance with the plaque biofilm. The various phagocytes (PMNs in the gingival sulcus and macrophages in the tissues) are unable to eradicate the microbial challenge, for plaque bacteria seldom invade the host tissues. The subsequent, prolonged nature of the inflammatory response results in gingivitis becoming chronic in nature. While in most people the immune response is able to contain the microbial challenge, it is only with mechanical cleaning that the microbial challenge can be eradicated. Collagen is degraded in the stable lesion but there is no loss of attachment. When the plaque is removed, gingival tissues repair and remodel, and there is no permanent damage or alteration of tissue architecture.

4.1.3 The established or progressive lesion. In some people, either due to environmental factors or their own innate susceptibility, or both, the stable lesion changes to a B cell / plasma cell response with the production of high levels of Interleukin-1 (IL-1) and Interleukin-6 (IL-6) and subsequent connective tissue breakdown and loss of bone. As the connective tissue attachment to the tooth breaks down, the junctional epithelium migrates in an apical direction and a periodontal pocket forms, which becomes lined by pocket epithelium with in-growth of rete pegs into the surrounding connective tissue. Increased permeability of this pocket epithelium allows continued ingress of microbial products, the continued production of inflammatory cytokines such as IL-1, TNF-a, Prostaglandin E2 (PGE2), leukotrienes, and chemokines, and perpetuation of the inflammatory process leading to continued tissue destruction. The main identifying feature of the progressing, established lesion is the predominance of plasma cells within the periodontal connective tissues indicative of a B cell adaptive immune response.

4.1.4 The advanced lesion. The main difference between the advanced and the established lesions is the overt loss of attachment that is evident clinically and histologically. It is now generally accepted that the mechanism of tissue destruction is via the effects of the immune response. Fibroblasts and macrophages are stimulated by the inflammatory cytokines IL-1, TNF-α and PGE_2 to produce matrix metalloproteinases (MMP), which are a family of proteinases whose primary purpose is the degradation of the extracellular matrix. Collagen molecules are cleaved into smaller fragments, which then become denatured in the extra-cellular environment or are phagocytosed by surrounding fibroblasts. With the advancement of the lesion, alveolar bone loss becomes apparent. However, a non-infiltrated fibrous band remains adjacent to the crystal bone, which effectively encapsulates the progressing lesion. With advanced periodontitis, plasma cells occupied 31% of the lesion volume, while the proportion of lymphocytes varied between 5% and 10%. Macrophages and polymorphonuclear (PMN) cells were found in densities of 1–2% and fibroblasts in 5%. Thus, the volume occupied by plasma cells was three times larger than the proportion of lymphocytes. Other inflammatory cells occurred only in small numbers (figure 4), containing substantial numbers of immunoglobulin (IgG, IgM).

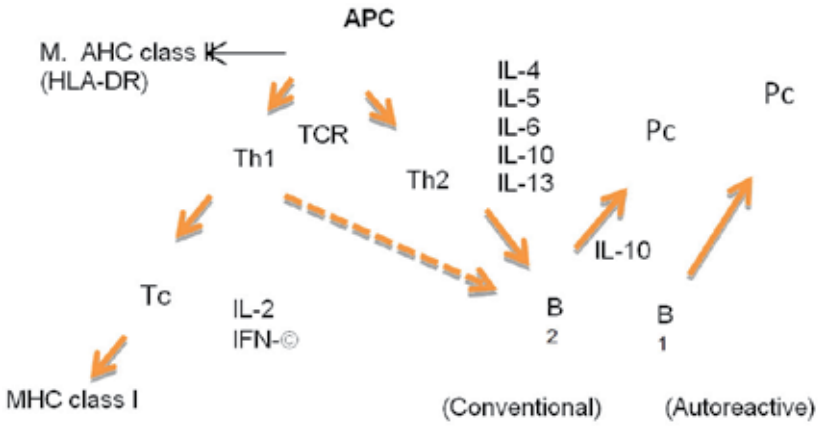

Fig. 4. Schematic outline of regulatory components of adaptive host response in periodontitis.

5. Conclusion

Treatment planning in gingivitis, as with any disease, must be based on an understanding of the aetiology and pathogenesis of the disease. While plaque is the cause of the disease, it is the innate susceptibility of the host that determines the ultimate outcome of the disease process. Innate susceptibility, in turn, is determined by the nature of the immune response to the specific periodontopathic complexes comprising the plaque biofilm. The innate immunity include physical barriers, such oral mucous, as well as the production of lysozyme, collectins, blood proteins, secretory Immunoglobulin, complement components, cytokines, adhesion, chemokines and others substances that regulate and coordinate many activities of the cells of innate immunity. The elimination (if possible) of any known cause of increased susceptibility and improvement in oral hygiene leads to the decrease of the dental plaque, which is responsible for the inflammatory alterations. Products containing antiseptics such as chlorhexidine or triclosan are effective against both gram-positive and gram-negative bacteria and reduced likelihood of gingivitis progressing to periodontitis, arrest progression of periodontitis, prevent supragingival calculus, and reduce oral malodor. Most self-administered plaque control programs are ineffective unless periodic professional reinforcement is also provided; a single session of ultrasonic prophylaxis associated with oral hygiene instructions is an effective method of reversing gingivitis, reducing bleeding upon probing, as well as the subgingival microflora. Through constant flushing activity during sonic instrumentation, disrupting the bacterial cell.

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7

Gingival Tissue and Pregnancy

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1. Introduction

During pregnancy, many changes take place in both systemic and local environments leading to a significant increase in the severity of gingivitis. In recent years, many studies have assessed the changes that occur within the gingival tissue during pregnancy and their potential impact on the foetus.

Pregnancy is defined as a state that includes fertilization, implantation and embryonic and fetal growth that ends with the birth of a baby after 280 days or 40 weeks. Pregnancy gingivitis presents as gingival erythema, enlargement and bleeding. Epidemiological studies have shown that the prevalence of gingivitis increases during pregnancy when compared to control groups. The evidence indicates that hormonal changes affect the rate of cell turnover of the gingival tissues, inducing several microbiological changes in the subgingival flora and immunosuppression of the immune system.

New evidence supports an association between the periodontal status and complications during pregnancy. Some countries have developed policies and practice guidelines that recommend oral care and the control of inflammation of the periodontal tissues throughout pregnancy.

This chapter will review the effect of pregnancy on gingival status and the potential impact of gingival inflammation on the unborn child.

2. Pregnancy gingivitis

Pregnancy is accompanied by an increase in the production of estrogen and progesterone. Initially, the ratio of estrogen and progesterone is 100:1 but during the final months this changes to 1:1. Following birth the hormones reach their normal levels within 2 to 3 days (Mariotti 1994, Laine 2002).

Two theories have been proposed for the actions of the hormones on the cells of the periodontal tissues: 1) a change in the effectiveness of the epithelial barrier to bacterial insult and 2) an effect on collagen turnover (Markou *et al*. 2009). There are several receptors for estrogen and progesterone within the gingival tissue. The estrogen receptors (ERs) exist as two subtypes: ERalpha and ERbeta. ERbeta is widely expressed at high levels in oral tissues (Välimaa *et al*.2004). ERbeta is involved in important physiological processes, such as cell differentiation, extracellular matrix organization and stromal-epithelial communication

(Morani *et al*. 2008). Estrogen firstly decreases collagen production and keratinization of gingival epithelium and secondly induces proliferation of fibroblasts and decreases the collagen and no collagen proteins, blocks the turnover of the gingival tissue, thereby reducing the capacity of gingival tissue to repair. The result is an increase in the permeability of the epithelial barrier and an increased response to plaque bacteria. (Markou *et al*. 2009). Liu *et al.* (1999) observed a decrease in cellular proliferation of the periodontal ligament (PDL). In addition, a decrease in the rate of collagen is mediated by ERbeta. In contrast, no immunoreactivity was expressed in these cells for progesterone receptors, implying that progesterone does not have a direct effect on the function of PDL cells (Jönsson 2007).

The reactivity for progesterone receptors was observed in gingival fibroblasts (Kawahara & Shimazu 2003). Progesterone also has effects on the vascular system favoring an increase in gingival exudate and vascular permeability and proliferation; this is possibly due to progesterone receptors present in the gingival tissue (Markou *et al.* 2009). Other factors associated with the tissue changes during pregnancy are those related to the fibrinolytic system. Fibroblast and macrophages from gingival tissue produce plasminogen activator inhibitor type-2 (PAI-2). Several studies had suggested a hormonal influence on the PAI-2 that disturbs the balance of the fibrinolytic system. A lower inhibitory capacity in terms of a low production of PAI-2 associated with progesterone during pregnancy in women with a higher inflammatory reaction has been observed and could contribute to gingivitis during pregnancy (Kinnby *et al*.1996).

3. Pregnancy and the subgingival microflora

Pregnancy is accompanied by many changes in the composition of the subgingival microflora. Jensen *et al*. (1981) and Korman and Loesche (1980,1982) showed that *Prevotella intermedia* and *Prevotella meloninogenica,* use either estradiol or progesterone as a substitute for napthaquinone (Jensen *et al.* 1981) and vitamin K (Korman & Loesche 1982), as essential growth factors which encourage a proliferation of these microorganisms in subgingival plaque during pregnancy.

The correlation between the concentration of hormones in saliva and changes in oral microflora during pregnancy are summarized in Table 1. These data provide further support for the fact that hormonal changes during pregnancy promote microbiological changes in the subgingival flora. Most of these studies found a positive correlation between an increase in estradiol and progesterone and an overgrowth of *P.intermedia* (Korman & Loesche 1980, Muramatsu & Takaesu 1994, Gursoy *et al*. 2008, Carrillo-de-Albornoz et *al*. 2010). A positive correlation was also observed between an overgrowth of *Porphyromonas gingivalis*, *Tannerella forshytia* (Andriens *et al*. 2009) and *Campylobacter rectus* and an increase in estradiol concentrations. (Yokoyama *et al* 2008).

It has been suggested that microbial changes observed during pregnancy may also occur at other sites in the body. High microbial counts in the vagina have been correlated with gingivitis in pregnant women when compared with patients without gingival inflammation (Person *et al.* 2009). Correlations between microbiological changes and increased gingival inflammation have also been extensively studied. The subgingival microflora increased from week 12 of pregnancy, was maintained during the second quarter and reduced during the third trimester to postpartum (Korman & Loesche 1980, Muramatsu & Takaesu 1994,

Yokoyama *et al.* 2008, Andriens *et al.* 2009). However, some studies had shown that these changes can be maintained throughout pregnancy (Gürsoy *et al.* 2009, Carrillo-de-Albornoz *et al.* 2010). Most of the studies agree that the increase in gingival inflammation occurs in the second quarter and is associated with an overgrowth of *P. intermedia*, (Korman & Loesche 1980, Muramatsu & Takaesu 1994). A correlation was also observed between an increase in gingival bleeding and an increase in *P. gingivalis* and *T. forsythia* (Andriens *et al.* 2009). After delivery, the levels of bacteria are reduced although, these changes can be sustained between 4 to 6 weeks postpartum (Gursoy *et al.* 2008, Andriens *et al.*2009). The evidence supports the fact that clinical changes during pregnancy are associated with changes in the subgingival flora. However, other factors such as an impairement of cellular function and immunological changes may also contribute to the increased severity of gingivitis in pregnancy. (Figure 1, Figure 2).

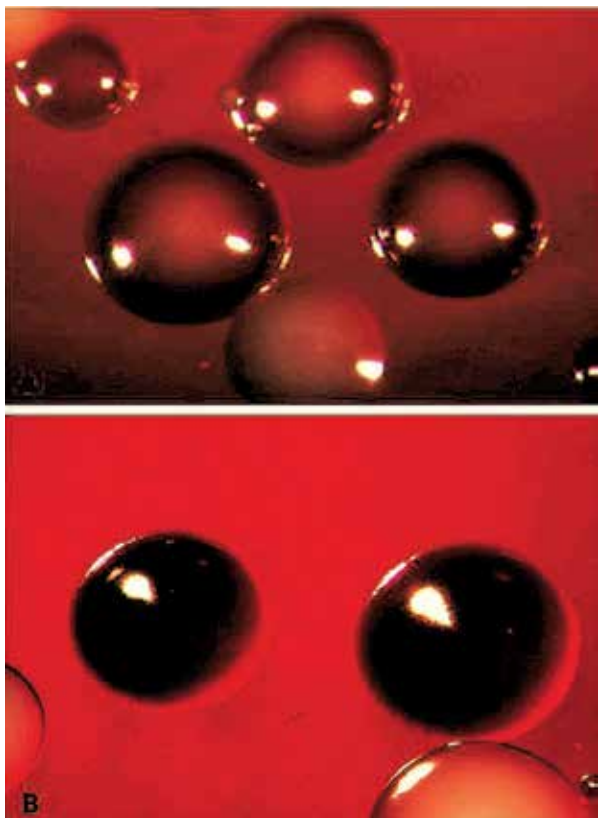

Fig. 1. A. *Prevotella intermedia* isolated from a pregnant patient. (Oral Basic Research Unit-UIBO, 2010 used for reseach purposes)

Fig. 2. Severe gingival changes in a woman with heavy plaque deposits during the second trimester (School of Dentistry, Universidad El Bosque, 2010. Used for academic resources)

Authors	Population	Design of Study	Hormone	Clinical changes	Time	Microorgnims
Korman & Loesche 1980	USA	Cross Sectional	Estradiol progesterone	Gingivitis	Second quarter	*P. intermedia* *P.melanogenica*
Muramatsu & Takaesu 1994	Japan	Cross Sectional	Estradiol progesterone	Bleeding	3 -5 month	*P. intermedia*
Yokoyama *et al 2008*	Japan	Cross sectional	Estradiol	Gingivitis	Second quarter	*C. rectus*
Gursoy *et al*. 2008	Finland	Cohort	No report	Gingivitis	12 weeks to end of pregnancy	*P. intermedia*
Andriens *et al.* 2009	Switzerland	Cohort	Estradiol progesterone	Gingivitis	12 – 28 weeks	*P. gingivalis* *T. forsythensis*
Carrillo de Albornoz *et al* 2010	Spain	Cohort	No report	Gingivitis	12 weeks to the end of pregnancy	*P. gingivalis* *P. intermedia*

Table 1. Clinical and Microbiological changes in subgingival plaque associated with hormonal changes during pregnancy.

4. Immunological changes during pregnancy

Increases in progesterone and prostaglandins modulate the immune system during pregnancy. Some degree of immunosuppression occurs during pregnancy which minimizes the risk of fetal rejection (Hansen 1988). Maternal tolerance of the fetal allograft could be the result of the integration of numerous mechanisms promoted by different cells presented in the decidua. Decidual macrophages and dendritic cells, which are found in close association with T lymphocytes are the most potent activators of T-lymphocyte responses and could play a sentinel function for the immune system, initiating antigen-specific T cell responses to fetal antigens (Piccini 2005). Progesterone and glucocorticoids share important anti-inflammatory and immunosupressive properties and both hormones have potent anti-proliferative effects in mitogen activation and cytotoxic T cell generation (Stites *et al*. 1983). There is a decrease in CD4/CD8 ratio and peripheral blood lymphocytes and a low expression of HLA class I during pregnancy (Szekeres-Bartho *et al.* 1985). T cell cytokine profile could be modulated by the hormones present in the microenvironment; high doses of progesterone present at the feto-maternal interface and in the cumulus induce the production of IL-4, a strong inducer of Th2 profile which is anti-inflammatory. Progesterone also upregulates HLA Class I type G gene expression, which is the NK inhibitory ligand (Yie *et al*. 2006), supresses the proliferation of CD4+ lymphocytes (Bainbridge *et al*. 2000) and induces apoptosis in activated CD8+ Lymphocytes (Fournel *et al*. 2000). Progesterone also down-regulates IL-6 production, rendering the gingiva less efficient at resisting the inflammatory challenges produced by bacteria (Lapp *et al*. 2003). Estradiol and progesterone levels increase in saliva during pregnancy, reaching a peak in the third trimester. Levels of IL-1 beta and PGE2 showed no significant change in their concentration in crevicular fluid during pregnancy. Although their concentrations were higher than non-pregnancy women, they did not correlate with clinical changes in gingival tissue (Figuero *et al*. 2010). However, there was an increased concentration of IL1-beta, IL6 and PGE2 in plasma in pregnant women with periodontitis, indicating that the presence of the disease can lead to increased systemic inflammatory markers (Offenbacher *et al.* 1998, Ebersole *et al*. 2010). A decrease in neutrophil chemotaxis has been observed during pregnancy, which may be due to the effects of sex hormones (Miyagi *et al.* 1992). These changes in the immune system may explain the susceptibility to infection during pregnancy and can support the clinical observation of complications in pregnant women with periodontitis.

5. Gingival changes during pregnancy and their treatment

The prevalence of gingivitis during pregnancy has been studied in different populations. (Ainamo *et al*. 1982). Studies that have assessed the prevalence of gingivitis during pregnancy using the CPITN index are shown in Table 2. The prevalence varies between 67 to 100%. In some studies, the prevalence and severity is higher in pregnant women when compared with non-pregnant women (Nuahma & Annan 1998, Rakchanok 2010). However, Miyazaki *et al*.1991 observed no difference between pregnant and non-pregnant. Studies using the CPITN index reported that most pregnant women have calculus and gingival inflammation and few women have healthy gingiva during pregnancy. Most pregnant women have non-surgical treatment needs and oral hygiene instruction but very few require complex periodontal treatment (Table 2). Cohort studies support the evidence that pregnancy is associated with gingival changes, (Tilakaratne *et al*. 2000, Gürsoy *et al.* 2008, Carrillo-de-Albornoz *et al*. 2010). The clinical indicators evaluated in these studies were bleeding on probing and pocket depth without loss attachment, which indicated that this

may be due to a more pronounced gingival overgrowth in the proximal surfaces of anterior teeth (see figure 2). Few studies have determined the correlation of detectable levels of plaque and gingival clinical changes. However, two studies reported the same level of dental plaque in pregnant and controls; however, pregnant women responded more severely to dental plaque than non pregnant women (Gürsoy *et al.* 2008, Carrillo-de-Albornoz *et al*. 2010).

The period between weeks 12 and 28 of pregnancy can be characterized by increased susceptibility to plaque bacteria and an inflammatory response in the gingiva (Adraiens *et al.* 2009). Studies have not observed loss of attachment during pregnancy, but any pre-existing periodontitis is exacerbated.(Amar & Chung 1995). Progesterone can reduce local production of matrix metalloproteinases and may explain why pregnancy gingivitis may not necessarily progress to periodontitis (Laap *et al.* 1995, Gursoy *et al*. 2010). Several cohort studies had shown that the loss of attachment can be observed during pregnancy and the predictor variables are depth of the pockets> 4 mm before week 26 weeks and presence of bleeding on probing (Moss *et al*. 2005). Thus, the loss attachment during pregnancy was not associated with pre-existing gingivitis, but with the presence of periodontal pockets when women became pregnant. During pregnancy, a significant change in tooth mobility can be also being observed. The initial mobility is dependent on the degree of vascularization and vascular volume of the periodontal ligament. When the female sex hormones act at high concentrations for prolonged periods, an increase in the permeability within the periodontal vascular system could occur. The resulting edema in the periodontal ligament may result in an increase in horizontal tooth mobility in the absence of any loss of periodontal support (Mealey & Moritz 2003).

Authors	Population	Sample size pregnant	Sample size control	Index	Prevalence % Pregnant/control	CIPTN % Periodontal Treatmet Need			
						0	1	2	3
Miyazaki et al. 1991	Japan	2424	1565	CPITN	95/96		**Pregnant**		
						5	5	70	20
							No Pregnant		
						4	4	75	20
Vasiliauskiene I.2003	Lintuania	1070	No control	CPITN	93	14	15,1	58	19.2
Agbelusi et al 2000	Laghos	250	No control	CPITN	67.8	32.2	13.6	50	18.9
Wandera et al 2009	Uganda	713	No Control	CPITN	67.3	32.7	3.3	63.4	0.6
						CIPTN Mean Periodontal Status				
						0	**1**	**2**	**3**	**4**
Acharya &, Bhat 2009	USA	259	204	CPITN	100%			**Pregnant**		
						0.66	1.91	2.66	0.70	0.10
								No Pregnant		
						2.41	1.71	0.99	0.29	0.09
Miyazaki H et al 1991	Japan	2424	1565	CPITN	95/96			**Pregnant**		
						2.4	0.8	2.4	0.4	00
								No Pregnant		
						1.9	0.6	3.2	0.3	00

Table 2. Prevalence, Periodontal status and periodontal treatment needed in pregnant women.

Another clinical feature that may arise during the latter stages of pregnancy is the so-called pyogenic granuloma, (pregnancy tumor or epulis). This occurs in 1 to 5% of pregnant women (Amar & Chung 1995). These result from angiogenesis, caused by the increased levels of progesterone and the stimulatory effects of estradiol on the connective matrix. They usually arise in sites with pre-existing gingivitis (Jafarzadeh *et al.* 2006), and often involve the interdental papilla of one of the maxillary anterior teeth and rarely exceed 2 cm in diameter. When they persist they can be surgically remove and their removal should be delayed until after delivery. (Jafarzadeh *et al*. 2006, Rader *et al*.2008). Their removal during pregnancy may be justified if they are continuously traumatized. Ideally they should be removed using CO2 or Nd:YAG laser. (Powell *et al*.1994, Lindenmüller *et al*. 2010). Surgery should only be done when the plaque and gingival inflammation have been controlled.

6. Knowledge, attitudes and practices of pregnant women

Poor prenatal care during pregnancy is often associated with women from deprived backgrounds. Such women have practices, attitudes and knowledge based on beliefs, myths and cultural tradition. These false beliefs that pregnancy per se has an adverse effect on the teeth and periodontal tissues lead to a lack of self care and deterioration of the oral health of these women. Evidence suggests that poor oral health can lead to obstetric problems such as low birth weight and preterm delivery (D'Angelo *et al*.2007, Luce *et al.* 2011).A number of factors influence visits to the dentist during pregnancy such as social, personal and financial factors and lack of knowledge of the possible connection between oral health and pregnancy outcome (Machuca *et al*. 1999, Gaffield *et al*. 2001, Yalcin *et al*. 2002, Sarlati *et al*. 2004, Acharya & Bhat 2009). However, an assessment of pregnant women with a relatively high socioeconomic status in the U.S. found that 49% of respondents reported having attended a dentist, and 43% were aware of the connection between oral health and pregnancy outcomes. (Huebner et al.2009. This suggests that better education focused on the importance of dental care before and during pregnancy should be provided to prevent future complications. (Al Habashneh *et al.* 2005).

We assessed the level of knowledge and oral health practices in pregnant women in a low socioeconomic population in Bogota, Colombia. The study showed that most pregnant women had good knowledge of the major oral diseases and how to prevent them; however, knowledge about the associations between pregnancy and perinatal complications were limited. Over 60% had attended the dentist during pregnancy and more than 50% claimed that they had received advice from their doctors and had been recommended to visit their dentist. However, they were not informed about the potential effect of changes in the mouth during pregnancy and the risk for pregnancy complications. In general, these women were from lower socioeconomic communities and the majority of women claimed to brush their teeth frequently and 35% used dental floss. These women had not perceived significant changes, such as gingival bleeding and swelling, during pregnancy. Women being treated at the community clinic, expressed fears of having radiographs, extractions and local anaesthesia during pregnancy. This study observed a significant improvement in the perception of oral health in a population that regularly attended public services and showed the impact of oral health care guide implemented in these public hospitals and emphasizes the importance of pre-conceptional preventive practices that improve the oral health of

women before they enter the pregnancy to minimize gingival and periodontal changes associated with this condition.(Lafaurie *et al*. 2011, unpublished data)

Another factor that influences health care during pregnancy is the low awareness amongst health care professionals of the changes that can occur in the oral cavity during pregnancy.There is clearly a need to provide health personnels with information on the importance of oral care during pregnancy (Al-Habashneh *et al.* 2008, Huebner *et al.* 2009, Salama *et al*. 2010).

7. Treatment of gingivitis during pregnancy

Few studies have assessed the effect of treatment on gingivitis during pregnancy. Most studies have evaluated the safety and effect of periodontal treatment on pregnant women with periodontitis. However, Lopez *et al*. (2005) assessed the safety and effect of treatment of gingivitis during pregnancy and found an increased risk of complications such as pre-term birth (PPT) and low birth weight (LBW) in women who were not treated.

The American Heart Association (AHA) guidelines for prevention of infective endocarditis report that bacteremia associated with oral bacteria was often associated with the patient's routine activities, such as chewing, tooth brushing and after therapeutic procedures (Wilson *et al*. 2008). Several studies have evaluated the presence of bacteremia in patients by mechanical stimuli such as brushing and flossing (Lockhart *et al*. 2008, Castra *et al*. 2009).), chewing (Geerts *et al*.2002, Ide *et al*.2004) and ultrasonic scaling (Forner *et al*. 2006, Kinane *et al*. 2005). The studies showed that pregnant women with high levels of gingival inflammation can be exposed to bacteremia arising from the mouth.

Once oral bacteria gain access to blood vessels they can cross the placental barrier. Subgingival bacteria increase in pregnancy gingivitis, as it had been reported that *Streptococcus* ssp. and *Fusobacterium nucleatum* have been cultured from amniotic fluid in pregnant women (Bearfield *et al*. 2002). An increase of IgM antibodies to *F. nucleatum, P. intermedia and C. rectus* in fetal cord blood, were significantly higher for preterm as compared to full-term neonates. It has been suggested that fetal infections by these microorganism may be related to prematurity (Madianos *et al*. 2001). Fardini *et al*. (2010) injected bacteria obtained from human saliva and dental plaque into pregnant rats. They demonstrated that a wide range of oral microorganisms may be associated with intrauterine infection.

Given the evidence that periodontal status is associated with complications of pregnancy, some countries have started the implementation of technical standards and guidelines of care which includes oral care during pregnancy. The guideline developed by the State of California has been widely used in clinical practice and has been adapted by other states in the USA (Kumar J, Samuelson 2009). Such guidelines emphasize the need for the care of pregnant women and include the time the treatments to be performed, medications given during pregnancy and indications and contraindications for certain procedures.

The recommendations for periodontal treatment are as follows:

7.1 Dental treatment should be as frequent as possible during the second trimester (weeks 12 to 28) of pregnancy. However, some studies emphasize that gingival inflammation should be reduced early in pregnancy. Treatment, such as scaling and root planing can be performed from week 8 without increased risk for pregnancy (Lopez *et al*. 2002, 2005).

7.2 Diagnostic X-rays during pregnancy are safe and can improve the assessment of periodontal status. A panel of experts from the Food and Drug Administration (FDA)

concluded that the recommendations regarding the use of oral radiographs should not be altered because a patient is pregnant (American Dental Association, 2010). The number and type of radiographs depends on the clinical condition and the patient's medical history. As a standard practice for taking radiographs the patient should be protected with a vest that covers the neck and abdomen.

7.3 Most periodontal therapy can be performed with local anesthesia and it is important to note that this does not increase adverse fetal outcomes. Most of the anesthetics used in dentistry are classified as Class B by the FDA. However, mepivacaine and bupivacaine are classified as class C and are contraindicated in pregnancy. Local anesthetics cross the placental barrier by passive diffusion but are not teratogenic (Hool 2010). Although epinephrine is not teratogenic intravascular injection can be avoided by correctly aspirating with the syringe. (Martin & Varner 1994). Before the use of vasoconstrictors the blood pressure should be measured because of hypertension during pregnancy occurs between 6 and 10% of pregnancies.

Although these treatment guidelines exist for pregnant women, there are no specific protocols for the treatment of gingival inflammation in pregnant women. All the evidence, including work from our group, indicates that periodontal treatment can induce a bacteremia in patients with severe periodontal disease (Lafaurie *et al*. 2007, Castillo *et al*. 2011). Although most pregnant women have gingivitis during pregnancy the degree of inflammation can vary from mild to severe. The needs for management protocols for pregnant women with have not yet been defined. There are no clear regulations on the use of antiplaque products during pregnancy. Toothpastes containing triclosan have been reported to reduce gingivitis during pregnancy (Kraivaphan *et al.* 2009). Clorhexidine has been associated with a reduction in cellular proliferation in vitro (Marioti *et al*. 1999) and desquamation of the mucosa had been reported (Jones 1997). Mouthwashes, such as chlorhexidine, have been used only once a day as part of periodontal therapy in women with gingivitis during pregnany (Lopez *et al*. 2002, 2005). Based on this evidence it seems beneficial to use mouthwashes and toothpastes containing anti-plaque agents in patients with high levels of gingival inflammation.

8. Gingival status, gestational diabetes and perinatal complications

A perinatal risk factor is any biological property, environmental or social, which when present is associated with increased probability of an adverse event, either in the fetus, the mother, or both. Early identification of risk factors may allow strategic planning of prenatal care. Prematurity is the leading cause of neonatal morbidity and mortality worldwide and the frequency varies between populations. The World Health Organization (WHO) in 2000 estimated the prevalence of LBW is approximately 15.5% (7% for most developed countries, 16.5% for those in developing counties. The prevalence determined for each of the regions established by the United Nations was: Africa 14.3% Asia 18.3%, 6.4% Europe, Latin America and the Caribbean 10%, 7.7% North America, and Oceania 10.5%. The risk of a premature infant dying is 180 times greater than that of a full term baby and those that survive have an increased risk of disability. The World Health Organization in 1976 defined low birth weight (LBW) as below 2,500 grams (5.5 pounds) regardless of gestational age and defines pre-term delivery (PPT) as before 37 weeks of gestation.

Many factors have been associated with LBW and PPT. They include socio-demographic factors, maternal age <18 and> 35 years, black race, low socioeconomic status and lack of

access to health services. All have shown to increase the risk for these perinatal complications (Elster 1984. Buescher & Mittal 2006, Rosenthal & Lobel 2011). Other associated factors include a history of PPT prior to 35 weeks gestational age, placental ischemia, premature rupture of membranes and cervical incompetence and complications during pregnancy ,such as genito-urinary infections, multiple gestation, bleeding in the second half of pregnancy and polyhydroamnios have been considered as predictors for LBW and PPT (McCormick 1985, Ferraz *et al*.1990, Alexander &. Korenbrot 1995, Kramer *et al*.1987, Heaman *et al*. 2008). Aspects related to the lifestyle, the consumption of snuff, alcohol and psychoactive substances and nutritional and psychosocial factors also increase the risk of perinatal complications. (Kogan 1995, Farrell *et al*.2006, Lederman 2011).

Periodontal status during pregnancy has also been associated with perinatal complications. Numerous cohort studies have been conducted to evaluate the risk association between periodontitis and pregnancy complications (Lopez *et al*. 2002, Moreu *et al*. 2004, Pitiphat *et al*, 2008. Saddki *et al*. 2008, Agueda *et al*. 2008). Three systematic reviews demonstrate an increased risk for PPT, LBW and low birth-weight with preterm birth and intrauterine growth restriction in individuals with periodontitis (Xiong *et al*.2007, Khader & Ta'ani 2005, Vergnes & Sixou M 2007). The risk varies between populations but in most studies the presence of periodontitis shown to be an independent risk factor for these complications.

Several studies have identified microorganisms associated with periodontal infections such *as P. gingivalis*, *Actinobacillus actinomycetemcomitans*, *F. nucleatum*. , *P. intermedia*, *T. forsythia* and *Treponema denticola* in samples of placenta of women with preterm labor and preeclampsia. (Barak *et al*. 2007, León et al. 2007). The proteolytic capacity of these microorganisms and their ability to activate systemic proinflammatory mechanisms, facilitate these perinatal complications. Two periodontal organisms have been specially associated with LBW; C. *rectus* and *P. gingivalis*. Rat fetuses exposed to infection with *C. rectus* showed a decreased size and weight compared to a control group (Yeo *et al*. 2007, Simor *et al*. 1986, O'Sullivan *et al*. 1988). Associations between periodontal disease and LBW and PPT were explained also by relating the physiological processes that lead to the time of delivery as periodontal infection lead to increased serum levels of PGE2, TNF, IL 1 ß, IL6, which trigger a series event such as cervical dilation, uterine contractions start and the activation of metalloproteinases that generate the breakdown of amniochorion. It seems likely, that periodontal disease could lead to preterm delivery (Damare *et al*. 1997, Offenbacher *et al*. 1998, Dörtbudak *et al*. 2005, Ebersole *et al*. 2010).

Although a risk association between periodontitis and pregnancy complications is accepted, intervention studies comparing individuals treated with scaling and root planing with a control non treated group have proved to be conflicting. Three trials reported that treatment reduced the complications of pregnancy, (Lopez *et al*. 2002; Tarannum &, Faizuddin 2007; Offenbacher *et al*. 2006). 4 studies in developed countries, however, failed to find an effect on pregnancy complications (Jeffcoat *et al*. 2005, Michalowicz *et al*. 2006, Newnham *et al*. 2009 Offenbacher *et al*. 2009). However, the studies differed in many respects: The type of population, sample size, presence of other perinatal risks, treatment received and by whom, and the weeks of gestation when the treatment took place. A systematic review (Polyzos *et al*. 2008) which evaluated 7 intervention studies found that scaling and root planing showed a protective effect for PPT OR 0.55 (0.35 to 0.87) but not for LBW OR 0.48 (0.23 to 1). The effect of treatment was significantly higher in patients without a history of PPT or LBW OR

0.48 (0.29 to 0.77) and a mild disease OR 0.49 (0.28 to 0.87). In 2010, the authors included another 3 studies in highly developed countries and show a loss of the protective effect of the periodontal treatment on perinatal complications (Polyzos *et al*. 2010).
Although the evidence is controversial, the periodontal treatment is a safe procedure and should be performed early in pregnancy between weeks 8 to 28. However it is important to further evaluate the treatment of advanced periodontal disease in patients with high perinatal risk. The most evidence-based studies show that periodontal treatment during pregnancy reduces further loss of attachment and improves the clinical status of the periodontium during pregnancy.

9. Periodontal status in high-risk women

Three conditions must be reviewed in relation to periodontal status and treatment needs during pregnancy.

9.1 Previous history of preterm delivery (PPT) and low birth weight (BWT)

The history of PPT and BWT is the most important risk factor for perinatal complications. The evidence indicates that periodontal treatment fails to reduce the incidence of pregnancy complications in patients with a history of PPT and LBW, It, It is necessary to review the protocols for periodontal management in patients with high levels of gingival inflammation. The use of antibiotics in chronic infections during pregnancy has been studied. The evidence indicates that antibiotics are safe during pregnancy and reduce perinatal complications (McDonald et al. 2007). Since gingival inflammation is associated with a polymicrobial flora, broad-spectrum antibiotics may be preferable in the treatment of pregnant women at perinatal risk. Beta-lactam antibiotics such as penicillin and cephalosporins are the drugs of choice. They are categorized as class B drugs by the FDA, cross the placenta, but are considered safe for the fetus (Nahum et al. 2006) If the patient is allergic to penicillin a macrolide antibiotic such as erythromycin, clindamycin and azithromycin, which are rated B by the FDA may be prescribed (Crider et al. 2009). However, antibiotics are only indicated in high-risk perinatal patients with high levels of inflammation and requiring extensive scaling and root planing. The use of antibiotics in these patients is warranted to control the high degree of bacteremia during mechanical treatment. The American Gynecology and Obstetrics Association suggest the following scheme for the prevention of infective endocarditis in pregnant women:

- Ampicillin 2 g intravenously 1 hour or 30 minutes before the procedure
- Cefazolin 1 g IV 1 hour or 30 minutes before the procedure
- Amoxicillin 2 g orally 1 hour or 30 minutes before the procedure
- Clindamycin 600mg 1 hour 30 min or earlier in patients who are allergic to penicillin

9.2 Hypertensive disorders of pregnancy

The prevalence of hypertension during pregnancy is between 6 and 10%. Hypertensive diseases and preeclampsia in particular are a cause of morbidity and perinatal and maternal mortality. They are more common in developing countries. The effect of periodontal infection on the incidence of preeclampsia has been evaluated in cohort studies. A systematic review showed an OR 1.76, 95% CI: 1.43-2.18, in women with preeclampsia compared with pregnant women without preeclampsia; the presence of periodontitis

increased the risk of perinatal complication (Vergnes *et al.* 2008). Because of the association between periodontitis and preeclampsia it is important that the periodontal status of pregnant women with preeclampsia risk are assessed in early pregnancy and treated before 20 weeks before presenting the early signs of this condition.

9.3 Gestational Diabetes

Gestational Diabetes Mellitus (GDM) is a carbohydrate intolerance of variable severity first recognized during pregnancy. Prevalence of GDM has been reported between 8 al 16%. (Moses et al. 2001, Chodick *et al.* 2011). Women with diabetes and with a past history of gestational diabetes, age > 40 and a body mass index > 35 Kg/m (BMI) are the most important factors for GDM during pregnancy (Teh *et al.* 2011). Women with gestational diabetes had a higher mean plaque index and higher mean gingival index than healthy pregnant women (Mittas *et al.* 2006). Patients with GDM and pregestational obesity had significantly more gingivitis and periodontal attachment loss that those with normal pregestational BMI. (Guthmiller *et al.* 2001, Chapper *et al*. 2005). Periodontal treatment should be considered when establishing future recommendations for metabolic control for this group of patients. However, in many cases, the periodontal treatment may be required by patients without good metabolic control with high levels of glycated hemoglobin (HbA1c) ≥ 8.0). In these uncontrolled diabetic patients with active periodontal disease, the first phase of treatment is to reduce levels of inflammation and prevent hyperglycemia produced by pain and stress of infection. The patient should be referred to a physician for a review of their metabolic control (Vermillo 2003.). Well controlled patients may be treated with conventional periodontal treatment. The use of antibiotics for periodontal treatment should be considered in these patients after consultation with their physician. Clinical protocols for gestational Diabetes Mellitus should be developed.

10. Conclusion and recommendations

Gingivitis during pregnancy is exacerbated by the hormonal changes that affect the host's responses to dental plaque. Ideally oral health policies and guidelines should be developed for the oral care of women before becoming pregnant. The treatment of gingivitis should commence at an early stage of the pregnancy to minimize the risk of more severe disease at a later stage. However, in high risk patient's treatment of periodontal disease should be undertaken with caution and the use of antibiotics may be recommended. Periodontal treatment improves the oral conditions of pregnant women and could help reduce perinatal complications.

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8

Diagnosis and Monitoring of Gingivitis in vivo Using Non-Invasive Technology - Infrared Spectroscopy

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1. Introduction

Plaque-induced gingivitis is a localized inflammation affecting marginal periodontal soft tissue (Armitage, 1999). It is considered to be a reversible periodontal disease. In contrast, periodontitis is an irreversible destructive periodontal condition, which is usually preceded by gingivitis although not all gingivitis develops into periodontitis. Why some gingivitis sites transition to periodontitis sites is not well understood, although there are some indications of "at risk" populations such as smokers and poorly controlled diabetics (Burt, 2005). It is also understood that development of chronic periodontitis only occurs in areas of long-standing gingivitis and furthermore that teeth with consistently inflamed gingival tissues are at a significantly higher risk of attachment and tooth loss (Lang et al, 2009). Consequently being able to non-invasively and closely monitor gingivitis sites would be very helpful in the prevention of periodontal disease. The basic clinical measures for periodontitis are gingival bleeding, radiographic bone loss, clinical attachment loss and clinical probing depths (Burt, 2005). Current clinical diagnostic measures are unable to identify gingivitis with high risk of transition to periodontitis since not all sites with gingivitis actually progress to periodontitis (Armitage, 1996). Therefore, the search for more accurate periodontal diagnostic instruments is continuing and a number of non-invasive diagnostic modalities such as optical and infrared spectroscopy, optical coherence tomography (OCT) and ultrasound have been evaluated for their potential in periodontal diagnosis. An illustration to better visualize the overall features of currently used clinical methods and emerging optical and infrared based diagnostic methods for periodontal diseases including gingivitis is presented in Figure 1. In principle, these diagnostic methods can be classified into three categories based on their features and clinical aspects. Clinical examination which is the mainstream of current practice and the gold standard, primarily measures clinical parameters such as bleeding on probing (BOP), probing depth (PD), and clinical attachment loss (CAL) as well as bone loss with the use of dental radiographs.

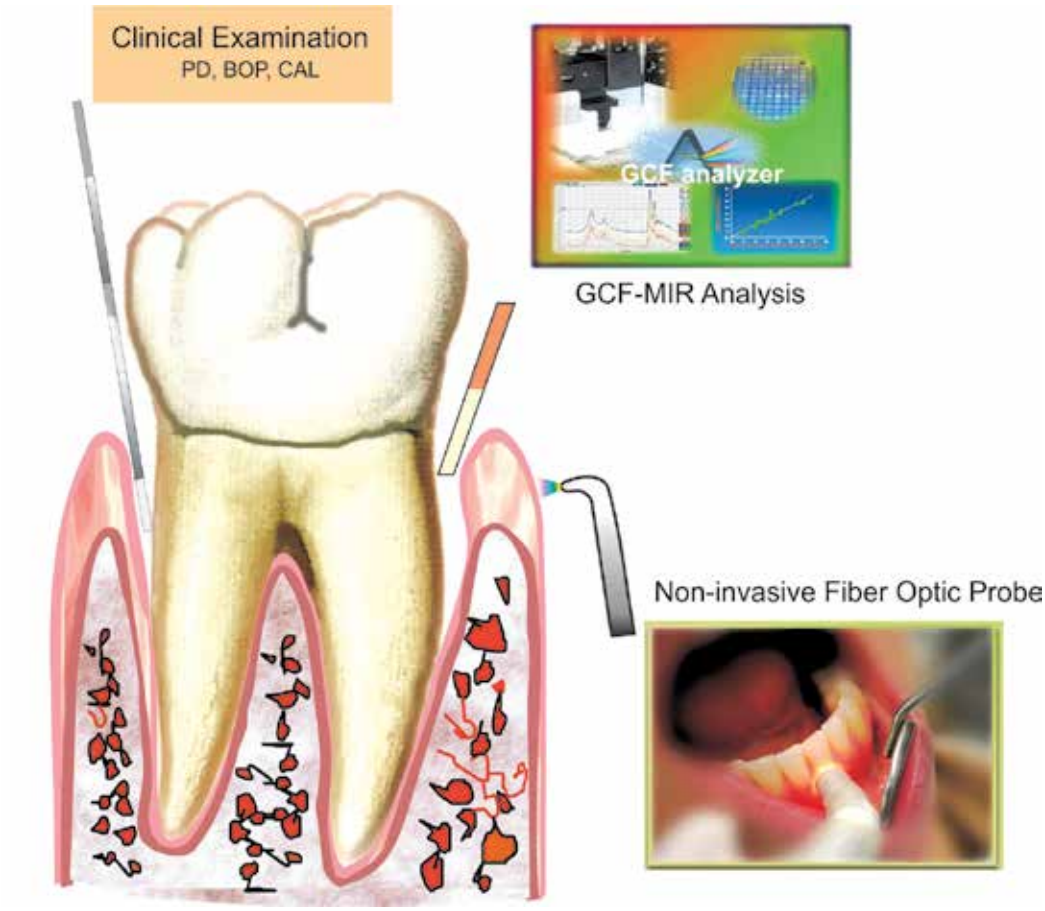

Fig. 1. Summary of current and proposed infrared spectroscopy based diagnostic methods for gingivitis.

The second group consists of molecular finger printing or finding molecular markers in gingival crevicular fluid (GCF). In addition to regular genetic analysis and laboratory type tests that measure fundamental aspects of oral biochemistry and microbiology, mid-infrared spectroscopy (MIRS) has shown some promise in providing molecular profiles of GCF related to periodontal disease. The last group consists of methods suited to non-invasive in vivo monitoring such as optical spectroscopy, OCT and ultrasound imaging. OCT and ultrasound are generally used to delineate anatomical features of the gingival and surrounding tissues which are affected by disease, whereas optical spectroscopy can simultaneously detect local alterations in tissue hemodynamics and thereby accurately differentiate inflamed periodontal sites from healthy sites.

Non-invasive diagnostic methods that do not employ ionizing radiation are of particular interest for routine use in the diagnosis and monitoring of gingivitis as well as in predicting disease progression. Therefore, methods based on optical and infrared spectroscopy that are being explored as complementary diagnostic tools in periodontal diagnostics will be primarily reviewed in this chapter.

2. Clinical diagnostic criteria for gingivitis and their limitations

Gingivitis is defined as gingival inflammation in the absence of clinical attachment loss or in the presence of reduced but stable attachment levels (Mariotti, 1999). It is one of the most common human diseases and occurs in all ages of populations. The prevalence of gingivitis is high in both high income developed countries and low and middle income developing societies, affecting 50 – 90 % of adults worldwide (Albandar & Rams, 2002). For instance, only 6.1% of American adults showed mean gingival index (GI) <0.50; most (93.9%) were > or = 0.50 (Li et al, 2010). The average GI in 97.9% of Chinese adults was 0.5 or higher, and only 2.1% of them had a GI lower than 0.5 (Zhang et al, 2010). Most people have clinical signs of gingival inflammation, such as redness, edema and bleeding on gentle probing, but the extent and severity of inflammation vary from one population to another and are closely related to bacterial dental plaque.

In gingivitis, inflammation is confined to the periodontal soft tissues and diagnosis of most gingivitis can be readily made on clinical presentation and visual examination. Common signs of gingival inflammation include redness, partly due to the aggregation and enlargement of blood vessels, swelling and loss of texture and bleeding on gentle probing or sweeping on gingival margin (Lang et al, 2009). However, for clinicians, these key clinical parameters are largely subjective observations and difficult to stage gingivitis. Thus, assessment of disease progression and the effect of treatment are often inaccurate and subjective since it relies on clinical monitoring and comparing of these clinical parameters. Some local and systemic factors may further complicate the precise measurement of gingival inflammation. For instance, cigarette smoking is a well established risk factor for periodontal diseases, but clinical signs of periodontal inflammation are reduced in a dose dependent manner in smokers (Scott & Singer, 2004; Dietrich et al, 2004; Erdemir et al, 2004). Unlike many other infections, painless bleeding often presents as an early, easily recognizable sign of gingivitis, in particular at its early stage when it is easy to treat and maintain. If left untreated, however, some gingivitis will develop into a more destructive irreversible form of periodontal disease, i.e., chronic periodontitis, leaving permanent damage to tooth supporting tissues. Longitudinal studies showed that teeth with chronically inflamed gingiva had 70% more attachment loss than healthy sites and a much higher risk of tooth loss as well (Heitz-Mayfield et al, 2003; Schatzle et al, 2003). Once chronic periodontitis has established, more invasive treatment approaches and life long professional maintenance are required for periodontal health. Therefore, inaccurate diagnosis of periodontal diseases can result in either under-treatment, if one fails to identify progressing gingivitis or over-treatment if treatment is delivered to stable sites. It is thus important to identify the sites and subjects at risk of progression in their earliest stage of development, particularly in cases with high risk of progression. Unfortunately, currently used periodontal diagnostic methods, such as periodontal probing and radiography, are not sensitive measurements in this regard. Neither method is able to differentiate between reversible gingivitis and early but irreversible periodontitis, nor identify progressing periodontitis until significant periodontal tissue has been lost. For instance, the standard deviation for the measurement of attachment level with conventional periodontal probes is reported to range from 0.62 to 1.17 (Glavind & Loe, 1967; Goodson et al, 1982; Aeppli et al, 1985). Error of this magnitude requires a measured change of 2 to 3 mm in order to safely conclude that a change did occur. A more sensitive means is needed to precisely identify disease progression at the early stage (Haffajee et al, 1983, Ranney, 1991). As an adjunct to

periodontal probing, our group has recently explored the potential of using optical - near infrared spectroscopy to measure site specific hemodynamics in relation to periodontal diseases, including gingivitis. The previously published results are elaborated in the following sections, which clearly demonstrate that optical spectroscopy is emerging as a powerful diagnostic tool for inflammatory periodontal diseases.

3. Diagnosis of gingivitis by optical spectroscopy

Indeed, a simple, user friendly, chair-side, diagnostic test for periodontal inflammation would be an invaluable addition to the dental clinic. To this end, optical spectroscopy has been extensively explored as noninvasive modality for the diagnosis of periodontal diseases including gingivitis.

The most attractive feature of a fiber optic optical spectroscopy measurement of periodontal inflammation is that it offers a rapid, non-invasive means of assessing the balance between tissue oxygen delivery and utilization. In the methodology pursued by our group, the measurement is made by positioning a fiber optic probe over the area of tissue under investigation but does not require a measurement within the periodontal pocket, unlike conventional periodontal probing. This poses less discomfort for the patient with measurement times on the order of a few seconds; one can envision optical spectroscopy as a practical chair-side tool for the practitioner.

It is generally known that the visible - near infrared spectral region of the electromagnetic spectrum covering the wavelength range from 400 to 2500 nm, conveys information on a few key inflammatory markers of periodontal disease (Sowa et al, 2006; Sowa et al, 2001). The electronic transitions stemming from the heme ring and central metal iron ion of hemoglobin are particularly strong absorbers of visible light as well as absorbing light in the near infrared region of the spectrum. For instance, the short wavelength region, 500 – 600 nm is dominated by the absorption from oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (Hb) in the capillary bed of gingival tissue while the absorption from water results in an increased attenuation at longer wavelengths in the 900 – 1100 nm region (Fig. 2) (Sowa et al, 1999; Hanioka et al, 1990). By fitting optical attenuation spectra to the known optical properties (extinction coefficients) of HbO2 and Hb, optical spectroscopy can measure relative concentrations of HbO2 and Hb (Hanioka et al, 1990; Attas et al, 2001). Furthermore, the 960 nm water band is known to shift with tissue temperature and changes in electrolyte concentration (Otal et al, 2003). Thus, optical spectroscopy provides a measure of the hemoglobin oxygen saturation of tissues and the degree of tissue perfusion as well as a measure of tissue edema.

Based upon these principles, visible – near infrared spectroscopy has been widely applied to biomedical problems, including cancer diagnostics, the early prediction of inflammation-related treatment failures in burn victims (Sowa et al, 2001; Liu et al, 2005; Sowa et al, 1999) , and monitoring ischemic conditions in urology such as testicular tissue perfusion and oxygenation of testicular torsion (Capraro et al, 2007; Stothers et al, 2008). Commercially, some near infrared based cerebral oximetry monitors, i.e., NIRO and INVOS, have been employed in the clinical settings for the surveillance of the cerebral oxygen balance under CO_2 challenge (Yoshitani et al, 2002).

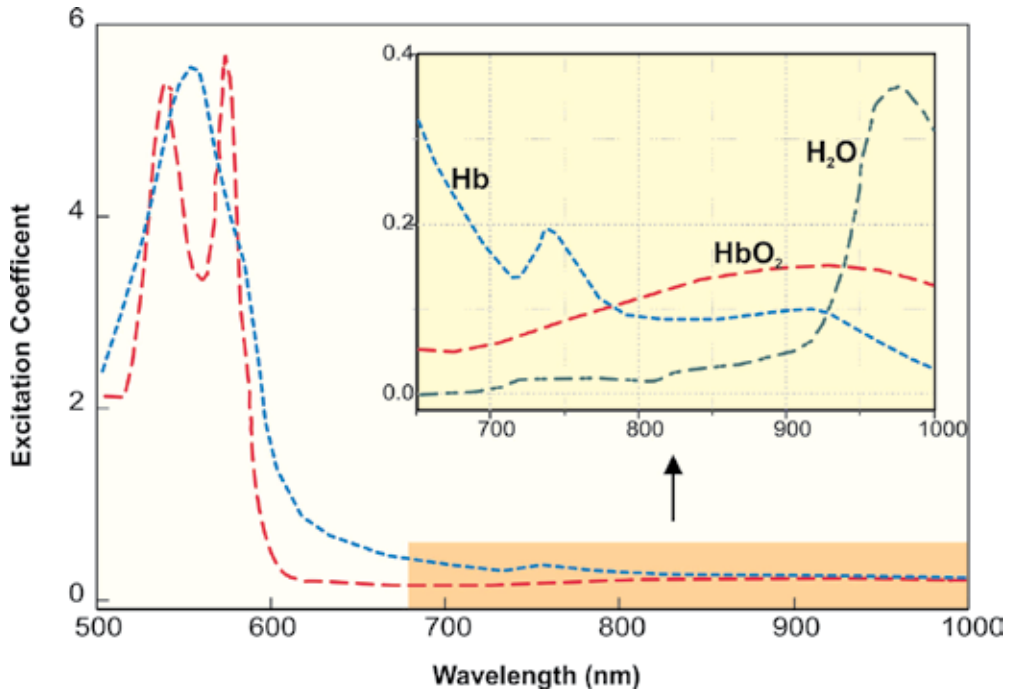

Fig. 2. Near infrared reference spectra (500–1000 nm) for water, deoxygenated hemoglobin (Hb) and oxygenated hemoglobin (HbO_2). The extinction coefficient data for water have been multiplied by a scaling factor of 10. (Reproduced from J Perio Res, 2009;44:117-24 with permission).

Likewise, hemoglobin and oxygenation indices have also been previously measured in periodontal tissues with the data suggesting that the increase in blood supply during inflammation is insufficient to meet the oxygen demand in inflamed gingivae (Hanioka et al, 1990). In addition, tissue edema, an index that is commonly used as a marker of gingival inflammation (Loe et al, 1963; Scott et al, 2004) can also be measured using near infrared spectroscopy (Liu et al, 2009; Sowa et al, 2001). Consequently, monitoring the intensity of the water bands in gingival tissues should provide an index of tissue hydration representing a simple indicator of inflammation at specific periodontal sites.

Furthermore, we have recently demonstrated, using optical spectroscopy, that tissue oxygenation at gingivitis sites was significantly decreased ($p<0.05$) compared to healthy controls as shown in Figure 3 (Liu et al, 2009). Such decreased oxygen saturation likely reflects tissue hypoxia resulting from an ongoing inflammatory response leading to increased oxygen consumption (Hanioka et al, 2000). It is well known that in destructive periodontal diseases, anaerobic microorganisms predominate in the periodontal pocket and diminished oxygen tension in deep pockets would be expected to promote growth of anaerobic bacteria (Amano et al, 1988; Loesche et al, 1969). Interestingly, it has been shown previously that tissue oxygen saturation correlates well with oxygen tension in periodontal pockets (Hanioka et al, 1990). In particular, in chronic gingivitis (stage III), the blood vessels become engorged and congested, venous return is impaired, and the blood flow becomes sluggish. The result is localized gingival anoxemia, which superimposes a somewhat bluish hue on the reddened gingiva (Hanioka et al, 1991).

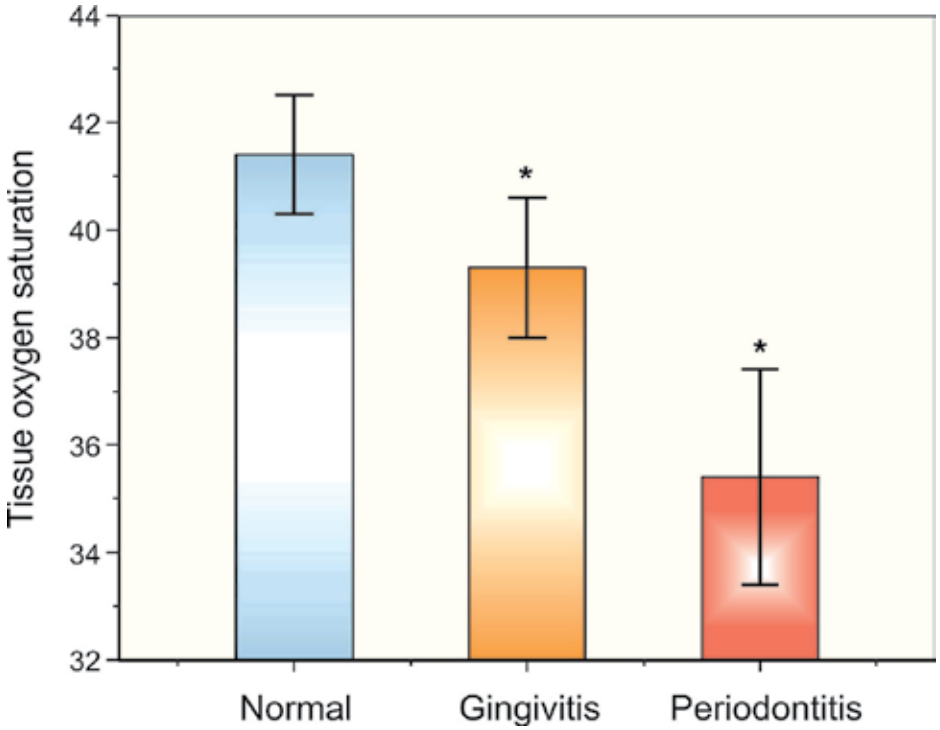

Fig. 3. Percent tissue hemoglobin oxygen saturation derived from the relative concentrations of Hb and HbO_2 from distinct two locations. Indices are compared between healthy, gingivitis and periodontitis sites. * Represents a significant difference from healthy sites, $p<0.01$. Vertical bars denote 0.95 confidence intervals.

We have recently attempted to establish a model to predict risk index of gingivitis based on spectral data from several independent studies (Liu et al, 2009; Ge et al, 2011; Nogueira et al, 2011). The method of Fort and Lambert-Lacroix, using partial least squares with penalized logistic regression was applied directly to the measured visible reflectance spectrum (510 – 620 nm) of the gum with a subject-out bootstrap cross validation approach to select classifier parameters. The probabilistic classification model was calibrated using the spectral data from healthy sites and sites with periodontitis and the model was then used to predict the sites with gingivitis that have optical properties that are more indicative of periodontitis. Figure 4 shows a risk index applied to cases that were deemed to be gingivitis based on clinical assessment. This method would allow us to stratify the gingivitis cases into those that have spectroscopic characteristics closer to healthy sites and those that were similar to periodontitis.

Comparing the risk score between sites with or without plaque (Figure 5) revealed that the risk score of the gingivitis sites with plaque were on average higher than the risk score of gingivitis sites without plaque ($p=0.02$). Both results (Fig. 4&5) strongly indicate that based on the hemodynamic information embedded in the optical spectra, one can readily develop prediction models or risk scores to further stratify gingivitis.

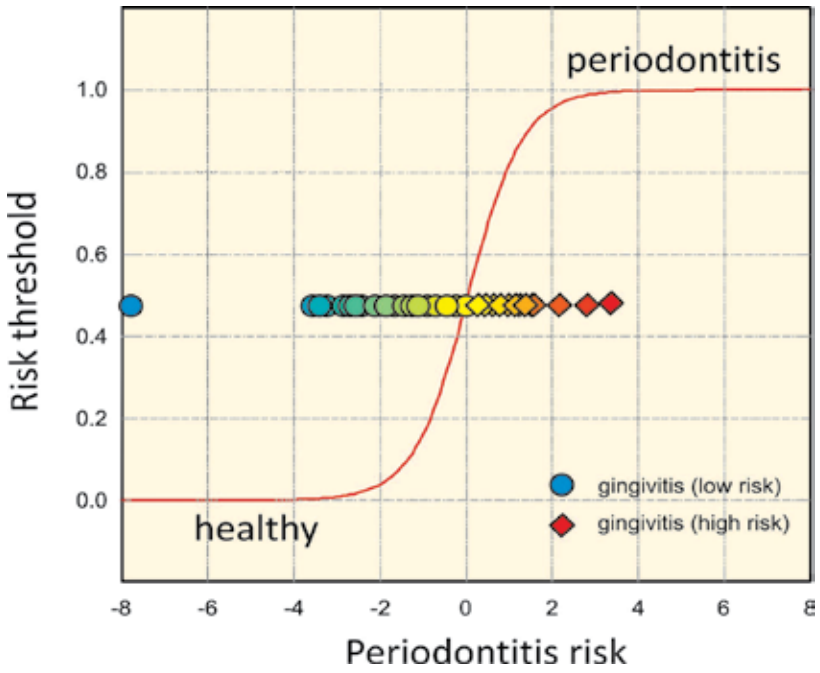

Fig. 4. Logistic regression model that weights sites exhibiting signs of gingivitis towards healthy sites (negative periodontal risk values) or periodontitis (positive risk values). Model: Logistic regression. Y=exp(-.07661+(1.57564)*x)/(1+exp(-.07661+(1.57564)*x))

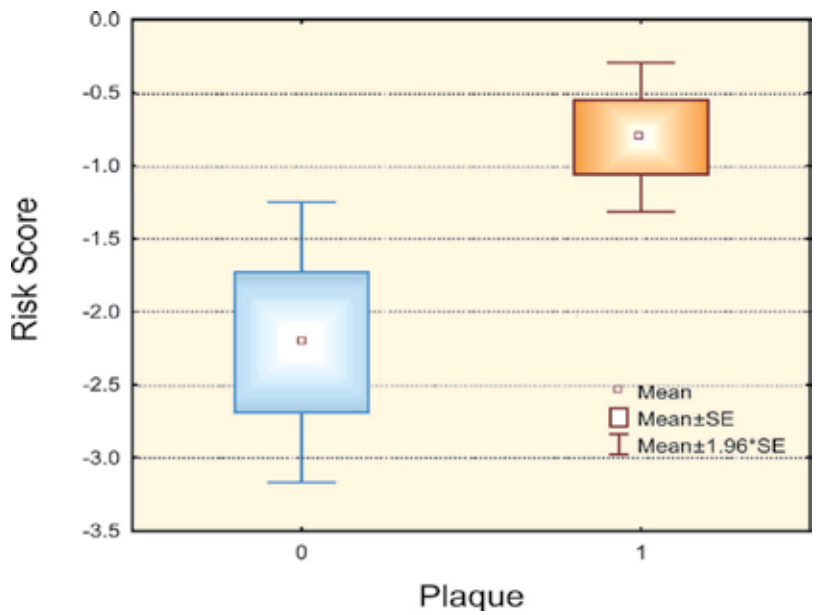

Fig. 5. The risk scores of gingivitis sites with or without plaque**.** T-test indicates a significantly higher risk score for gingivitis sites with plaque compared to gingivitis sites without plaque (p=0.02).

As tissue oxygen saturation is not measurable clinically, optical spectroscopy can provide a further index of inflammation that may prove useful to the periodontist. In other words, after future studies, the intra-oral optical probe may be able to determine sites at which disease has not yet progressed clinically, but whose biochemically-defined profile suggests that a particular site has pathogenic potential, such as the anaerobicity required to establish a pathogenic micro flora.

4. Molecular fingerprinting of gingivitis GCF by MIR spectroscopy

Another important aspect in evaluating gingivitis is to fully utilize the molecular and biochemical information embedded in GCF. In fact, studies on GCF have extended over a period of about 60 years. Originally proposed by Alfano (Alfano, 1974), GCF represents the transudate of gingival tissue interstitial fluid but in the course of gingivitis and periodontitis, GCF is transformed into a true inflammatory exudate (Veli-Jukka, 2003). The composition of GCF is the result of the interplay between the bacterial biofilm adherent to the tooth surfaces and the cells of the periodontal tissues. GCF contains several cellular and molecular components of the immunologic response present in serum, as well as mediators and by-products of tissue destruction generated within the tissues. These substances possess a great potential to serve as indicators of periodontal disease, the healing process after therapy or as a window to periodontal disease. Therefore, GCF provides an easily collected fluid containing inflammatory mediators released during disease processes that affect periodontal tissues (Champagne et al, 2003).

Gingivitis is a form of periodontal disease in which gingival tissues present with inflammation but in which tissue destruction is mild and reversible. Gingivitis affects more than 90% of the population, but only 7–15% of the adult population is affected by a more severe form of the disease, chronic periodontitis (Brown & Löe, 1993). The histological presentation of gingivitis includes vascular changes with increased vasopermeability and vasodilatation, and the presence of an exudate of polymorphonuclear neutrophils, migrating from the tissue into the gingival crevice (Tsai et al, 1998; Page et al, 1976). Gingivitis is thought to be a neutrophil-dominated response, as mostly neutrophil mediators are identified in GCF, including leukotriene B_4, platelet activating factor, prostaglandin E_2, interleukin-1, thromboxane B_2, elastase and collagenase (matrix metalloproteinases-8) (D'Ercole et al, 2008; Munjal et al, 2007; Lamster et al, 2007; Kinane & Mark, 2007). Thus, inflammatory cytokines can be detected within the GCF and serve as an indicator of local immuno-regulatory and inflammatory status. Although in gingivitis the tissue destruction is mild and reversible, the tissue damage products like hydroxyproline/collagen fragments, have also been identified as biomarkers (Bowers et al, 1989; Huynh et al, 2002). Therefore, it is obvious that GCF provides a unique window for analysis of periodontal condition.

Several tests have been developed that are aimed at specifically and sensitively revealing the pathologic and metabolic status of periodontal tissues (Armitage, 2003). Some of them have shown good specificity and sensitivity values as well as potential for predicting disease progression (Jeffcoat & Reddy, 1991; Jeffcoat, 1992; Magnusson et al, 1996; Bader & Boyd 1999; Teles et al, 2009). Unfortunately only a handful of GCF tests have made their way into clinical practice. Clinicians are still missing a practical test based on enzymes,

tissue degradation products or cytokines that accurately indicates the initial periodontitis process, active disease periods or effective healing. However, despite the complex nature of periodontal diseases which involves a multifaceted immune and inflammatory reaction to a polymicrobial flora, and inter-individual variation in inflammatory response, such potential biomarkers are generally studied individually or rarely in small numbers (Kinane & Mark, 2007). This may explain why the predictive value of potential biomarkers studied to date has not been sufficient for effective routine clinical use (Lamster et al, 2007).

Different from analyzing one or more particular biomarkers in tissue or body fluid, IR spectroscopy analyzes complex biological systems by capturing the entire IR spectrum which represents the sum of the contributions of the biomolecules present such as proteins, lipids, sugars and nucleic acids (Petibois & Déléris, 2006). Essentially, the IR spectrum of a tissue or cell sample can be regarded as molecular fingerprint of the tissue or cells. If this molecular fingerprint is modified by a disease process, which is normally the case, then IR spectroscopy can be used to detect and monitor the disease process.

Therefore, IR spectroscopy can distinguish differences in the characteristics of diverse molecules by probing vibrations of chemical bonds and using these molecular and sub-molecular profiles to define and differentiate "diseased" and "healthy" tissues (Jackson et al, 1997). As covalent bonds vibrate, they absorb energy in the form of IR light (Hynes et al, 2005; Liu et al, 2006). The wavelength of light that is absorbed depends on the nature of the covalent bond (e.g., C=O, N-H), the type of vibration (bending, stretching, etc.), and the environment of the bond. In the last fifteen years, IR spectroscopists have taken advantage of this molecular information, in combination with pattern recognition/classification methods, to explore its potential as a powerful tool for the diagnoses of various diseases based upon the spectra of biological fluids, including amniotic fluid, lipid profiles, synovial fluid, saliva and gingival crevicular fluid to predict fetal lung maturity (Liu et al, 1998), diagnose heart disease (Liu et al, 2002) and rheumatoid arthritis (Eysel et al, 1997), assess global diabetes-associated alterations (Scott et al, 2010) and evaluate periodontal inflammations (Xiang et al, 2010), respectively.

The IR spectrum of saliva and GCF is a rich source of information regarding the oral cavity and associated inflammation. In a recent study by Scott et al, they have assessed global, diabetes-associated alterations to saliva at the molecular and sub-molecular levels by using infrared spectroscopy (Scott et al, 2010). For instance, by evaluating the difference spectrum a great deal of molecular information embedded in the saliva from diabetic patients can be distilled as shown in Figure 6. Following Fourier self-deconvolution (FSD), the most striking difference between the spectrum of diabetic saliva and that of control were vibrations arising from sugar moieties and/or glycosylation products, such as AGEs (advanced glycation end products). This can be visualized by examining the spectral range 950-1180 cm^{-1} that originated from various C-C/C-O stretching vibrations in sugar moieties. The 1020 cm^{-1} band is usually attributed to the C-O stretch vibration in glycogen while the bands at 1070 and 1169 cm^{-1} can be assigned as C-O-C symmetric and asymmetric vibrations of sugar moieties and phospholipids. Obviously, therefore, the contribution of AGEs and ALE's (advanced lipoxidation end products) to diabetic spectra may be large. This is consistent with previous reports that found that stimulated or unstimulated salivary glucose concentrations are higher in diabetic patients than in control subjects (Garay-Sevilla et al,

2005; Sola-Penna, 2008). These findings are also in keeping with numerous studies that have shown increased salivary AGE content in the development of diabetes complications (Bilous, 2007).

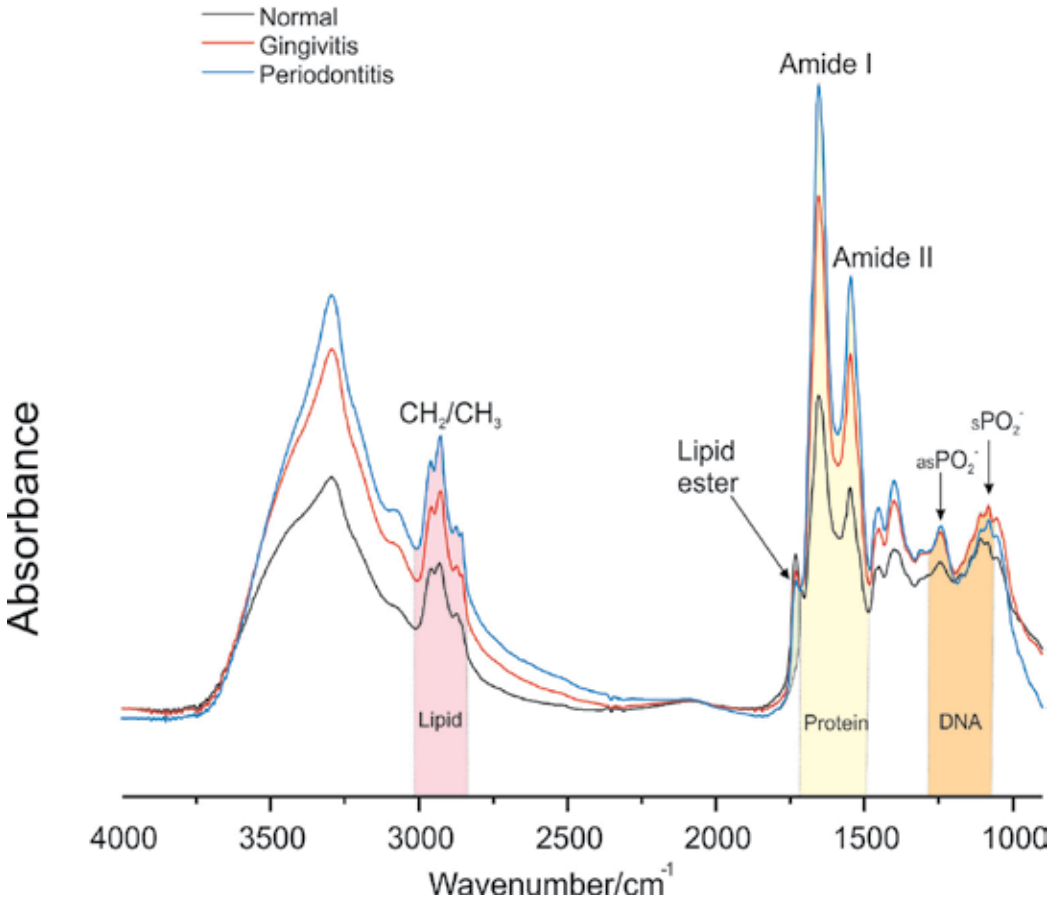

Fig. 6. General features of FSD-processed mean IR spectra of control and diabetes (bottom) subjects and the difference spectrum (diabetes minus control, top). Note: Although some non-highlighted bands exhibit pronounced differences, they do not convey significant meaning in terms of biological significance. (Reproduced from Diabetology & Metabolic Syndrome 2010, 2:48 (1-9) with permission).

More relevant to gingivitis, our group recently has employed IR spectroscopy to characterize GCF from healthy, gingivitis and periodontitis sites and determined specific spectral signatures that clearly demarcate healthy and diseased tissues (Xiang et al, 2010). With the FSD method which can narrow effective bandwidths, enhance resolution, and increase available discriminatory data (Surewicz et al, 1988), we were able to reveal subtle differences in spectral band intensity and positions arising from the three major

components, i.e., lipid, protein and DNA observed in GCF from healthy, gingivitis and periodontitis groups. For instance, by integrating the three major DNA sensitive bands - the bands at 1087 and 1240 cm^{-1} arising from symmetric and asymmetric PO_2^- stretching vibrations of phosphodiester groups in DNA and the 1713 cm^{-1} band - we can see that GCF DNA concentrations in diseased subjects are increased compared to healthy subjects (Fig. 7). GCF contains a diverse population of cells, which include bacteria, desquamated epithelia and transmigrating leukocytes (Delima et al, 2003; Palmer et al, 2005). The increased DNA component in GCF from gingivitis and periodontitis sites, relative to healthy controls, is likely due to a combination of an inflammation-driven increase in leukocyte migration into the GCF, particularly neutrophils; an increase in epithelial turnover, reflecting ongoing tissue remodeling; and of the inflammatory stimulus itself, i.e., plaque bacteria.

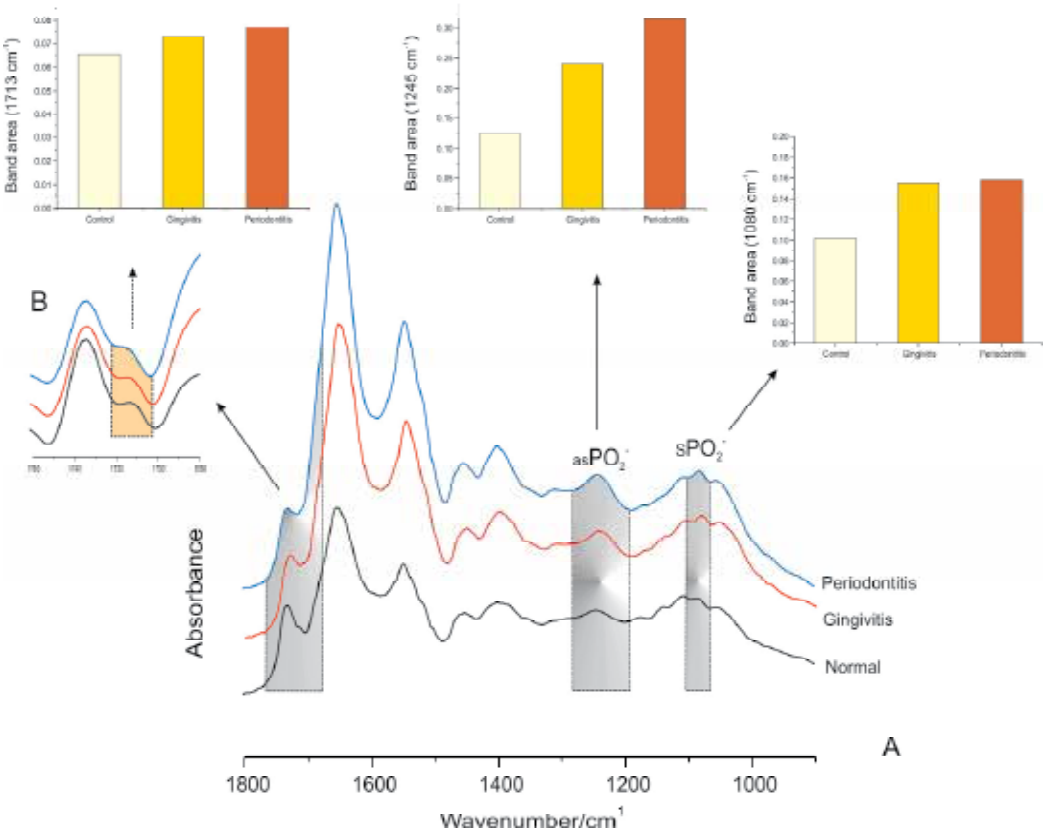

Fig. 7. Relative DNA contributions are increased in diseased GCF groups. The shade areas highlights DNA-specific signals in GCF. The enlarged area of another important DNA band, 1713 cm^{-1}, arising from DNA pair base vibration after Fourier self-deconvolution (FSD). The histograms representing the integrated area (relative DNA content) in the spectra from the three groups. (Reproduced from J Perio Res, 2010; 45: 345-352 with permission).

Increased protein (Amide I at 1652 cm^{-1}) and lipid (symmetric CH_2 stretching vibration at 2853 cm^{-1} from the fatty acyl chains) signals are also evident at diseased sites (Figure *8*). In particular, disease-specific cellular and molecular alterations to the composition of GCF are clear, most obviously the increased intensity of the 1652 cm^{-1} Amide I band at inflammatory sites (gingivitis and periodontitis) compared to healthy sulci. This indicates that the protein concentrations in both disease groups were significantly higher than in controls, in agreement with prior reports of increased total protein levels in periodontitis GCF (Akalin et al, 1993); and a significant correlation between total GCF protein concentration and disease severity (Baltacioglu et al, 2008). Many GCF proteins have been extensively explored as potential diagnostic markers that define periodontal inflammation. They include inflammatory mediators, particularly cytokines and matrix metalloproteinases, and tissue breakdown products, such as, fibronectin, collagen fragments and hydroxyproline, which should reflect the extent of underlying tissue destruction.

In addition, the integrated area of the =CH band at 3012 cm^{-1} has been used as an index of the relative concentration of double bonds in lipid structures from unsaturated fatty acyl chains (e.g. linolenic, arachidonic, etc.) arising from lipid peroxidation (Severcan et al, 2005; Liu et al, 2002). Interestingly, lipid oxidation is increased in the inflammatory groups, as evidenced by the olefinic =CH band at 3012 cm^{-1} providing further evidence of the importance of lipid peroxidation in periodontal disease pathogenesis (Tsai et al, 2005; Sheikhi et al, 2001).

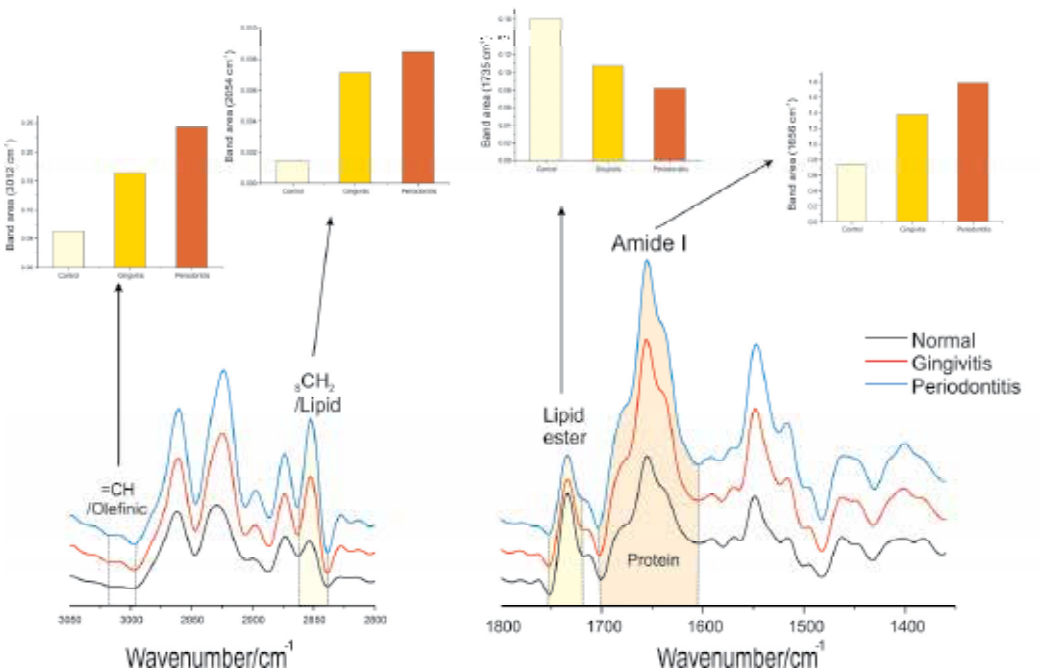

Fig. 8. Relative concentration of protein and lipid components derived from GCF MIR spectra after FSD procedure. The histograms representing the integrated area (relative protein, lipid and lipid peroxidation content) in the spectra from the three groups. Clear differences in protein and lipid content of GCF from diseased and healthy sites are apparent. (Reproduced from J Perio Res, 2010; 45: 345-352 with permission).

Besides the unique capability of IR for capturing the composite molecular content of GCF, it may also provide qualitative diagnosis of periodontal inflammatory status. This could be achieved by using linear discriminant analysis (LDA), to correlate observed spectral differences of GCF from inflammatory conditions (gingivitis and periodontitis) and normal healthy status. This is primarily due to the fact that periodontal disease is clearly multi-factorial and our LDA analyses consider multiple components in the GCF as the basis to designate individual spectra as healthy or diseased. As shown in Table 1, LDA could classify GCF from gingivitis and healthy control sites that the overall accuracy for the classification of GCF samples as controls or gingivitis was 91.4% for the training set and 72.4%, in the validation set. Comparing to the better overall accuracy for the classification of GCF samples in periodontitis, 98.4% for the training set and 93.1% for the test set, this would suggest that the gingivitis-specific molecular alterations to GCF are less profound than in periodontitis.

In a nutshell, there are several advantages to using IR spectroscopy of GCF for screening and diagnosis of periodontal inflammation. Namely, IR spectroscopy is reagent-free requiring only small sample volumes; GCF samples are essentially unprocessed; the process is readily automated; IR spectroscopy is straightforward requiring minimal training for operators; and GCF samples are easily collected by clinicians with sample collection targeted to specific sites or to a representative set of teeth.

Classes			Accuracy(%)	SP (%)	PPV (%)
Training Set					
Control	**32**	1	97.0	84.0	88.9
Gingivitis	4	**21**	84.0	97.4	95.5
Validation Set					
Control	**12**	2	85.7	60.0	66.7
Gingivitis	6	**9**	60.0	85.7	81.8

Diagnosis of gingivitis was determiined by linear discriminant analysis of the infrared spectra. Overall accuracy was 91.4% on the training set and 72.4% on the test set. Bold numbers indicate accurate classifications. SP=specificity; PPV=positive predictive value.

Table 1. Diagnostic accuracy of gingivitis based on IR spectra of GCF

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9

Gingivitis Control

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1. Introduction

The term periodontal disease generally includes both chronic periodontitis and gingivitis. Gingivitis is the inflammatory response in the gingival tissues to the presence of a plaque biofilm on the tooth surface at the dento-gingival junction. When gingivitis has developed the marginal gingival tissues show the clinical signs of inflammation which include redness, swelling due to the formation of oedema, and bleeding on probing. Although inflammation is present there is no alveolar bone loss and no apical migration of the junctional epithelium beyond the cementum enamel junction (CEJ).

Since the experimental gingivitis studies of Harald Loe and colleagues in 1965, dental plaque has been recognised as the sole cause of gingivitis. Treatment therefore must involve the removal of plaque, the re-establishment of a healthy oral environment by removal of the factors which retain or hinder the removal of plaque, and the maintenance of this healthy environment by proper oral hygiene procedures.

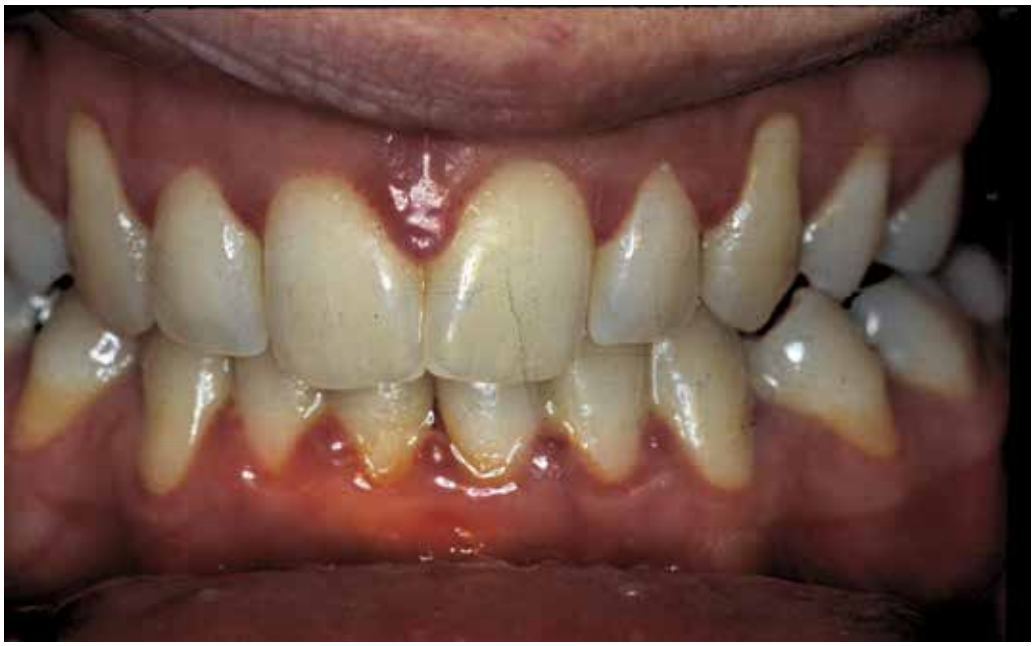

Fig. 1. Gingivitis

2. Aetiology of gingivitis

Dental plaque is the sole aetiological agent in the initiation and progression of gingivitis. It can be defined as;

"the soft, adherent, structured deposits that form on teeth and other hard surfaces in the mouth, consisting of continually growing microbial colonies in an inter-microbial matrix."

This definition is virtually identical to that of a microbial biofilm and in this context dental plaque is now considered to be a biofilm, The prevention and treatment of gingivitis therefore must be aimed at the regular removal and disruption of this continually forming biofilm (Slots, 2002). Poor oral hygiene and lack of plaque control not only leads to the development of gingivitis but may also increase the risk of its possible progression to chronic periodontitis. There have indeed been many studies on the importance of adequate oral hygiene on the long-term maintenance of periodontal health (Axelsson et al 1981,1991). The pivotal study of Loe and colleagues (1965) showed that the cessation of oral hygiene measures in individuals with clinically normal gingival tissues resulted in gross accumulation of plaque and the development of gingivitis. The time to develop this clinical gingivitis varied from 10-21 days. They further showed that the removal of this accumulated plaque led to the resolution of the inflammation.

The development of gingivitis was first described by Page and Schroeder (1976). Broadly speaking, soon after the initial colonization of the acquired pellicle by Streptococci, bacterial enzymes and metabolic end products increase the permeability of the junctional epithelium and the so-called Initial Acute Lesion develops. This subclinical lesion is characterized by the formation of oedema, the accumulation of polymorphonuclear leukocytes (PMNs) and the loss of connective tissue immediately subjacent to the junctional epithelium. Approximately 2-5 days after, the the formation of the Initial Acute Lesion the so-called Early Lesion develops in which the nature of the lesion changes to one with increased numbers of lymphocytes and macrophages. At the same time the vascular changes become more pronounced and perivascular inflammatory infiltrates develop. Immunohistological analysis has shown that the development of gingivitis follows a similar pattern to that of a controlled delayed type hypersensitivity (DTH) response and that it is primarily a T cell/macrophage lesion. In contrast chronic periodontitis is characterised by large numbers of B cells and plasma cells (reviewed in Ohlrich et al., 2009).

3. Management rationale

Gingivitis is a ubiquitous oral disease. Many of those affected are unaware since it is painless and in its early stages not associated with obvious clinical symptoms. Its effect on the periodontium however, can be reversed with adequate plaque control.

Effective plaque control remains the cornerstone of disease control but it can be difficult and depends on factors such as motivation, knowledge and manual dexterity (Robinson et al,.2009).

Dental plaque must be physically removed. It cannot be rinsed off and regular tooth brushing, flossing and other interdental cleaning practices are required for effective removal. Chemical or antimicrobial agents, which aid in plaque and gingivitis reduction, should be thought of as supplementary home care practices. Gingivitis prevention however, demands an holistic patient view as successful uptake of oral health advice depends largely on individual behavioural variables.

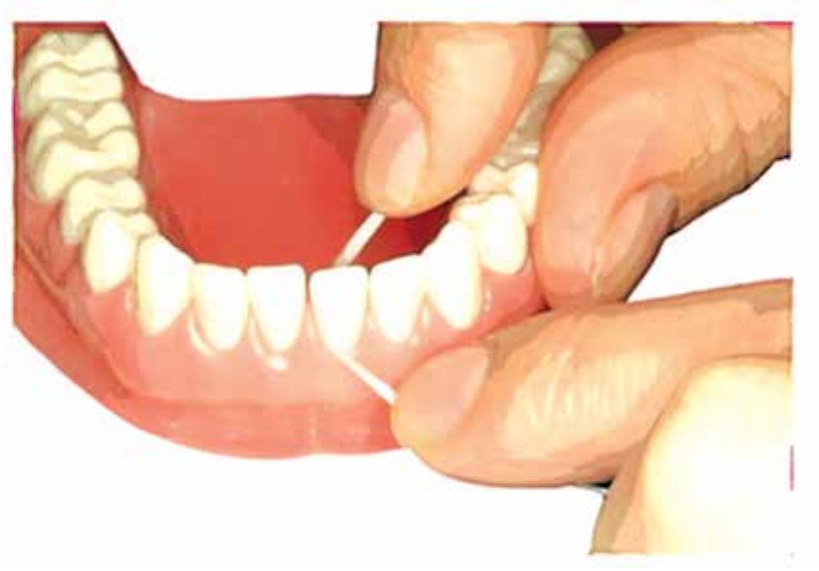

Fig. 2. Flossing

4. Mechanical plaque control

The toothbrush remains the principal method for the mechanical removal of dental plaque. The toothbrush when used correctly removes dental plaque and food debris. It may also remove stained pellicle through the addition of an abrasive dentifrice. Efficacy and efficiency of tooth brushing is influenced by three main components; tooth brush design, the skill of the individual using the brush, and the frequency and duration of its use (Frandsen et al., 1972).

Various tooth brushing techniques are documented (Echeverria and Sanz, 2003), however it is the Bass technique and its adaptation that seem most favoured by dental professionals. Studies comparing the plaque removal efficacy of different tooth brushing techniques have typically shown little or no difference between them (Echeverria and Sanz , 2003). There is little then to be gained from introducing particular tooth brushing techniques unless the patient's current method is proving inefficient in the removal of plaque or is causing trauma to the teeth or gingival tissues. Advice and professional recommendations are better directed at modifying a patient's existing technique to improve plaque removal in neglected areas (Frandsenet al., 1972) and in correcting traumatic techniques. This may include addressing the stiffness of brush bristles. Current consensus for optimal tooth brushing frequency is that twice per day is consistent with maintenance of gingival health (Davies et al, 2003). Optimal duration of tooth brushing is two minutes (Cancro and Fischman, 1995), however it is recognised that most individuals rarely brush for longer than 60 seconds (Davies et al, 2003). With the addition of timers to many powered tooth brushes users can be aware of the duration of their tooth brushing (van der Weijden et al, 1993).

4.1 Manual tooth brushes

There are several important considerations in the selection of a well designed manual tooth brushes. These include bristle stiffness (soft is optimal for the removal of plaque and

minimizes gingival trauma), handle size (should be appropriate to the size, age and hand dexterity of the user), head size (should be appropriate to the size of the users' mouth), and bristle pattern (should enhance plaque removal in the approximal spaces and along the gingival margin) (Egelberg and Claffey, 1988).
To date there is little evidence to indicate any one manual tooth brush is more effective than another (Jepsen, 1998). There are suggestions however that higher plaque scores are associated with natural bristles, whereas nylon bristles gave significantly better plaque removal. Lower plaque scores, less gingival recession and tooth brush abrasion have also been found in patients using toothbrushes with soft bristles (Anaise, 1976).

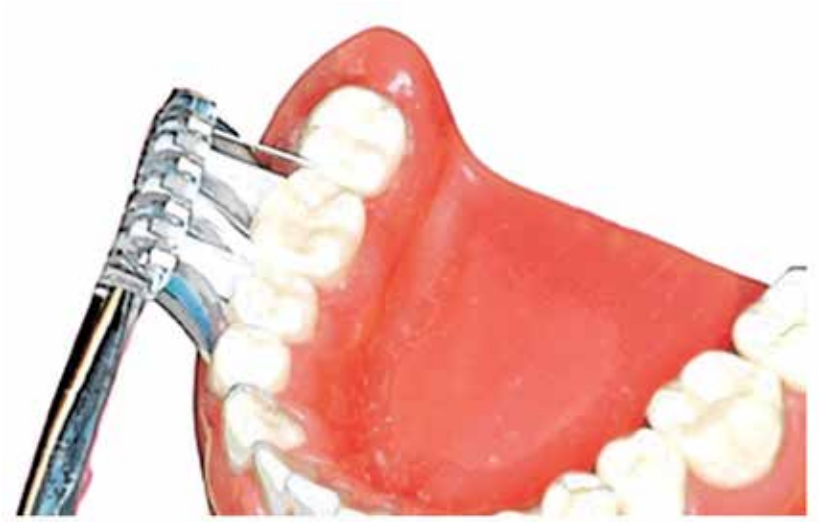

Fig. 3. Manual Tooth Brushing

4.2 Interdental cleaning aids

Interdental cleaning aids are important in controlling dental plaque and hence gingivitis since dental plaque accumulation and growth occurs on the interproximal surfaces of all teeth including the molars and premolars (Lang et al, 1973; Quirynen and Steenberghe, 1989). Interdental aids should be easy to use and efficient in removing dental plaque without causing damage to the hard or soft tissues. Interdental aids include dental floss or tape, toothpicks, automated interdental devices and interdental brushes. All of these aids have been shown to remove plaque and hence reduce inflammation (Axelsson, 1991; Lang et al, 1994).
The use of dental floss in conjunction with tooth brushing results in twice as much dental plaque removal than tooth brushing alone (Kinane, 1998). The use of floss has been shown to remove 80% of proximal dental plaque including subgingival plaque when correctly used (Waerhaug, 1981). Different types of floss have not been shown to be more effective than others. Some patients find floss difficult to manoeuvre without damage to the soft tissues. This is likely to include patients with poor dexterity, large hands or small oral cavities and those in whom the teeth are crowded or malaligned. Automated flossing devices may be easier for some patients to use. Flossettes and floss holders may enhance interdental cleaning by reducing the required level of manual dexterity. Interdental brushes may also be

easier for some patients to manage as long as the gingival tissue can accommodate them without causing trauma. While current concensus is that interdental aids are crucial in the control and prevention of plaque induced gingivitis and the choice of aid is dependent on individual patient requirements and needs, but none are universally superior (Kinane, 1998).

Interdental brushes are designed specifically to target the interproximal spaces between the teeth. Early research found they were able to remove plaque from 2-2.5mm subgingivally (Waerhaug, 1981). Interdental brushes are available in a variety of sizes and forms, with individual small handles or long handles. The choice of handle is usually based on patient preference or dexterity requirements. These manual tooth brushes also have novel designs and filaments of different sizes and shapes.

Early research in these brushes showed evidence that a V-shaped brush better removes interdental plaque than a straight multi-tufted variety over a 12 day period (Bergenholtz et al, 1984). It is important to note that in the healthy mouth where teeth are aligned and interdental papillae completely fill the interdental area, interdental brushes are contraindicated because of the blunting trauma they can cause to the tip of the papilla. In these cases dental floss is the preferred interdental aid.

Interdental rubber-tipped stimulators are also widely available and have been shown to reduce bleeding scores and gingivitis (Yankell et al, 1992). Another variety of brush designed for specific, difficult to access, areas of the mouth is the 'end-tufted brush'. This can be used effectively in the distal and lingual aspects of molars, posterior to the last molar, in quadrants where third molars are present and in furcation areas.

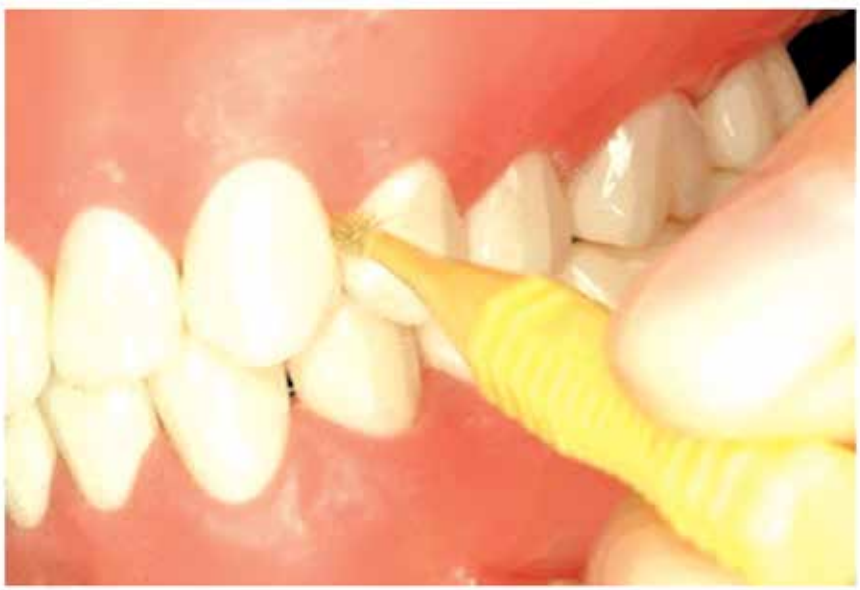

Fig. 4. Interdental Cleaning Brush

Toothpicks can provide effective plaque and food debris removal in wide open interproximal spaces. A triangular rather than rectangular design is better suited to the interdental space (Mandel, 1990). These aids must be used correctly to avoid potential blunting or depression of the interdental papilla (Echeverria and Sanz, 2003).

4.3 Powered tooth brushes

Powered tooth brushes first became available in the 1960s (Deacon et al, 2009) and early models had a simple back and forth action. Later designs were rotary action brushes and most recently ultrasonic powered tooth brushes have been developed with higher frequency vibrations (van der Weijden et al, 1998). There are no definitive conclusions regarding the superiority of one mode of powered tooth brush over any other (Deacon et al, 2009). The main benefit of powered tooth brushes is considered to be the rotary head movement which it is claimed will increase interdental cleaning (Walmsley, 1997).

The relative effectiveness of manual versus powered tooth brushing has recently been summarised, with powered brushing thought to have a higher efficacy in the removal of plaque and a greater reduction of gingivitis (Robinson et al., 2009). This review also found that powered tooth brushes whose action is rotation-oscillational reduced plaque and gingivitis by 11% and 6% respectively in the short term, with a reduction of gingivitis of 17% at more than three months (Robinson et al., 2009). This appears to be related to the capacity of the brush to reduce plaque and in particular with the counter-rotational and oscillating-rotating brushes rather than sonic brushes (Sicilia et al, 2002).

A systematic review by the Deery et al., (2004) categorised powered tooth brushes into six groups dependent on their design and mode of action. They were assessed for their plaque and calculus removing efficacy along with how well they maintained gingival health compared with manual tooth brushes. There was no statistical difference between manual and powered tooth brushes in plaque reducing capabilities, however rotation oscillation powered tooth brushes did show a greater degree of plaque and gingivitis reduction over both short and longer term periods. (Deery et al,. 2004)

The effect of an oscillating/rotating/pulsating powered tooth brush on plaque and gingivitis compared with manual tooth brushing found the powered toothbrush maintained statistically significant lower plaque levels for nine months compared with the manual tooth brush (Rosema et al, 2008). The powered tooth brush also showed significant benefits in preventing gingival bleeding versus manual brushing alone. Deacon et al, (2009) suggest there is no evidence that any particular mode of powered tooth brush is superior.

Perhaps the ultimate advantage of powered versus manual tooth brushes lie in the belief of the population that they are easy and simple to use. Many studies have shown high compliance rates even six months after purchase (Stalncke et al,. 1995). The role of the dental professional remains important regardless of the type of brush but if individuals are more likely to accept powered tooth brushes or believe they are easier to operate this may enhance uptake of oral hygiene advice. Irrespectively, powered tooth brushes will remain important when manual dexterity is compromised. Deacon et al., (2009) further suggests individuals may choose to use powered brushes for reasons unrelated to clinical outcomes such as avoidance of bad breath, improving the appearance of teeth and because they like to use technology.

As powered tooth brush bristle movement is not able to be directly controlled by the user, these brushes tend to lead to a greater probability of damage to the gingival tissues. Nevertheless a number of studies have reported that any trauma is transient (Deacon et al,. 2009).

5. Oral health behavioural change

Oral hygiene advice is largely aimed at personal plaque control efforts through tooth brushing and interdental cleaning (Suomi et al 1973). This process however, is dependent on behavioural changes and changes in relation to thoughts and beliefs regarding oral health (Tedesco, et al., 1991). Patient compliance is necessary for the successful outcome of preventive or treatment recommendations (Blinkhorn, Non-compliance with oral self-care recommendations is the key problem in the prevention of poor oral health (Widström, 2004). Unwillingness to perform self-care (Weinsteinet al,. 1983), lack of motivation (Syrjala, et al., 1994), and poor dental health beliefs (Kuhner & Raetzke, 1989) may all contribute to poor oral health outcomes. Interventions aimed at improving compliant oral health behaviour must therefore be supported by strategies that enable implementation of behavioural change. If these changes can be made, oral health promotion, in the form of individual oral hygiene instruction is effective for plaque removal (Dahlen, et al., 1992; Kay & Locker, 1997). Evidence suggests however, that these behavioural changes are short-term and not sustained (Kay & Locker, 1997).

Behavioural science theory provides the basis for understanding why people do the things they do. It is only through conceptualising these principles that professionals are then able to provide the motivation to facilitate behavioural change. Health behaviour theories provide a framework on which to base professional guidance. As motivation has been defined as "the impulse that leads an individual to action" (Darby & Walsh, 1995, p. 85), effective health advice must first identify and utilise that impulse.

The most basic theory of health behaviour change assumes that knowledge of healthy behaviour can directly effect changes in attitudes and behaviour (Kallio, 2001). Traditional educational interventions have been shown to be of little help in achieving long term behavioural change (Renz et al, 2008). In the changing social context of medicine the oral health professional's message must shift from "expert pronouncement" toward informed choice, non-directive counselling and a non-judgemental perspective. Because of this shift in focus in oral health education practitioners must attempt to both explain process and predict outcomes in order to influence oral health behaviour. Intrapersonal factors and characteristics such as prior knowledge and experience, attitudes and belief systems make this a complex and individualised process.

Behaviour change is a process - not an event. Individuals are at varying levels of motivation or readiness to change, and interventions must be tailored to an individual's current status. This helps explain why standard, routine and rigid oral hygiene instruction does not always produce effective compliance with home care instruction. If patients are actively involved in their oral health, the responsibility to adopt appropriate oral health behaviours is personalised. Oral health professionals can enhance this process and improve maintenance of the gingival tissues by giving patients positive feedback about the success of their plaque removal efforts (Tedesco et al 1991). Studies have shown that patients who receive sporadic care may deteriorate over time. In a study of periodontal health status in young US Navy personnel it was reported that appropriate preventive therapies should be provided and

repeated at intervals specific to individual need (Diefender et al, 2007). Individually tailored oral health educational programmes have been shown to be efficacious in improving long-term adherence to good oral hygiene practice (Jonsson et al., 2010). Targeted planning in interventions to increase compliance to flossing has also been successful (Schuz, et al., 2006), but continued monitoring and reinforcement is also required to maintain this level (McCaul et al., 1992).

Rarely will behaviour change on the basis of good advice alone (Watt, 2002). Positive reinforcement from an oral health professional is pivotal in building the patient confidence and self-esteem to maintain and improve oral health enhancing behaviours (Kallestai et al., 2000; Macgregor et al., 1997). Hope of success and satisfaction with life should be considered as predictors of good oral health behaviour and status (Dumitrescu et al., 2010). In children, psychological predisposition and family environment can significantly influence tooth brushing behaviour (Ayo-Yusuf et al., (2009) and in young adults preventive programmes can demonstrably help reduce plaque and gingival inflammation (Hugosen et al., 2007).

Psychological models may provide the basis for intervention studies relating to oral health behaviour. It is noted however that for clinical benefit to be measured, changes in oral hygiene behaviour must be maintained over long periods of time and to date most studies have had short follow-up periods (Renz et al, 2008).

6. Chemotherapeutic / antimicrobial agents

Chemical or antimicrobial agents which reduce plaque and gingivitis should be thought of as supplementary to the principal home care practices of regular tooth brushing and interdental cleaning. The patient who is unable to manage mechanical cleaning or is reluctant to perform this may benefit from the use of chemotherapeutic agents. The use of mouthwash together with mechanical oral hygiene, health orientation and motivation assisted in the maintenance of oral hygiene in orthodontic patients (Alves et al, 2010). As adjuncts chemotherapeutic agents may assist in the prevention of gingivitis by changing plaque composition in such a way that health cannot convert to disease (Kornman, 1979).

In order for an antimicrobial agent to be effective in the elimination or reduction and control of subgingival plaque micro-organisms it must reach the target without being diluted by saliva and then remain at sufficient concentration without being washed away by the gingival crevicular fluid. Despite the dilution action of saliva antimicrobial and antiseptic agents can provide excellent prevention of supragingival plaque accumulation (Walker et al, 2004). Modes of delivery of chemotherapeutic agents include toothpaste, chewing gum and varnishes.

6.1 Mouthwashes

Mouthwash use dates back over 6000 years as evident in recipes of Ebers Papyrus of 1500 BC. They were concocted from ingredients which included mice intestines, honey, white wine and stale urine (Addy, 2003). Historically they have been used to reduce plaque formation and so prevent or delay the onset of gingivitis. Recent evidence suggests mouth washes in conjunction with tooth brushing is more beneficial than daily flossing with respect to interproximal plaque reduction (Zimmer et al, 2006). Specifically tested were 0.06% chlorhexidine and 0.025% fluoride and 0.1% cetylpyridinium chloride and 0.025% fluoride.

Chlorhexidine is regarded as the gold standard among mouthwashes for its plaque inhibitory, anti-plaque and anti-gingivitis ability (Parnell et al, 2010). It is the most effective antimicrobial agent available for reducing gingivitis and plaque in humans (Marsh, 1972; Jones, 1997). Chlorhexidine is a bisbiguanide originally developed as a disinfectant for skin and mucous membranes. Clinical trials consistently show a 60% reduction in plaque and gingivitis in short-term studies and a 55% reduction in plaque and a 45% reduction in gingivitis.(Cianco, 1989). The mode of action of chlorhexidine is to bind to hydroxyapatite and glycoprotein to prevent pellicle formation. It alters the bacterial cell wall causing cell lysis and disrupts adsorption of bacteria. It is highly substantive which means it binds to both hard and soft surfaces in the mouth and remains active for up to 12 hours. It is poorly absorbed by the gastrointestinal tract and considered to have low toxicity. Possible side effects include staining of the teeth, tongue and anterior restorations, taste alteration, soft tissue ulcerations and increased calculus formation. Some formulations contain alcohol and this may be a consideration in its use. It is best used short term but is safe in the longer term except for aesthetic issues due to staining and possible taste alteration (Perry & Beemsterboer, 1996).

Phenolic Compounds (Essential Oils) – Listerine® is the most thoroughly studied of these mouth rinses. Long-term studies showed it to be effective in reducing plaque by 25-28% and gingivitis by 30% (Ciancio, 1989). More recently Tufekci et al., (2008) found Listerine® use can reduce the amount of plaque and gingivitis in patients undergoing orthodontic treatment. It was also found to have greater antiplaque and antigingivitis efficacy than a cetylpyridinium chloride containing mouthwash (Amini et al., 2009). In subjects with mild to moderate gingivitis essential oils have reduced the effects of orally induced bacteraemia (Fine et al, 2010).

Listerine® is a mixture of three phenolic-derived essential oils, thymol, menthol and eucalyptol which are combined with methysalicylate. The mechanism of action is to alter the bacterial cell wall (Ciancio, 1987). It has been found effective against both supra and subgingival plaque (Fine et al, 2010). Listerine® mouthwash has low substantivity and side effects include burning sensation, bitter taste and possible staining of the teeth.

Mouth rinses containing essential oils often have significant amounts of alcohol, have a strong flavour and are often less costly than chlorhexidine mouth washes.

Quaternary Ammonium Compounds – Daily use of Cetylpyridinium chloride has been found to reduce plaque and gingivitis in short-term studies (Silva et al, 2009). Its mechanism of action is by increasing bacterial cell wall permeability, decreasing cell metabolism and reducing cell attachment to tooth surfaces (Ciancio, 1987). It significantly reduces the anaerobic bacteria of supragingival plaque (Hu et al.,, 2009), and at 0.05% has been demonstrated to provide 12-hour protection against plaque and gingivitis. (Silva et al, 2009).

6.2 Toothpastes

The use of toothpaste in conjunction with tooth brushing is to facilitate plaque removal and to apply therapeutic or preventive agents to the tooth surface (Echevarria and Sanz, 2003). The addition of abrasive agents further enhances plaque and stain removal. The addition of these agents cannot negatively influence the balance of normal flora, as this could increase the risk of bacterial resistance, the development of super infections and could also result in hypersensitivity reactions (Seymour & Heasman, 1992; Paraskevas & van der Weijden, 2006)

The most common additive ingredient in toothpaste is fluoride but as this does not benefit plaque removal or gingivitis per se, other antibacterial agents are added.

As chlorhexidine's highly active cationic structure is inactivated by detergents and flavouring agents it cannot be formulated into a toothpaste (Sanz et al, 1994). Triclosan is a phenolic compound and also used as a toothpaste additive. It is usually combined with either zinc citrate or a copolymer of methoxyethylene and maleic acid, in which form it is effective in reducing plaque and gingivitis (Ciancio, 1987). A non-ionic phenol derivative, Triclosan has a broad spectrum antimicrobial activity against gram positive and gram negative bacteria and has been shown to be beneficial in toothpaste (Pires et al., 2007).

Triclosan acts on the microbial cytoplasmic membrane to induce leakage of cellular constituents and bacteriolysis (Rolla et al., 1996). Triclosan is most effective when combined with a copolymer. Gunsolley (2006) found that the 2.0% addition of the copolymer Gantrez (methoxyethylene and maleic acid) was crucial as triclosan preparations without this ingredient were not as effective. The copolymer enhances the antibacterial activity of Triclosan through improved binding to the tissues of the oral cavity and a subsequent significant increase in oral retention. Triclosan has been shown to significantly reduce new supragingival plaque development and moderately reduce existing plaque levels and established gingivitis (Linde et al., 1993). It appears to have more effect on gingivitis in cases where oral hygiene is poor. Effects increase over time with maximum results seen 3-6 months after its use is initiated (Saxton et al, 1987).

Stannous Fluoride – Well known in dentistry for its caries-inhibiting effects, there is evidence that stannous fluoride has properties which altering cell metabolism and cell adhesion (Tinanoff, 1990). Stannous ion enters the cell and affects the growth and adherence of the bacteria. Paraskevas & van der Weijden (2006) in a systematic review of the effects of stannous fluoride on gingivitis, concluded that stannous fluoride toothpaste resulted in a reduction in gingivitis and plaque compared with the control, sodium fluoride toothpaste. This effect however was relatively small. Gunsolly (2006) reports that although stannous fluoride shows a statistically significant antiplaque and anti-gingivitis effect, only the anti-gingivitis effect is clinically significant. He suggests the main action of stannous fluoride is its ability to alter the effect the plaque has on gingivitis, rather than its composition or virulence.

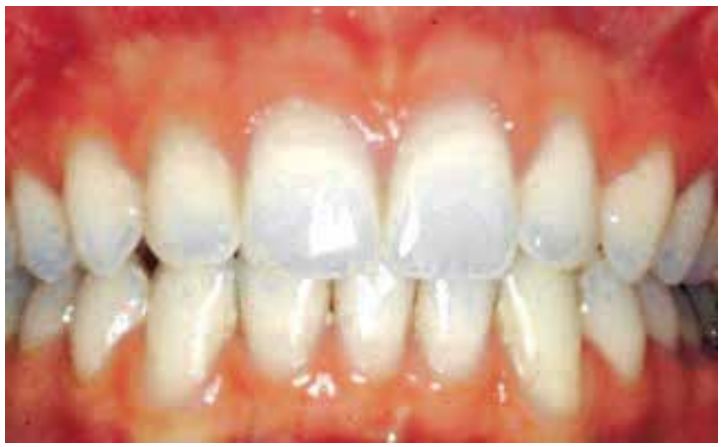

Fig. 5. Healthy Gingivae

7. Acknowledgment

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8. Conclusion

Gingivitis is a reversible disease of the oral cavity. Many adjuncts may assist in the control of pathogenic dental plaque to prevent disease or to reduce its expression. Appropriate behavioural change however is a prerequisite to improving oral health and reducing gingivitis. Mechanical plaque control through effective oral health behaviour remains the essence of effective management of the disease.

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10

Periodontal Inflammation: From Gingivitis to Systemic Disease?

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1. Introduction

There has been a resurgence of interest in recent years in the systemic effects of oral infections such as periodontal diseases. The study of the various means by which periodontal infections and inflammation may influence a variety of systemic conditions is collectively referred to as periodontal medicine. The periodontium responds to tooth-borne biofilm (dental plaque) by the process of inflammation. Dental biofilms release a variety of biologically active products, such as bacterial lipopolysaccharides (endotoxins), chemotactic peptides, protein toxins, and organic acids. These molecules stimulate the host to produce a variety of responses, among them the production and release of potent agents known as cytokines. These include interleukin-1 beta, interleukin-8, prostaglandins, and tumor necrosis factor-alpha. There is a spectrum of periodontal response to these molecules, from mild gingivitis to severe destructive periodontitis. These and other host products and responses may influence a variety of important disease pathways, including atherosclerosis, mucosal inflammation, and premature parturition. The purpose of this chapter is to review the possible biological pathways by which periodontal diseases may influence these disease processes.

There has been increasing attention paid in recent years to the possibility that oral bacteria and oral inflammation, particularly periodontal diseases, may influence the initiation and/or progression of several systemic disease processes. This, of course, is not a novel concept. Indeed, the focal-infection hypothesis, which grew from the principles of infectious disease first established by Koch and Pasteur in the mid-19th century, put forth the notion that the invasion of the bloodstream by bacteria from a localized infection (such as periodontal diseases) could spread to distant organs and tissues to cause disease.[1-3] In fact, this hypothesis was so convincing to practitioners of the time that tonsillectomy and full-mouth extraction enjoyed widespread implementation to treat many diseases, regardless of whether or not infection could be proven to be the cause. However, because it became clear that it was impossible to correlate with confidence a particular systemic disease with a preceding oral infection or dental procedure, the focal-infection hypothesis fell from favor by the middle of the 20th century. Yet, interest in the systemic effects of periodontal infection was reignited in the early 1990s by a series of case-control and other epidemiologic studies that demonstrated statistical associations between poor oral health and several

systemic diseases. The goal of this chapter is to describe the biologically plausible circumstances that underlie these potential associations. The reader is further referred to recent definitive reviews on the pathogenesis of periodontal disease for specific details that are beyond the scope of this chapter.[4,5]

The periodontium responds to the tooth-borne biofilm, long known as dental plaque, by the process of inflammation. Plaque is composed of numerous bacteria, comprising over 700 species, which tenaciously adhere to the tooth surface.[6] Scientists are now beginning to understand the complex molecular interactions that occur, for example, between the bacteria and salivary pellicle that coats the tooth, and between gram-positive cocci of early plaque and gram-negative filamentous bacteria that populate the tooth as plaque matures.[7] Recent work has elucidated complex signaling pathways (referred to as quorum sensing) between bacteria, mediated by soluble chemicals produced by the bacteria that control biofilm development.[8] It is anticipated that this knowledge will eventually yield sophisticated strategies to limit the pathogenic potential of dental plaque.

Within a few hours of meticulous tooth cleaning, bacteria colonize the tooth surface primarily around the gingival margin and interdental spaces (Figure 1).[9] The developing biofilm releases a variety of biologically active products, including lipopolysaccharides (endotoxins), chemotactic peptides, protein toxins, and organic acids.[4] These molecules diffuse into the gingival epithelium to initiate the host response that eventually results in gingivitis and, in some circumstances, inflammatory periodontal diseases.[4] Clinically, gingivitis is characterized by a change in color—from normal pink to red—with swelling and, often, sensitivity and tenderness.[10] Gentle probing of the gingival margin typically elicits bleeding.[10] Because gingivitis is often not painful, it may remain untreated for many years.

Epidemiologically, the prevalence of gingivitis in non-Hispanic whites is approximately 50% of the population, with up to 63% in Mexican Americans showing clinical signs of the disease.[11] It is quite possible that this rate is somewhat understated because it is possible that gingivitis, in its most nascent form, is clinically undetectable. Periodontitis affects approximately 35% of dentate US adults 30 to 90 years of age, with 21% having a mild form and 12% having a moderate or severe form of the disease.[12] Thus, gingivitis is much more widespread than periodontitis in the US population.

Histopathologically, gingival inflammation presents as a spectrum of severity in humans.[7] In a relatively small subset of the population, the gingiva are virtually devoid of inflammatory infiltrate, the so-called "pristine gingiva" (Figure 2).[7] These subjects practice impeccable oral hygiene and demonstrate no clinical signs of inflammation. More widespread would be the "normal healthy gingiva," which demonstrates a mild-to-moderate inflammatory infiltrate. Clinically, these two conditions would appear indistinguishable in that the tissues would appear quite healthy.

Probably most prevalent in the population is established gingivitis that is associated with a more widespread biofilm and clear clinical symptomology (redness, swelling, and bleeding), and histopathologically showing significant inflammatory infiltration (Figure 3).[7] The most severe form of periodontal diseases results in the destruction of the periodontal ligament and supporting osseous tissue and, ultimately, exfoliation of the teeth. Periodontitis is associated with extensive formation of biofilm dominated by anaerobic, gram-negative bacteria and spirochetes.[13]

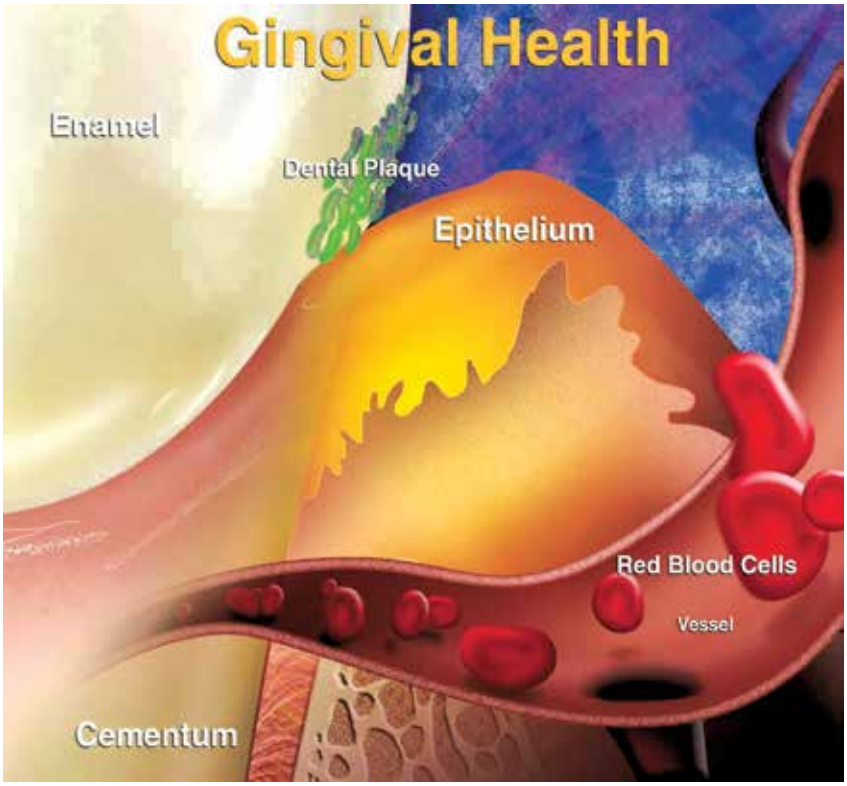

Fig. 1. Biochemical events in periodontal disease. Pristine gingiva are not exposed to significant numbers of plaque microorganisms to yield a host response. Few signs of acute inflammation or cellular infiltrate are noted.

Fig. 2. Left panel: Pristine gingiva is found in subjects with impeccable oral hygiene and minimal plaque. Gingival tissues are free of clinical signs of inflammation, and tissues are essentially free of inflammatory infiltrate. Right panel: Early gingivitis is found in subjects with some plaque formation. While the gingival tissues are free of clinical signs of inflammation, a mild inflammatory infiltrate is evident, consisting of vasculitis and the presence of neutrophils.

Fig. 3. Left panel: In the absence of effective plaque control, a robust inflammatory response results in clinical signs of inflammation (redness, edema, bleeding) and a significant inflammatory infiltrate, including neutrophils, lymphocytes, and evidence of collagen breakdown. Signs of periodontal attachment loss or alveolar bone loss are not evident. Right panel: The inflammatory response results in marked collagen breakdown, periodontal attachment and alveolar bone loss, and clinical signs of inflammation.

As mentioned previously, initial dental plaque bacteria (typically gram-positive cocci and filaments) release a variety of chemical compounds during their normal metabolism (organic acids, chemotactic peptides, etc). These products are soluble and penetrate the superficial layers of the sulcular epithelium. These substances signal the epithelium of the gingiva to produce a variety of biologically active mediators, most prominently cytokines such as interleukin-1 beta (IL-1β), interleukin8 (IL-8), prostaglandins, tumor necrosis factor-alpha (TNF-α), and matrix metalloproteinases (Figure 4). These products influence a number of cellular processes, including the recruitment and chemotaxis of neutrophils to the site, with increased permeability of the gingival vessels that results in extravasation of plasma proteins from the blood vessels into the tissue. The epithelium also responds by induction of innate defense systems, which include the production of antimicrobial peptides, such as defensins, calprotectin, etc.[14] In addition, the salivary defense system works to limit bacterial growth through the flushing action of simple fluid flow that clears bacteria from the oral surfaces, bacterial-aggregation factors, antimicrobial proteins, etc.[15]
Should the dental plaque biofilm continue to grow and expand to populate the subgingival space, these noxious compounds will stimulate the epithelium to produce bioactive mediators, resulting in further recruitment of a variety of cell types, including neutrophils, T-cells, monocytes, etc (Figure 5). The resulting established or chronic gingivitis is the most prevalent type of gingival inflammatory lesion in the population as a whole. Thus, continued exacerbation of the process results in signaling of underlying cell types, including fibroblasts, to increase production of proinflammatory cytokines in the tissues. Host systemic responses to this insult also can be documented. For example, evidence of specific antibodies to oral organisms can be demonstrated in peripheral blood. Also, the acute-phase response is associated with gingival inflammation, including the production of C-reactive protein (CRP), fibrinogen, complement, etc, by both local cells and the liver.[16,17] These proteins not only possess biological activities that may further exacerbate the inflammatory response, they may also impact the initiation or progression of systemic disease processes, such as atherosclerosis.[18,19]

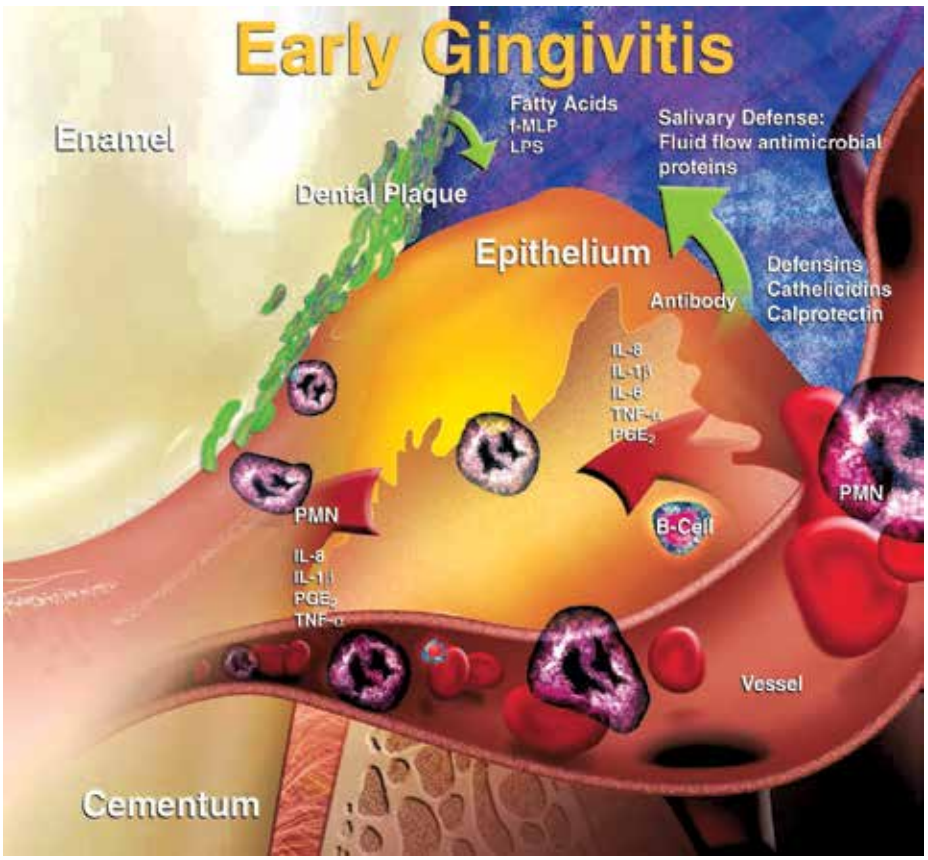

Fig. 4. Bacteria in dental plaque release biologically active components, including lipopolysaccharides, chemotactic peptides, and fatty acids. These components signal gingival epithelial cells to release proinflammatory cytokines that diffuse into the underlying connective tissues to stimulate acute vasculitis, which leads to dilation of blood vessels and extravasation of plasma components into the connective tissue compartment. Chemotactic peptides signal white cells to interact with and stick to vascular endothelium, after which the neutrophils enter the connective tissues. In addition to the inflammatory response, the host attempts to clear itself of microorganisms by responding to these signals with epithelial production of antimicrobial peptides. Saliva also affords numerous antimicrobial mechanisms to protect the host

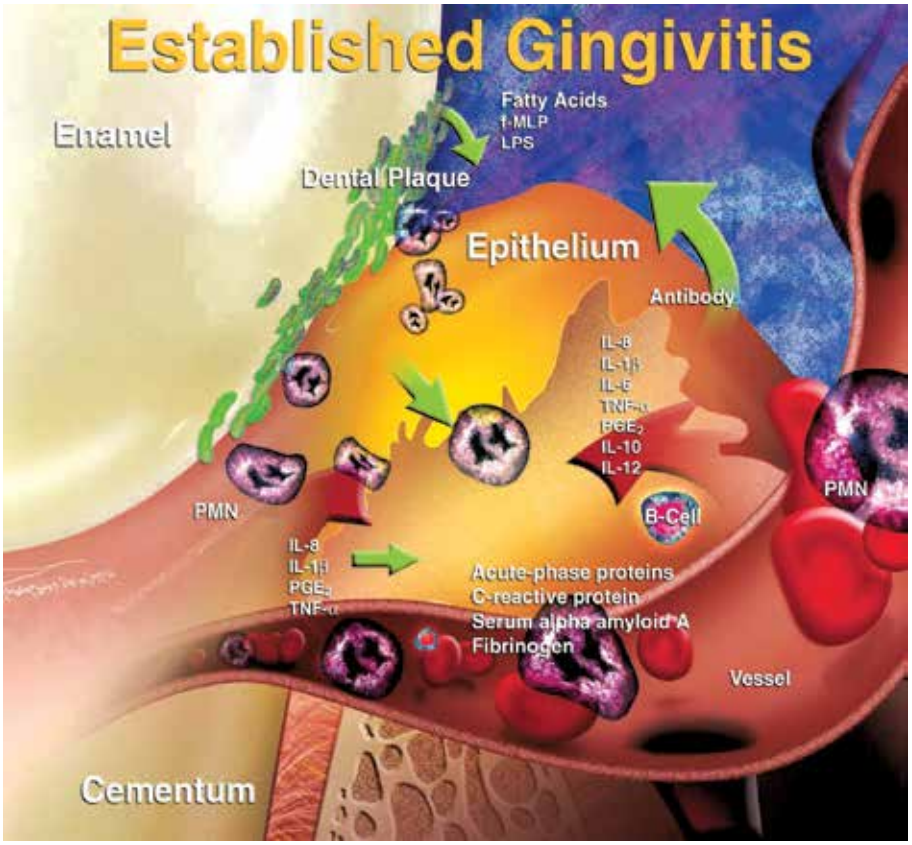

Fig. 5. Increased numbers and increasing diversity of bacteria in dental plaque continue to release biologically active components that increase the intensity and spread of the inflammatory response. Increased numbers of neutrophils, monocytes, and macrophages infiltrate the tissues to release more diverse cytokines and prostaglandins that exacerbate the inflammatory response. Lymphocytes (T-and B-cells) and plasma cells also infiltrate, the latter releasing antibodies against the microorganisms that may also cross-react with the host tissues. The acute-phase response (including production of acute-phase proteins such as CRP, serum alpha amyloid A, and fibrinogen) also is evident.

To this point, rigorous tooth cleaning and oral hygiene procedures would reverse the course of gingivitis and return the periodontium to a healthy state.[20] Unfortunately, however, many people fail to maintain adequate hygiene and so the process of inflammation often continues unchecked for years. In some individuals, for reasons that are not entirely clear, the inflammatory process expands to involve the breakdown of collagen in periodontal ligament and bone resorption, resulting in periodontitis (Figure 6). The rate of breakdown varies between individuals. It has been suggested that there are underlying genetic mechanisms or other risk factors (e.g., smoking, diabetes, stress, etc) that provoke these processes in certain people and not in others. We've heard lately of polymorphisms and various genes that control, for example, interleukin or fibrinogen synthesis. There is an ongoing scientific effort to determine the role of host genetics in the susceptibility to periodontal infection.[21]

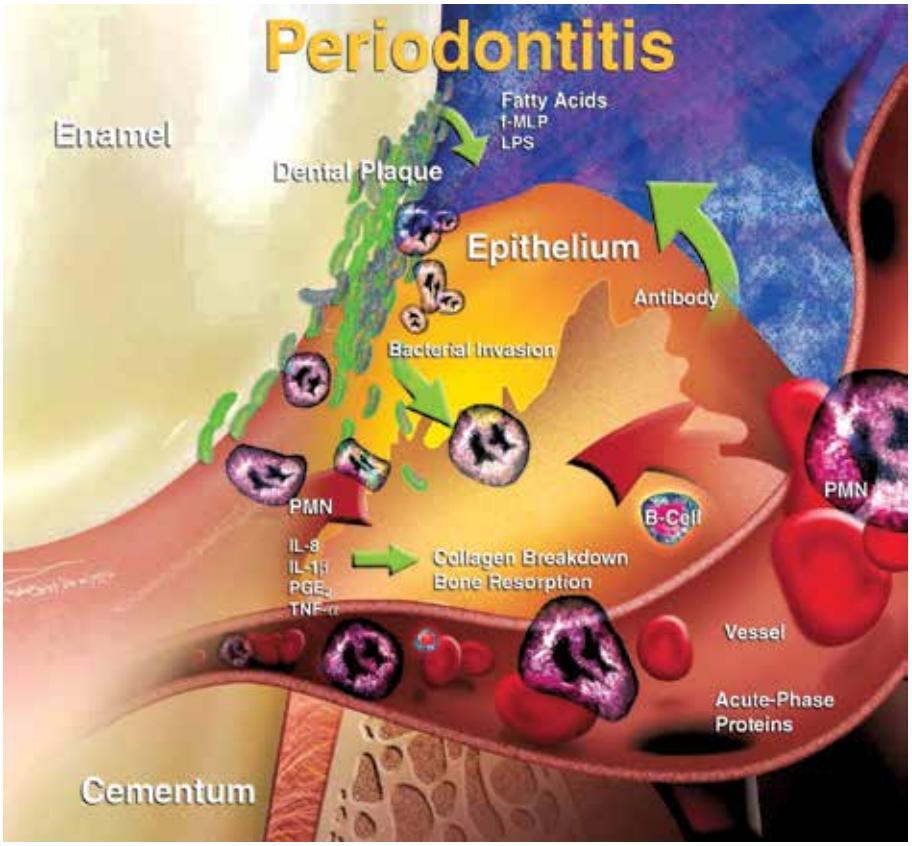

Fig. 6. In some subjects, for reasons that remain unknown, the chronic inflammation of established gingivitis spreads to provoke periodontal ligament and alveolar bone destruction.

2. Gingival health and bacteremia

A consequence of this inflammatory process is ulceration of the gingival sulcular epithelium, which allows bacterial translocation from the sulcus into the bloodstream. The surface area of the periodontal ligament has been calculated to cover about 75 square centimeters. Thus, a person having 50% horizontal bone loss and inflamed pocket epithelium would have a wound surface of approximately 30 to 40 square centimeters. Such a wound surface would likely increase the risk for bacterial translocation when compared to a healthy periodontium. In the most prevalent periodontal disease, established gingivitis, pockets of 4 to 5 millimeters may translate into a gingival wound surface area of 10 to 20 square centimeters. Considering that many people go a long time without having gingivitis treated, this chronic inflammatory condition may promote continuous, low-grade chronic bacteremia. Several studies have indeed shown that the incidence of bacteremia is elevated in subjects with increasing severity of gingival inflammation.[22,23] When using rather insensitive bacterial culture techniques, bacteremia could be detected even in subjects with clinically healthy gingiva. The use of more sensitive molecular techniques, such as the polymerase chain reaction,[24,25] would likely prove bacterial translocation from the periodontium to be even

more common than presently appreciated. While most studies of dentally related bacteremia have centered around purposeful activities such as tooth brushing, periodontal probing, and tooth extraction, it is possible that while participating in daily activities (chewing, speaking, habits, etc), minor disruptions to gingival integrity occur in a significant number of individuals with gingival inflammation.

3. Gingival inflammation: Pathways of systemic effects

Oral bacteria and gingival inflammation may theoretically influence systemic health through four potential pathways: bacteremia, systemic dissemination of locally produced inflammatory mediators, provocation of an autoimmune response, and aspiration or ingestion of oral contents into the gut or airway (Figure 7). Low-grade but persistent bacteremia may allow oral bacteria to aggregate platelets through receptor-ligand interactions. Studies have shown that infusing rabbits with aggregating bacteria caused significant hemodynamic changes, acute pulmonary hypertension, and cardiac abnormalities, including ischemia.[26] This very provocative work suggests that bacteremia of oral origin may have serious implications for systemic health.

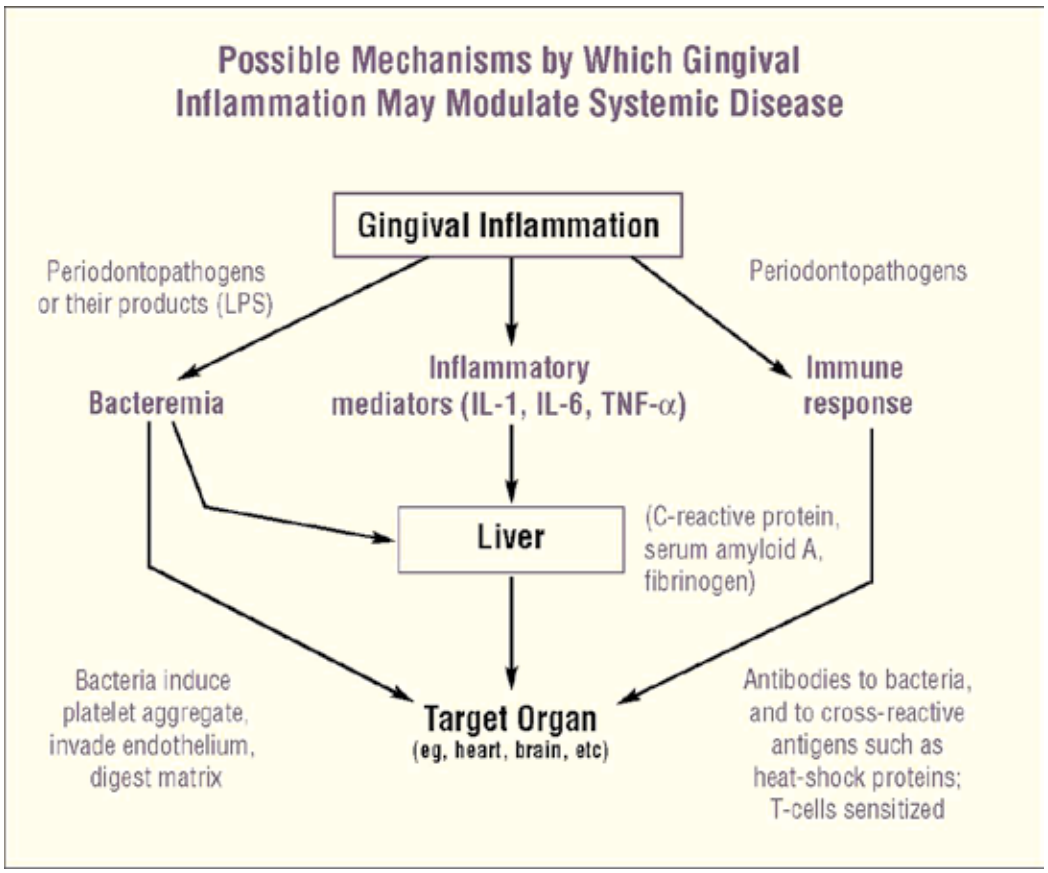

Fig. 7. Theoretical pathways by which the gingival inflammatory response may impact systemic inflammation and systemic processes such as atherosclerosis.

Several inflammatory mediators can be measured as being elevated in peripheral blood in subjects with periodontal disease,[17] suggesting that periodontal inflammation either contributes directly to the elevation of the concentration of these substances in peripheral blood or signals distant organs (e.g., the liver) to produce them. The liver could respond, for example, through the acute-phase response by producing CRP, fibrinogen, etc. These proteins may have deleterious effects on other target organs (e.g., heart, brain) by modulating disease processes such as atherosclerosis. Recent studies have suggested a connection between chronic infections, such as *Chlamydia pneumoniae* infection or periodontal diseases, and atherosclerosis.[27] It has been suggested that immunity to bacterial pathogens plays a role in the atherosclerotic process and that this response may involve autoimmunity.[28] It has been observed that almost all humans have immune reactions against microbial heat-shock protein 60 (HSP60). The human version of this protein is highly homologous with bacterial HSP60. It is possible that the immune response generated against the microbial version of this protein could cross-react with human HSP60 on arterial endothelial cells to influence the course of atherosclerosis.[28] Bacteria thought to induce gingival inflammation may also stimulate an autoimmune response by presentation of cross-reactive epitopes that stimulate autoantibody or T-cell response reactive with host antigens, such as HSP60, to drive a proinflammatory response with cardiovascular effects.[29,30]

Dental plaque and/or periodontal inflammation may influence pathogenic processes occurring in distally contiguous mucosal surfaces, for example, in the respiratory or digestive tracts.[31,32] Salivary hydrolytic enzymes, observed to be elevated in patients with periodontitis, can promote the adhesion of pathogenic bacteria to the oral surfaces, thereby altering oropharyngeal colonization patterns. It is also possible that periodontopathic bacteria stimulate the periodontium to release proinflammatory cytokines that, when aspirated or swallowed, alter mucosal surfaces to promote adhesion of pathogenic bacteria that cause diseases such as pneumonia or gastric ulcers.[31,32] Finally, cytokines released from inflamed periodontal tissues may enter the respiratory tract in aspirated saliva, triggering the sequence of neutrophil recruitment, epithelial damage, and infection.[31]

4. Gingival inflammation and systemic disease

Several case-control studies[33] published in the early 1990s found that patients with a history of myocardial infarction had worse oral health than control subjects (studies are summarized in the reference). This has led to a flurry of studies to verify these observations. While most of these studies support a modest association between periodontal diseases and the outcomes of atherosclerosis (of myocardial infarction, angina, or stroke), several studies have not supported this association. This is complicated by the absence of a standard definition or measures for periodontal diseases and that underlying mechanisms common to both periodontal diseases and atherosclerosis share common risk factors, such as lifestyle habits like cigarette smoking. It is possible that dental plaque stimulation of cytokine production in the periodontium may elevate levels of cytokines in the peripheral blood. This may in turn stimulate hepatic production of acute-phase proteins, such as CRP. These proteins could then induce vascular injury, atherogenesis , cardiovascular disease, and stroke. Several studies have shown that patients with periodontal diseases demonstrate elevated levels of CRP and fibrinogen, as well as peripheral white blood cells.[17,34] Elevated

levels of these proteins have been suggested to be risk factors for cardiovascular disease.[35-37] Additional evidence has been reported for the possible direct role of bacteria in atherosclerosis. It has been reported that chronic disease agents, such as *C pneumoniae*, play a role in atherosclerotic plaque development. Recently it has been reported that the DNA of oral bacteria could be amplified directly from atherosclerotic plaques. It is, therefore, possible that these pathogens may play a role in the development and progression of atherosclerosis leading to coronary vascular disease

Lung diseases such as hospital-acquired pneumonia and chronic obstructive pulmonary disease (COPD) also have been associated with poor oral health.[31,38] It is possible that oral biofilms on the teeth may serve as a reservoir of infection for respiratory pathogenic bacteria. In subjects admitted to hospital intensive care units or nursing homes, bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus*, and enteric bacteria have been shown to colonize the teeth. These bacteria may then be released into the oral secretions to be aspirated into the lower airway to cause infection. It is also possible that inflammatory mediators, such as cytokines produced by the periodontium, released into the secretions also can be aspirated to have pro-inflammatory effects in the lower airway.

Several epidemiologic studies have reported associations between poor oral health and COPD.[39,40] One interesting observation found that lung function measured through spirometry is associated with measures of periodontal disease.[40] In subjects stratified by periodontal attachment loss, those with more severe attachment loss tended to demonstrate less lung function than those with less attachment loss. Further research is necessary to dissect the contribution of periodontal inflammation from those of established etiologies, such as smoking on lung function.

There also has been interest in the association between periodontal inflammation and adverse pregnancy outcomes.[41,42] Unfortunately, adverse pregnancy outcomes, such as premature birth and low birth weight, are quite common events. This is a very significant public health problem in the United States, and has been associated with subclinical genitourinary or other infections. During parturition, the uterus is influenced by the hypothalamus through the production of oxytocin, which stimulates uterine contraction. Prostaglandins that are produced by the placenta also stimulate uterine contraction, which normally leads to birth in the third trimester (37 weeks). It is thought that chronic infections drive the inflammatory process, which leads to the release of inappropriate levels of prostaglandins and TNF-α, which prematurely stimulates uterine contraction to promote preterm birth.

It has been suggested that periodontal infection and the release of lipopolysaccharides and other biologically active molecules drive the process of inflammation, as described above. This results in the elevation of prostaglandins and TNF-α in the crevicular fluid. Lipopolysaccharides released from the oral cavity into the bloodstream may stimulate prostaglandins in the placenta, causing preterm birth. It is also possible, such as in atherosclerosis, that cytokines in the periodontium may lead to elevated peripheral blood cytokine levels and stimulate hepatic production of acute-phase proteins that may influence the birth process. Very recent work has also found that periodontal pathogens, such as *Fusobacterium nucleatum*, may travel from the gingival sulcus to the placenta to cause preterm birth.[43] Thus, it is possible that these bacteria may enter the bloodstream from the oral cavity to directly affect the birth process.

5. Summary

Dental plaque drives periodontal inflammation, with gingivitis being the initial manifestation of this process. With appropriate intervention, this process can be reversed and the periodontium returned to a state of health (Figure 8). However, an exuberant local host response, including the synthesis of cytokines and antibodies, in some cases results in the destruction of periodontal ligament and supporting bone (periodontitis). Periodontitis is typically treated by removing the etiology (dental plaque) and returning the gingival tissues to health. Unfortunately, in many cases, periodontal disease goes untreated for many years. It is possible, then, for the systemic host response to this insult to contribute to disease processes that result in cardiovascular disease and stroke, respiratory disease, and adverse pregnancy outcomes.

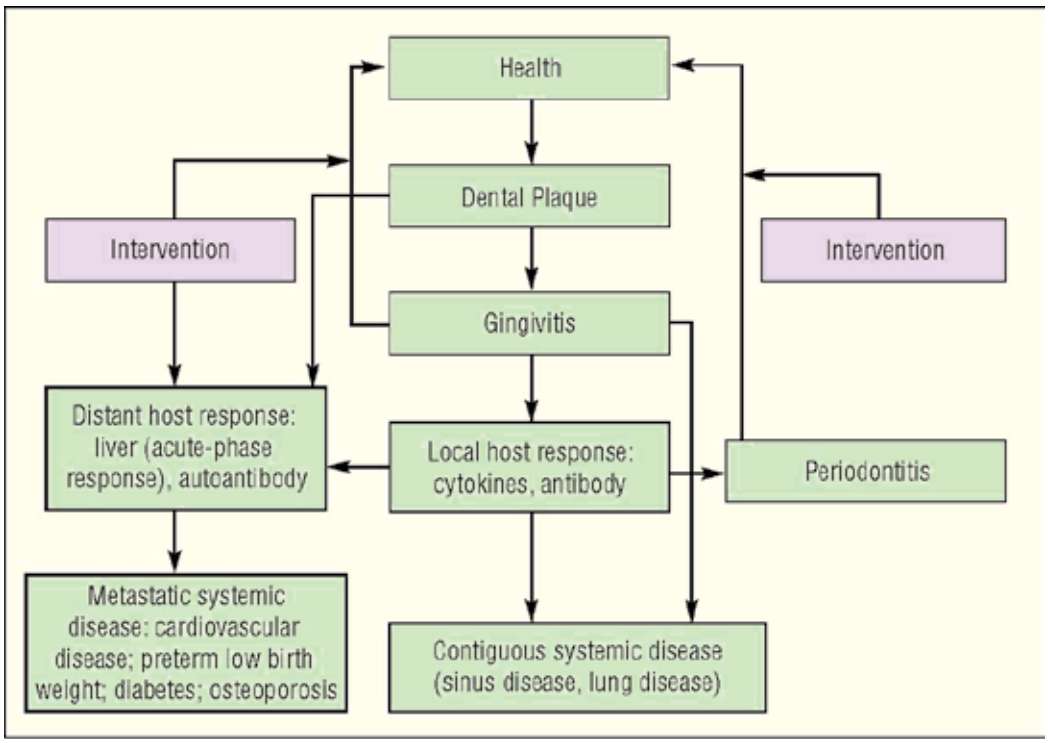

Fig. 8. Suspected interrelationships between gingival inflammation, systemic disease, and response to periodontal therapy.

What is the status of periodontal medicine today? While there are a number of preliminary studies that point to an association between periodontal inflammation and several systemic conditions, as mentioned above, the data are equivocal. In many cases, there has been an emphasis on linking periodontal attachment loss with systemic disease. It is possible that the use of this outcome measure, which represents "historical" evidence for the disease without indicating the temporal sequence or duration of disease activity, may cloud the role of periodontal inflammation in this process. Future investigations are needed that use better definitions for periodontal disease and measures of how gingival inflammation and tooth

loss may best determine the role this localized, chronic disease process plays in the progression and severity of important systemic diseases.

6. Acknowledgement

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Part 2

Non-Plaque Associated Gingival Disease

11

Diagnosis and Management of Desquamative Gingivitis

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1. Introduction

Desquamative gingivitis (DG) is characterized by erythematous gingiva, desquamation and erosion of the gingival epithelium, and blister formation. It is a clinical manifestation common to several diseases (Endo et al., 2008a; Lo Russo et al., 2008; Rees, 2011). It is seen mainly in adults, especially women, although rare cases have been observed in children (Barnett et al., 1981; Cheng et al., 2001; Leao et al., 2008; Lo Russo et al., 2009; Yih et al., 1998). Nisengard and Levine (1995) cited the following as the standard for the clinical appearance of DG: 1) Gingival erythema not resulting from plaque, 2) Gingival desquamation, 3) Other intraoral and sometimes extraoral lesions, and 4) Complaint of sore mouth, particularly after eating spicy foods. Nikolsky's sign often shows a positive reaction in patients with DG (Fig. 1). This sign involves the application of a shearing force on normal-appearing gingiva, producing epithelial desquamation. The specificity of Nikolsky's sign was higher (96.3%) than the sensitivity (46.7%), indicating that Nikolsky's sign is useful in the preliminary diagnosis of oral blistering diseases and may represent a simple clinical tool for oral health practitioners (Mignogna et al., 2008).

It was previously thought that DG was caused by a hormone imbalance since it often occurred in middle-aged and older women. However, advanced diagnosis using immunological techniques indicates that most cases of DG are caused by mucocutaneous diseases, the most common ones being lichen planus (LP), mucous membrane pemphigoid (MMP), and pemphigus vulgaris (PV) (Endo et al., 2008a; Leao et al., 2008; Lo Russo et al., 2008; Lo Russo et al., 2009; Nisengard & Rogers, 1987; Rees, 2011; Yih et al., 1998). Contact allergic reactions to various oral hygiene products have also been reported to present as DG (Endo & Rees, 2006, 2007; Endo et al., 2010; Lamey et al., 1990; Rees, 1998, 1999, 2011). Although a definitive diagnosis of the specific disease or disorder causing DG is required to provide proper treatment, it is almost impossible to do so based solely on clinical manifestations. Therefore, histopathological examination and direct immunofluorescence (DIF) testing are often required to establish the final diagnosis. According to a summary of results using DIF, 75.4% of the 174 cases that were clinically diagnosed as DG were caused by mucocutaneous diseases (Nisengard & Rogers, 1987), the most commonly recognized

ones being cicatricial pemphigoid (cicatricial pemphigoid was renamed MMP; 48.9%), LP (23.6%) and PV (2.3%). Recent studies of the clinical associations of DG found that LP seems to be the most frequent cause. Leao et al. (2008) evaluated 187 patients with DG, and found LP to be the most common (70.5%) while MMP (14%) and PV (13%) were less prevalent. When Lo Russo et al. (2009) evaluated 125 patients with DG, they found the most common cause was LP (75%), whereas DG due to MMP occurred in only a small percentage of patients (9%) .

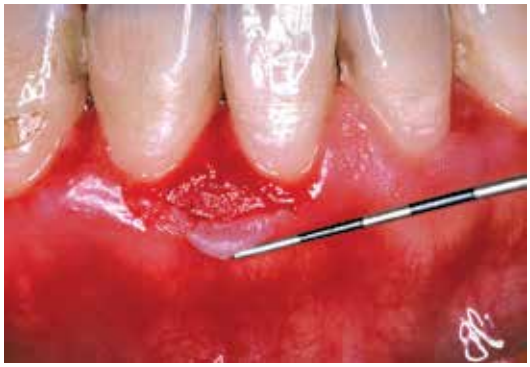

Fig. 1. Positive Nikolsky sign associated with PV. The epithelium could be peeled away easily by slightly scratching the surface of the gingiva.

The management of DG has been a major problem, largely because the etiology of the disease has been elusive. In this chapter we will review the current literature on the pathogenesis, diagnosis and management of DG.

2. Oral Lichen Planus

LP is a chronic inflammatory mucocutaneous disease caused by an unknown etiology (Mollaoglu, 2000; Roopashree et al., 2010; Scully et al., 1998). The disease commonly occurs in middle-aged and older people, and the morbidity rate of women is higher than that of men (Camacho-Alonso et al., 2007; Eisen, 2002; Ingafou et al., 2006.; Mignogna et al., 2005; Xue et al., 2005). DG is recognized in about 30% of oral LP patients (Mignogna et al., 2005) (Fig. 2). Lesions are found mainly in the skin, genitalia, or oral mucosa and may be found in multiple regions, although they are confined to the gingiva alone in some patients (Eisen, 2002; Ingafou et al., 2006; Mignogna et al., 2005; Mollaoglu, 2000; Scully et al., 1998; Xue et al., 2005).

Histopathologically, LP is characterized by band-like lymphocyte infiltration below the epithelium accompanied by basal cell liquefaction (Lo Russo et al., 2008; Rees, 2011). As pronounced basal cell liquefaction occurs, the epithelium may detach from the underlying connective tissue. DIF findings for LP are useful so as to rule out other mucocutaneous diseases. The findings are nonspecific but supportive if fibrin or fibrinogen deposition is found in the basement membrane zone (BMZ) in a linear pattern (Lo Russo et al., 2008; Rees, 2011; Rinaggio et al., 2007; Yih et al., 1998). It may not be necessary to routinely conduct DIF testing, since the histopathological findings of LP are usually diagnostic. Since LP is idiopathic, the therapeutic goal is the remission or suppression of the symptoms. Spontaneous remissions of oral LP are infrequent (Ingafou et al., 2006). Skin lesions are often

transient in nature, whereas lesions of oral LP may demonstrate a chronic and protracted clinical course (Al-Hashimi et al., 2007; Endo et al., 2008b; Ingafou et al., 2006; Plemons et al., 1999). Although it is still subject to some controversy, LP may have premalignant potential (Eisen, 2002; Ingafou et al., 2006; Mignogna et al., 2005; Xue et al., 2005). Therefore, it is important to provide treatment and long-term follow-up examinations for patients with LP.

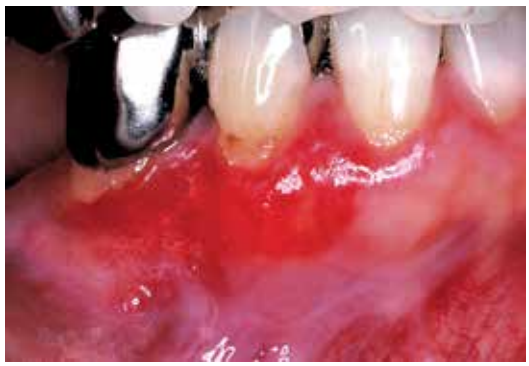

Fig. 2. Patchy erythematous attached gingiva associated with LP.

3. Mucous Membrane Pemphigoid

MMP is one of a group of autoimmune, subepithelial blistering diseases that predominantly affect the mucous membranes (Bagan et al., 2005; Chan et al., 2002; Fleming & Korman, 2000; Scully & Lo Muzio, 2008). Most patients with this disease are in the fifth or sixth decade of life, and the majority of them are women (Chan et al., 2002; Fleming & Korman, 2000; Scully & Lo Muzio, 2008). Oral lesions are observed in almost all cases, and the primary lesion often appears in the oral cavity (Chan et al., 2002; Fleming & Korman, 2000; Scully & Lo Muzio, 2008). DG is a common manifestation of MMP (Carrozzo et al., 2004; Endo et al., 2006a) (Figs. 3 and 4). The gingiva appears erythematous with a diffuse or patchy distribution. Vesiculobullous lesions on the gingiva break easily and form erosions with irregular margins. Other oral sites include the buccal mucosa, palate, alveolar ridge, tongue, and lip (Chan et al., 2002; Fleming & Korman, 2000). Extraoral mucous membranes including the conjunctiva, skin, pharynx, external genitalia, nose, larynx, anus and esophagus may also be affected (Chan et al., 2002; Fleming & Korman, 2000).

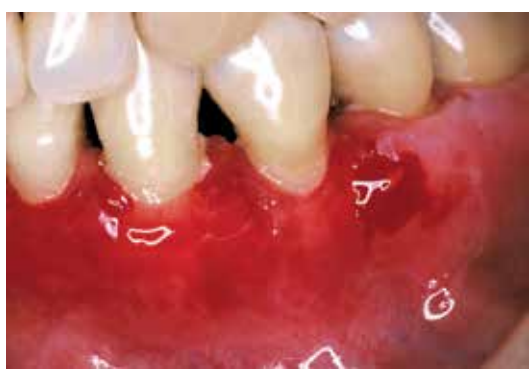

Fig. 3. Desquamative lesions with bleeding on the attached gingiva associated with MMP.

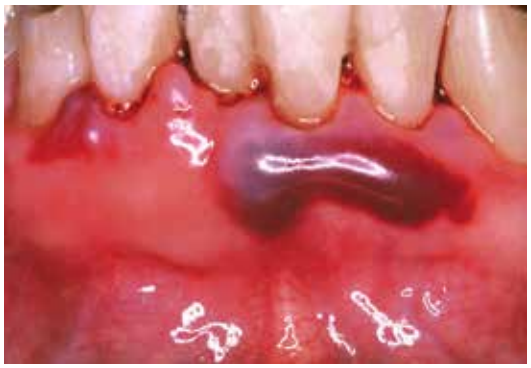

Fig. 4. Localized blood-filled bulla found on the gingiva associated with MMP.

Scarring and an associated loss of function are the major sequelae of some forms of MMP. Life-threatening airway obstruction and sight-threatening ocular scarring have been reported (Alexandre et al., 2006; Higgins et al., 2006, 2010; Thorne et al., 2004; Trimarchi et al., 2009). However, scarring is rarely seen on the oral mucosa. Early detection and early treatment of ocular lesions are especially important because the conjunctiva is the second most-frequent site of involvement (Fleming & Korman, 2000; Thorne et al., 2004). It has been reported that ocular lesions were observed in 64% of MMP patients and that MMP patients with oral lesions frequently have asymptomatic ocular lesions (Fleming & Korman, 2000; Higgins et al., 2006; Thorne et al., 2004). These observations indicate that all patients diagnosed with MMP should undergo ophthalmic examination. The early manifestation of ocular MMP includes chronic intractable conjunctival irritation in which the patients complain of a burning sensation, dryness, or a foreign body sensation. Scar-like adhesions of the eyelid to the conjunctiva (symplepharon) may occur after repeated conjunctival fibrosis and progressive symblepharon may lead to blindness (Fleming & Korman, 2000). Skin lesions are observed in up to 24% of MMP patients (Fleming & Korman, 2000). Fifteen percent of MMP patients experience nasal lesions, which may present in the form of crusty ulcers on the septum or turbinates (Alexandre et al., 2006; Fleming & Korman, 2000). Scarring and adhesion can also take place and may result in nasal airway obstruction (Alexandre et al., 2006; Trimarchi et al., 2009). Laryngeal MMP is a rare condition (12.2%) and the supraglottis is the most commonly affected site (Higgins et al., 2010).

Histopathologically, MMP is characterized by subepithelial bulla formation (Lo Russo et al., 2008; Rees, 2011). In the DIF testing of MMP patients, the linear deposition of C3, IgG or other immune globulin is observed in the BMZ (Lo Russo et al., 2008; Rees, 2011; Rinaggio et al., 2007; Yih et al., 1998). Multiple target antigens of MMP were identified in BMZ components by circulating autoantibodies in the patients' serum. These antigens include bullous pemphigoid (BP) 180, BP230, laminin 5, laminin 6, beta 4 integrin and unknown antigens (Chan et al., 2002). There is presently no firm correlation between specific clinical phenotypes and the target antigens in MMP patients (Carrozzo et al., 2004; Chan et al., 2002). To date, the most common target antigen associated with MMP is BP180 (Balding et al., 1996; Bedane et al., 1997; Calabresi et al., 2007). The extracellular domain of BP180 is considered to have a number of MMP-reactive antigenic sites of which at least two sites have been identified. One is the non-collagenous 16 a (NC16a) domain located in the upper lamina lucida, and the other is the carboxy-terminus domain at the lamina lucida/lamina densa interface (Bagan et al., 2005; Balding et al., 1996; Bedane et al., 1997; Calabresi et al.,

2007; Van den Bergh & Giudice, 2003). The authors evaluated circulating IgG autoantibody specific for BP180NC16a using enzyme-linked immunosorbent assay (ELISA) (Endo et al., 2006a). In five cases of MMP, the BP180NC16a ELISA was positive in three cases, although the antibody was present in low titers (Endo et al., 2006a). The presence or absence of the autoantibodies to BP180NC16a was inconsistent with the severity of the oral lesions or the presence of the extraoral lesions (Endo et al., 2006a). To date, there is no general consensus regarding the establishment of a prognostic indicator for MMP. There is also no known correlation between antigen-specific autoantibodies and the disease prognosis. Autoantibodies against various BMZ components probably play a role in the pathogenesis of MMP but there are still many questions about their pathogenic role.

4. Pemphigus Vulgaris

PV is an autoimmune disease characterized by acantholysis in the epithelium (Bystryn & Rudolph, 2005; Scully & Mignogna, 2008). Most patients with PV are in their fourth and fifth decade of life, and the disease is equally common in men and women (Bystryn & Rudolph, 2005; Scully & Mignogna, 2008). Early symptoms of PV develop in the oral cavity in about 80% of the patients (Sirois et al., 2000), so it is often detected first by dentists. PV is rare among the mucocutaneous diseases causing DG (Endo et al., 2008a; Leao et al., 2008; Lo Russo et al., 2009; Nisengard & Rogers, 1987), and it can be fatal as well.

In PV bullae rupture rapidly and intact bullae formation in the oral cavity is rarely seen. Oral PV is often regarded as difficult to diagnose in its early stages because less characteristic oral manifestations are produced than those associated with cutaneous PV. Diagnostic delays greater than 6 months are common in oral PV (Sirois et al., 2000). If the treatment is delayed due to misdiagnosis or inadequate initial management, the risk of the disease spreading or other complications may increase. Clinically, PV frequently begins with oral lesions and later progresses to skin lesions. Lesions may occur anywhere on the oral mucosa (Bystryn & Rudolph, 2005; Mignogna et al., 2001). On occasion, the gingiva is the only site involved in early lesions, and DG is a common manifestation of the disease (Endo et al., 2005, 2008c; Mignogna et al., 2001) (Figs. 5 and 6). Acantholytic (Tzanck) cells can be confirmed in cytologic smears obtained by scratching the gingiva (Endo et al., 2008a; Mignogna et al., 2001). Tzanck cells show degenerative changes, including round, swollen hyperchromatic nuclei with a homogenous cytoplasm (Endo et al., 2008a).

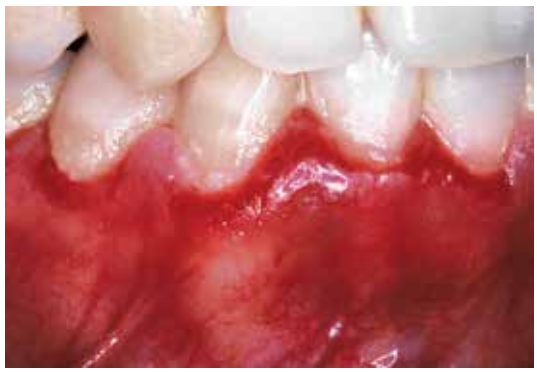

Fig. 5. Mild erythema and swelling of gingiva associated with PV.

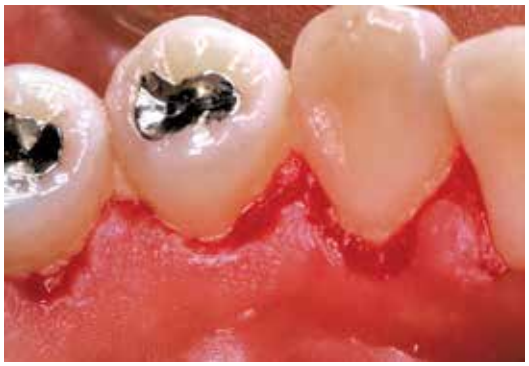

Fig. 6. Eroded gingival surface with ragged edges associated with PV.

In a histopathologic examination, PV is characterized by intraepithelial bullae formation (Lo Russo et al., 2008; Rees, 2011). In the DIF testing of PV patients, deposition of IgG and C3 is often found between the epithelial cells and is characterized by a fishnet pattern (Lo Russo et al., 2008; Rees, 2011; Rinaggio et al., 2007; Yih et al., 1998). The main target antigen of PV is desmoglein (Dsg) 3, a constituent of the extracellular region of desmosomes on keratinocytes (Amagai, 1999). In well developed disease almost all PV patients have circulating autoantibodies to Dsg3 (Amagai et al., 1999; Daneshpazhooh et al., 2007; Harman et al., 2000). More than 50% of PV patients also have Dsg 1 autoantibodies, the primary antigen of pemphigus foliaceus (Amagai et al., 1999; Daneshpazhooh et al., 2007; Harman et al., 2000). In some PV patients, the Dsg autoantibody profile correlates with the clinical manifestations (Daneshpazhooh et al., 2007; Endo et al., 2008c). The authors reported PV in a 31-year-old woman with only gingival involvement at the onset of the disease, but later she developed more extensive oral and skin lesions (Endo et al., 2008c). Using ELISA, the authors confirmed a change in the autoantibody profile of sera corresponding with the transition from mucosal PV type (lesions limited to the oral mucosa) to mucocutaneous PV type (lesions found in the oral mucosa and skin) (Endo et al., 2008c). When the lesions were limited to the oral cavity, the Dsg3 ELISA was high, and the Dsg1 ELISA was very low (Endo et al., 2008c). After having oral and skin lesions for 26 months, both the Dsg3 and Dsg1 ELISA levels of the patient were high (Endo et al., 2008c). It is believed that the autoantibodies to Dsg3 were produced initially for unknown reasons, causing the gingival PV lesions. As the autoantibody reaction spread, autoantibodies to Dsg1 as well as Dsg3 were produced, which led to the expression of mucocutaneous PV lesions. The pattern of the dynamic Dsg autoantibody profile displayed by this PV patient may be indicative of the phenomenon called "epitope spreading" (Endo et al., 2008c). The authors concluded that PV patients with oral lesions only should be closely followed and immediately referred to other experts (dermatologists, internists or other physicians) if they develop lesions on parts of their body other than the mouth.

5. Hypersensitivity reactions as cause of DG

Localized or generalized epithelial desquamation, erythema, ulceration, and/or vesiculobullous lesions of the gingiva is sometimes elicited by contact hypersensitivity reactions to various foodstuffs, preservatives, oral hygiene products and dental restorative materials (Endo & Rees, 2006; Endo et al., 2010; Lamey et al., 1990; Rees, 1998, 1999, 2011)

(Figs. 7 and 8). These reactions may appear identical to DG and be difficult to differentiate from mucocutaneous diseases. Non-specific histopathologic findings with submucosal perivascular inflammatory cell infiltration should raise suspicion of a contact hypersensitivity etiology (Endo & Rees, 2006; Endo et al., 2010). Patient maintenance of a 1 to 2 week food diary is often beneficial in identifying the causative agent(s). It is also recommended that the patients record the use and frequency of oral hygiene products. Patients are considered to have allergic reactions to a relevant allergen if their patch test results are positive. Eliminating causative agent(s) leads to disappearance of gingival lesions in most contact hypersensitivity cases.

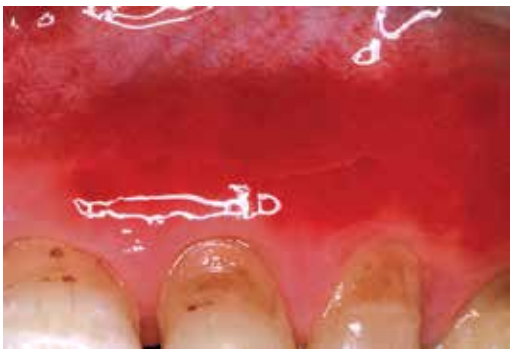

Fig. 7. Gingival erythema following use of a toothpaste

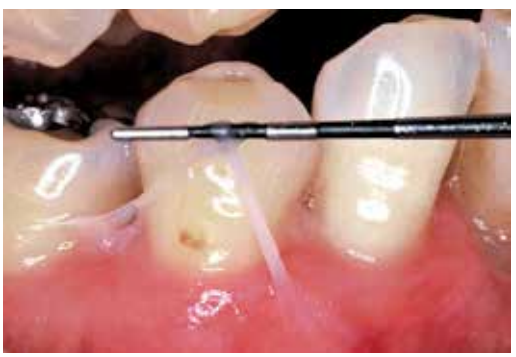

Fig. 8. Epithelial sloughing following use of a mouthrinse

6. Management of DG

The therapeutic approaches to DG are based on expert opinion rather than empirical evidence. Several treatment methods have been reported (Carrozzo & Gandolfo, 1999; Chan et al., 2002; Endo et al., 2008b; Fatahzadeh et al., 2006; Kirtschig et al., 2003; Lamey et al., 1992; Motta et al., 2009; Nisengard, 1996; Nisengard & Levine, 1995). However, treatment may achieve only a temporary effect if idiopathic or autoimmune diseases are causing the DG. A wide range of medications has been advocated for DG but few have been subjected to adequate placebo-controlled trials. The specific disease or disorder causing the DG, the severity of the DG lesions, the presence or absence of extraoral lesions, and the medical history of the patient are the key factors in determining the selection of a topical or systemic treatment (Figs. 9,10,11,12).

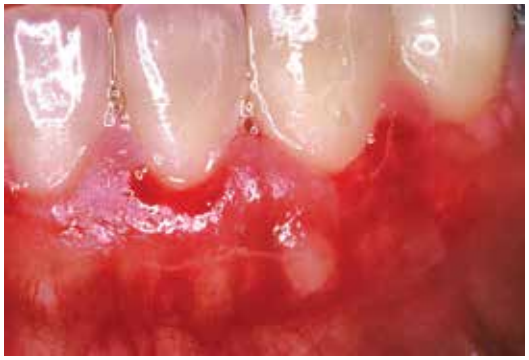

Fig. 9. Initial presentation associated with PV in a 31-year-old woman. The initial examination revealed localized erosions in the marginal gingiva, which is the only site involved in this patient.

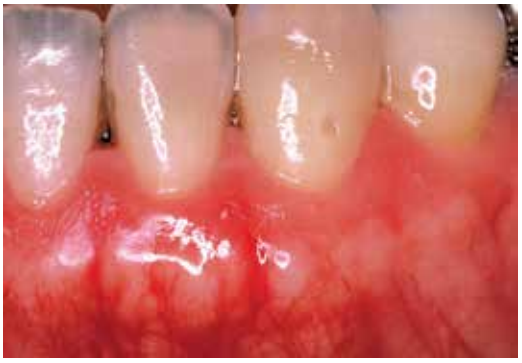

Fig. 10. Treatment response associated with PV in a 31-year-old woman. The gingival lesions went into remission with the topical corticosteroid therapy. However, the circulating autoantibody titer to desmoglein 3 was consistently high, so the patient should be closely followed.

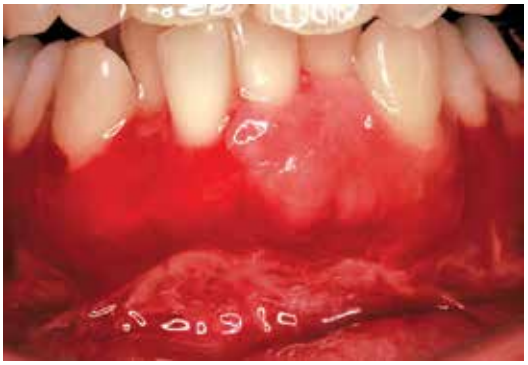

Fig. 11. Initial presentation associated with PV in a 46-year-old woman. The initial examination revealed severe desquamation and erosion on the gingiva. Lesions were also found on the tongue, soft palate, floor of mouth and skin. The patient complained of pain on swallowing.

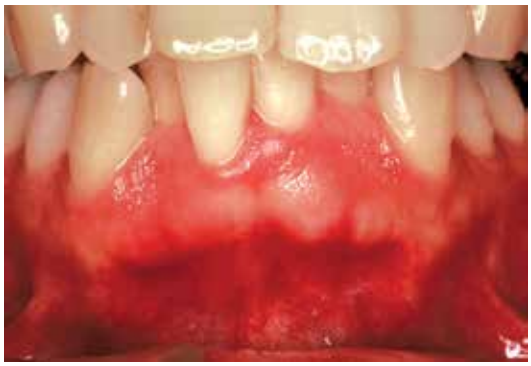

Fig. 12. Treatment response associated with PV in a 46-year-old woman. DG was successfully managed with systemic treatment by a dermatologist.

Treatment of DG requires elimination or control of local irritants. Rough restorations, ill-fitting dentures, traumatic oral hygiene procedures, and dysfunctional oral habits should be corrected (Endo et al., 2006b; Fatahzadeh et al., 2006). Prosthetic treatment for patients having DG should be limited to a fixed prosthesis, since wearing a tissue-borne prosthesis may be uncomfortable (Erpenstein, 1985; Fatahzadeh et al., 2006). In some cases, DG can be successfully managed with topical corticosteroids combined with effective plaque control (Endo et al., 2005; Endo et al., 2008b; Endo et al., 2008c; Guiglia et al., 2007) (Figs. 13 and 14). The symptoms of the gingiva were improved by meticulous oral hygiene habits in some DG patients with oral LP (Erpenstein, 1985; Guiglia et al., 2007; Holmstrup et al., 1990) (Figs. 15 and 16). Plaque accumulation may be a stimulus factor to make DG worse, but the plaque itself does not cause DG. Therefore, it should be noted that the underlying causes of DG cannot be eliminated by plaque control alone.

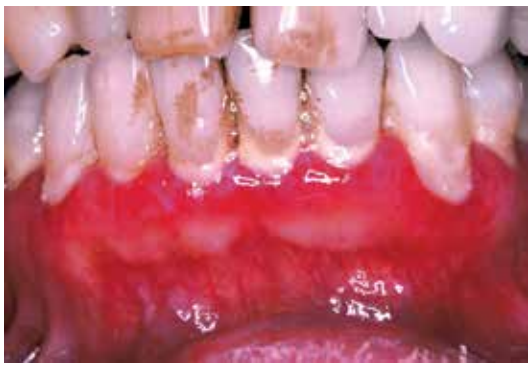

Fig. 13. Initial presentation associated with MMP in a 51-year-old woman. The initial examination revealed mild erythema and swelling of the gingiva with plaque and calculus deposits. The gingiva bled easily, and it was hard for her to brush her teeth.

Fig. 14. Treatment response associated with MMP in a 51-year-old woman. The symptoms of the gingiva improved due to a topical corticosteroid combined with effective plaque control.

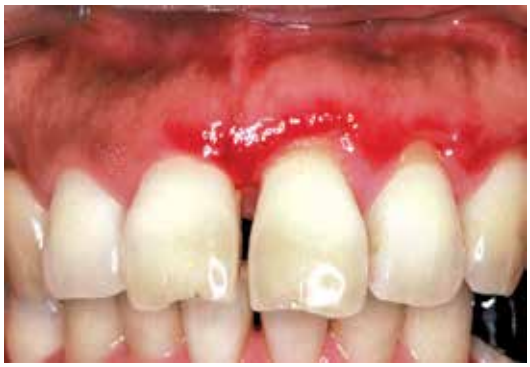

Fig. 15. Initial presentation associated with LP in a 55-year-old woman. The initial examination revealed moderate erythematous attached gingiva.

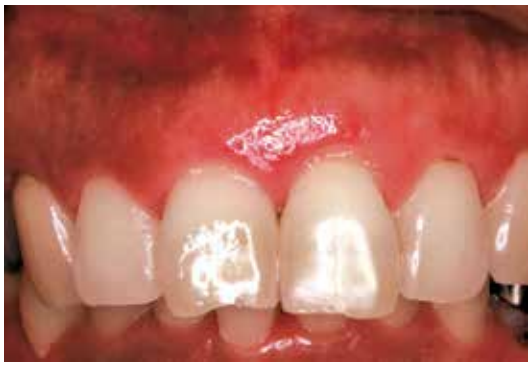

Fig. 16. Treatment response associated with LP in a 55-year-old woman. The condition of the gingiva improved with effective plaque control alone. The improved gingival condition was maintained for a long period.

Periodontal and dental problems are often observed in DG patients. However, little information is available regarding the periodontal and dental management of DG patients

(Damoulis & Gagari, 2000; Lilly et al., 1995; Rees, 1995). There are several case reports on periodontal surgery or implant therapy performed on patients with DG (Endo et al., 2005; Esposito et al., 2003; Fatahzadeh et al., 2006; Lorenzana et al., 2001; Penarrocha-Diago et al., 2000). Lorenzana et al. (2001) described the successful treatment of multiple gingival recessions in a patient with cicatricial pemphigoid. Following the elimination of the pemphigoid-associated lesions, the gingival recessions were treated using connective tissue grafts. During the surgical and healing phases of treatment, the patient continued with applications of a topical corticosteroid. After a follow-up period of 18 months, nearly 100% root coverage was evident with favorable esthetics (Lorenzana et al., 2001). Endo et al. (2005) described a PV patient with a favorable outcome after the effective treatment of oral lesions. A buccal frenulectomy was performed to improve the abnormal attachment position of the frenulum. There were no adverse effects on the wound healing, since a low potency corticosteroid was used for the treatment, and the treatment period was short. The patient maintained lesion-free oral mucosa for the following six months (Endo et al., 2005). Desquamation of tissue and a lack of tissue elasticity caused by active mucosal disease can disturb the manipulation of the mucosal flap (Brain et al., 1999). Increasing medication dosage and/or frequency and strict mucosal disease control prior to surgical intervention may facilitate tissue manipulation and reduce the surgical complications (Toscano et al., 2010). However, the long-term use of topical corticosteroids may adversely affect normal wound healing, which is a factor complicating the surgical management of these patients.

Implant therapy is likely to enhance the quality of life in patients with systemic diseases and may help them maintain long-term masticatory function (Nakadai et al., 2010). Published case reports indicated that DG patients associated with PV (Fatahzadeh et al., 2006), LP (Esposito et al., 2003) and epidermolysis bullosa (Penarrocha-Diago et al., 2000) can be successfully treated with osseointegrated implants. These reports indicate that the degree of disease control may be more important than the nature of the disease itself in regard to the effects on osseointegration.

It is difficult for patients with DG to brush their teeth due to pain and bleeding. Therefore, their oral hygiene is likely to be ineffective, making it difficult to treat this condition (Arduino et al., 2011; Pradeep et al., 2010; Ramon-Fluixa et al., 1999). Lack of correct oral hygiene and the accumulation of plaque may increase the long-term risk for plaque-induced periodontal diseases. However, there is some controversy about the relationship between the existence of DG and periodontal status (Akman et al., 2008; Arduino et al., 2011; Lo Russo et al., 2010; Ramon-Fluixa et al., 1999; Schellinck et al., 2009; Tricamo et al., 2006). Akman et al. (2008) evaluated the periodontal status of PV patients and compared it with that of healthy controls. The results showed that the periodontal condition is worse in PV patients. They concluded that PV patients should be encouraged to continue long-term periodontal follow-ups (Akman et al., 2008). Arduino et al. (2011) showed that patients diagnosed with MMP have higher levels of gingival and periodontal inflammation than healthy controls due to a substantial difference in the oral hygiene of the study groups. A pair of recent studies (Schellinck et al., 2009; Tricamo et al., 2006) showed that patients with MMP appeared to be no more at risk than their matched healthy controls for developing an increased progression of periodontal disease. In the first of these studies (Tricamo et al., 2006) the periodontal health of patients with MMP was compared to that of age and sex matched control patients without MMP or other forms of DG. MMP patients were found to have significantly more Class I furcation defects and more gingivitis but were otherwise

similar to controls. In a 5 year follow-up of the same MMP patients and the same controls (Schellinck et al., 2009), both groups experienced some progression of periodontal disease but there were no significant differences between groups. Ramon-Fluixa et al. (1999) reported that the periodontal status in patients with oral LP was no worse than that observed among healthy controls even if patients with atrophic-erosive gingival lesions exhibited higher plaque and calculus deposits. Furthermore, Lo Russo et al. (2010) showed that in sites where DG lesions are present, the probing depth and clinical attachment loss, full-mouth plaque score and full-mouth bleeding score are not significantly different from sites where DG lesions are absent.

7. Conclusions

The clinical and diagnostic features of DG were reviewed in this chapter. Although a definitive diagnosis is required to provide proper treatment, it is almost impossible to differentiate between the diseases and disorders reported to cause DG based solely on the clinical presentation. Both histopathological examination and DIF testing are often essential to establish a definitive diagnosis. If biopsy testing is inconclusive, other etiologic factors such as hypersensitivity reactions to oral hygiene products should be suspected. The management of DG can be challenging for oral health practitioners. Since it is possible for the lesions to recur after DG goes into remission, patients should be observed for a long period of time. Periodic follow-ups should be performed and treatment started immediately when gingival lesions recur.

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12

Genetic Disorders Associated with Gingival Enlargement

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1. Introduction

A number of genetic disorders present with gingival manifestations which may be in the form of desquamative, ulcerative lesions or an enlargement of the gingiva. Gingival enlargement is a broad term that refers to gingival overgrowth without cause suggestion i.e. a strictly clinical description of the condition avoiding the flawed pathologic implications of terms used such as hypertrophic gingivitis or gingival hyperplasia. In this chapter we will summarize gingival enlargement that can be attributed to gene pathology.

Gingival enlargement may present in some genetic disorders secondary to certain treatments not to actual gene expression e.g. Cystinosis secondary to treatment with cyclosporine-A, or epilepsy treated with phenytoin. This category of genetic disorders will not be discussed in this chapter, but should be considered in the differential diagnosis.

Genetic disorders associated with gingival enlargement fall into four main categories according to etiology, clinical presentation and histopathological findings (Table 1). This classification is suggested as a guiding tool in differential diagnosis. The first category is *Hereditary Gingival Fibromatosis* (HGF), which represents a heterogeneous group of disorders characterized by progressive enlargement of the gingiva. HGF may appear as an isolated entity i.e. as autosomal dominant Gingival Fibromatosis or as part of a syndrome. These syndromes are rather rare but they all have gingival fibromatosis as a constant feature. The second category is *Lysosomal Storage Disorders* which are a group of disorders characterized by deposition of macromolecules anywhere in the body including the gingiva leading to gingival enlargement. Gingival enlargement in this category is not always a constant feature. It ranges from being common to being a rare feature. The third category is referred to as *Vascular Disorders* while the last category includes syndromes characterized by the presence of *characteristic dental abnormalities*.

2. Hereditary gingival fibromatosis

Gingival enlargement may present as a specific entity, hereditary gingival fibromatosis (HGF), and may appear in an isolated form. However, there are several uncommon syndromes in which gingival fibromatosis can be a feature.

Hereditary Gingival Fibromatosis	Lysosomal Storage Disorders	Vascular Disorders	Disorders Associated with Dental Abnormalities
Gingival fibromatosis (isolated)	Hurler syndrome	Sturge Weber syndrome	Wilson syndrome
Zimmerman – Laband syndrome	Maroteaux-Lamy syndrome	Klippel-Trenaunay syndrome	Goltz syndrome
Ramon syndrome	Scheie syndrome		Regional Odontodysplasia
Systemic hyalinosis	Hurler/ scheie		
Jones syndrome	Hunter syndrome		
Rutherfurd syndrome	Sly syndrome		
Cross Syndrome	I- Cell disease		
Gingival fibromatosis, hypertrichosis and mental retardation.	Aspartylglucosaminuria		
Neurofibromatosis type I	Alpha Mannosidosis		
Schinzel – Giedion syndrome	Niemann – Pick disease		
Costello syndrome	Anderson – Fabry disease		
	Menkes Kinky hair disease		
	Ligneous periodontitis		
	Cowden syndrome		

Table 1. Classification of genetic disorders associated with gingival enlargement**.**

Clinically HGF develops as a slowly progressive, benign, localized or generalized enlargement of keratinized gingiva that, in severe cases, may cover the crowns of the teeth.Localized forms of HGF usually affect the maxillary tuberosities and the labial gingiva around the mandibular molars. However, the symmetric generalized form of HGF that affects the labial, lingual, and palatal gingiva is the most common (Baptista, 2002; Kelekis-Cholakis et al., 2002). Males and females are equally affected. (Xiao et al., 2001; Ye et al., 2005). Enlarged gingiva may be normal in color or erythematous and are firm and nodular on palpation. Although the alveolar bone is usually unaffected, gingival enlargement results in pseudo-pocketing and periodontal problems, due to difficulties in maintaining an effective level of oral hygiene. The overgrowth may also result in functional and esthetic concerns, create diastemas, impede or delay tooth eruption, and create changes in facial appearance as a result of lip protrusion. Severe overgrowth can result in crowding of the tongue, speech impediment, and difficulty with mastication, and can prevent normal closure of lips (Lynch et al., 1994; Shafer, 1983).The onset of gingival overgrowth usually coincides with the eruption of the permanent incisors, or, at times, with the eruption of the primary dentition. In very rare cases; it can be also present at birth (Anderson et al., 1969).

Since HGF has not been reported in edentulous patients, it appears that the presence of teeth is necessary for overgrowth to develop.

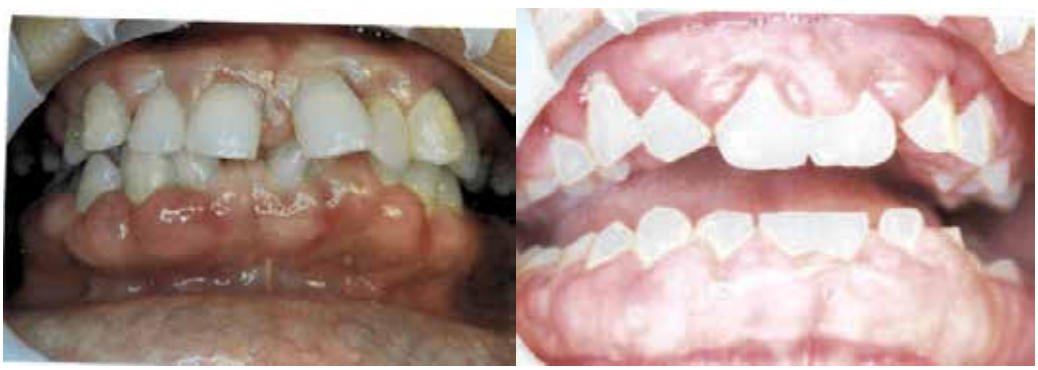
Histologically: HGF usually involves moderate hyperplasia of a dense, hyperkeratotic epithelium with elongated rete ridges (Araujo et al., 2003; Doufexi et al., 2005). Epithelial hyperplasia can also occur as a consequence of acanthosis, but this was found only in areas of chronic inflammation (Farrer-Brown et al., 1972; Raeste et al., 1978). HGF tissues show an increased amount of collagen fiber bundles running in all directions associated with few fibroblasts and blood vessels (Araujo et al., 2003; Doufexi et al., 2005; Martelli-Junior et al., 2000). Two populations of fibroblasts were identified in the lesions. One contains little cytoplasm around the nucleus, which is associated with dense collagen bundles. The other contains prominent cytoplasm with well developed organelles. Those fibroblasts have been considered inactive and active, respectively (Collan et al., 1982; Sakamoto et al., 2002). The connective tissue in HGF also exhibits an accumulation of elastic and oxytalan fibers (Baptista, 2002; Chavrier & Couble, 1979; Doufexi et al., 2005; Hart et al., 2000; Sakamoto et al., 2002). Although a rare finding, small osseous calcifications and abundant neurovascular bundles may also be present (Gunhan et al., 1995; Kelekis-Cholakis et al., 2002). HGF does not usually involve inflammation and local accumulation of inflammatory cells can be found only in cases where pseudo-pocketing resulted in plaque accumulation (Shafer, 1983).

Extracellular matrix production and degradation:

The hallmark of HGF is the accumulation of excess extracelluar matrix (ECM). Transforming growth factor (TGF) expression is up regulated in HGF (Häkkinen & Csiszar, 2007) . TGF can promote ECM accumulation by increasing ECM synthesis and can also inhibit ECM breakdown by down regulating matrix metalloproteinases (MMPs) expression and by increasing expression of tissue inhibitors of matrix metalloproteinases (Steffensen et al., 2001).

2.1 Isolated hereditary gingival fibromatosis

Isolated hereditary gingival fibromatosis (OMIM #135300; Gene Map locus 2p21; other loci reported on chromosomes 5q & 11p) is mainly autosomal dominant (Fig.1), though autosomal recessive inheritance has been reported. The enlargement affects both deciduous and permanent dentition. The gingiva appears firm, non hemorrhagic and large enough to interfere with speech and, in some instances, with mouth closure (Ramakrishnan et al., 2010).



(a)Affected mother (b)Affected son

Fig. 1. Isolated autosomal dominant hereditary gingival fibromatosis.

2.2 Zimmerman – Laband syndrome

Zimmerman – Laband syndrome or Laband syndrome (OMIM #135500; Gene Map locus 3p14.3) is an autosomal dominant disorder. Apart from gingival enlargement, it is characterized by abnormal fingers, nails, nose, and ears. Other findings include splenomegaly, hepatomegaly, and hyperextensible metacarpophalangelal joints (Hoogendijk et al., 2006).

2.3 Ramon Syndrome

Ramon Syndrome (OMIM #266270) is characterized by cherubism, seizures, mental deficiency, hypertrichosis, stunted growth and juvenile rheumatoid arthritis (Suhanya et al., 2010).

2.4 Systemic Hyalinosis

Systemic Hyalinosis is an autosomal recessive systemic disorder due to mutation in CMG2, or ANTXR2 gene. It is characterized by widespread deposition of hyaline material in all body tissues. Some tend to classify this entity into infantile systemic hyalynosis (OMIM #236490, Gene Map locus 4q21) and juvenile hyaline fibromatosis (Murray-Puretic-Drescher syndrome OMIM #228600) according to age of onset & disease severity. Individuals usually present with painful joint contractures, diffuse thickening of the skin with pearly papules and fleshy nodules and failure to thrive. Gingival enlargement is a constant feature and other oral structures may also be enlarged. Histopathologic features are the deposition of amorphous, eosinophilic hyaline material (fig.2) (El-Kamah & Mostafa, 2009 ; El-Kamah et al., 2010).

2.5 Jones syndrome

Jones syndrome (OMIM #135550) is autosomal dominant in inheritance. It is mainly characterized by gingival fibromatosis with progressive sensorineural deafness (Kasaboğlu et al., 2004).

2.6 Rutherfurd syndrome

Rutherford syndrome (OMIM #180900) is usually autosomal dominant in inheritance. Its key features are corneal opacity, mental retardation and aggressive behavior. Gingival fibromatosis in this syndrome may be associated with failure of tooth eruption (Raja et al., 2008).

2.7 Cross syndrome

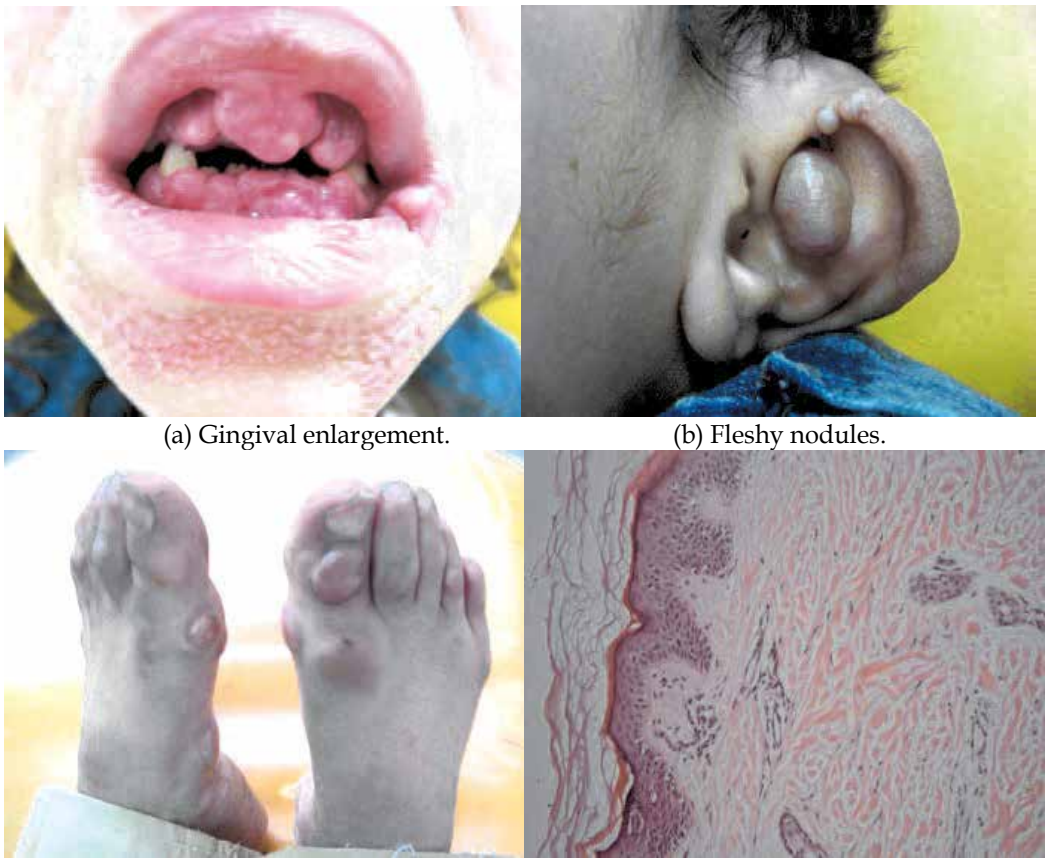
Cross- McKusick- Breen syndrome or Kramer's syndrome (OMIM #257800) is characterized by hypopigmentation, mental retardation and writhing movement of hands and legs (Witkop, 1971).

2.8 Gingival fibromatosis, hypertrichosis and mental retardation

Gingival fibromatosis, hypertrichosis and mental retardation **(**OMIM #605400) is autosomal recessive in inheritance. It is characterized by epilepsy, finger abnormalities, hirsutism, bulbous short nose and abnormal ears (Gohlich-Ratmann et al., 2000).

2.9 Neurofibromatosis type I

Neurofibromatosis or Von Recklinghausen disease (OMIM #162200, Gene Map locus 17q11.2) is an autosomal dominant neurocutaneous disorder caused by mutation in NF1



(a) Gingival enlargement. (b) Fleshy nodules.

(c) Multiple nodules on joints. (d) 200X Hyalinosis in dermal papillae.

Fig. 2. Systemic Hyalinosis.

gene. It is characterized by cafe-au-lait spots, Lisch nodules in the eye, fibromatous tumors of the skin with an increased risk of developing benign and malignant tumors. Orally, there is gingival neurofibroma. Characteristic histopathologic features: neurofibroma cells can be detected with their nuclei among the waved collagen fibers -beneath the oral mucosa. Epithelium and numerous tumor cells and capillary vessels can be seen among waved collagen fibers (El-Kamah et al., 2004).

2.10 Schinzel-Giedion syndrome

Schinzel-Giedion syndrome or Schinzel-Giedion mid-face retraction syndrome (OMIM #269150, Gene Map locus 18q21.1) is an autosomal recessive malformation syndrome characterized by severe mid-face retraction referred to as 'figure-of-eight' appearance, severe mental retardation and congenital heart defect. Neither the etiology nor detailed clinical course is known since most of the patients affected with Schinzel-Giedion syndrome die before the age of ten. However, a long-lived patient showed gingival hyperplasia that was progressive even after gingivectomy. Histopathologic examination revealed fibrous hyperplasia of the gingiva with mucoid depositions and no inflammatory changes (Kondoh et al., 2001).

2.11 Costello syndrome

Costello syndrome or Noonan like syndrome with nasal papillomata (OMIM #218040) is a rare disease characterized by fetal and neonatal macrostomia with slow postnatal growth due to the severe feeding difficulties, distinctive coarse facial dysmorphism and mental retardation. The most striking cutaneous feature is redundant skin of the neck, hands and feet. Nasal and perioral papillomas are also common between the ages of 2 and 15. Oral examination is important as Costello syndrome patients develop gingival hyperplasia usually within the first years of life and is considered as a quite distinct feature that can also aid in its differential diagnosis from Noonan syndrome and Cardiofaciocutaneous syndrome that phenotypically overlap with Costello syndrome (Digilio et al., 2008).

3. Lysosomal storage disorders

Lysosomal storage diseases are a heterogeneous group of disorders caused by lysosomal enzyme dysfunction including mucopolysaccharidosis, mucolipidosis and others. Individually they are very rare, but this group as a whole has a prevalence of more than 1:8,000 live births (Manger, 2010). The majority of lysosomal storage disorders (LSDs) result from defective lysosomal acid hydrolysis of endogenous macromolecules and their consequent accumulation. Over 40 disorders have been described. They tend to be multisystemic and are always progressive, although the rate of progression may vary. There are several potential ways in which accumulated substrate might cause the disease. The most obvious is enlargement of the affected cell, resulting in enlargement of the respective organ such as hepatosplenomegaly, cardiomyopathy etc. (Vellodi, 2005). The buildup of undigested material, secondary to lysosomal enzyme dysfunction, results in the formation of typical histochemical and ultrastructural changes. Light microscopy often reveals engorged macrophages with a characteristic appearance, such as that of 'sea-blue histiocytes' in Niemann–Pick disease (Vanier et al., 1988).

3.1 Mucopolysaccharidosis

Mucopolysaccharidosis (MPS) are a family of lysosomal storage disorders resulting from the partial catabolism of several glycosaminoglycans (GAGs). Depending on which particular enzyme is deficient, the MPS syndromes are defined into groups MPS I through VII, with several subgroups for a total of 10 disorders. In humans, clinical features include dysmorphic features, hepatosplenomegaly, hypertelorism, macroglossia, hypoplastic and irregularly shaped teeth, hyperplastic lips and gingiva, facial dysmorphia, corneal clouding, and mental retardation. Gingival enlargement is considered as one of the main oral manifestations of Maroteaux-Lamy syndrome, and a common feature in Hurler syndrome. It is rarely reported with Scheie syndrome, Hurler/ scheie compound syndrome, Hunter's syndrome and Sly syndrome. Gingival enlargement may not be previously reported with Sanfilippo syndrome and Morquio syndrome (Sheridan et al., 1994).

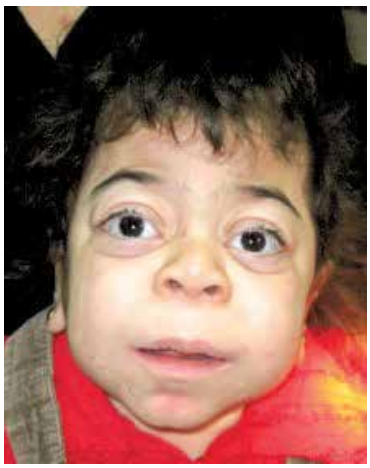
3.1.1 Hurler syndrome

Hurler syndrome (Mucopolysaccharidosis IH, OMIM #607014, Gene Map locus 4p16.3) is an autosomal recessive disorder caused by a mutation in the gene encoding for the enzyme alpha-L-iduronidase leading to deficiency of the enzyme and accumulation of glycosaminoglycans (heparan sulphate and dermatan sulphate) in various tissues (Hingston

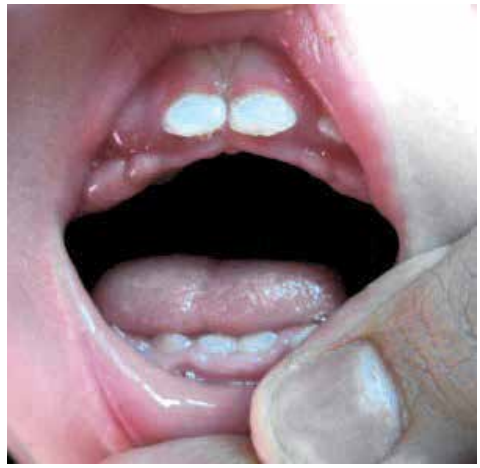
et al., 2006). Hurler sundrome is characterized by mental retardation, dwarfism, coarse facial features, flexion contractures, hepatosplenomegaly, hernias, corneal clouding (Leroy & Croeker, 1966; McKusick et al., 1965), respiratory infections and cardiac complications (Mckusick &Neufeld, 1983). Gingival hyperplasia is a common feature in this disorder. Other intraoral features include macroglossia, short mandibular rami with abnormal condyles consistent with limited opening of the mouth, spaced hypoplastic peg-shaped teeth with retarded eruption, and localized dentigerous cyst-like radiolucencies (Gardner, 1971; Keith & Weidmann, 1990; Thomas &Tandon, 2000; Worth, 1966). Histopathological reports showed Hurler cells in the gingival tissue (Gardner, 1968).

3.1.2 Maroteaux-lamy syndrome

Maroteaux-lamy syndrome or Mucopolysaccharidosis typeVI (OMIM #253200, Gene Map locus 5q13) is a lysosomal storage disorder inherited as an autosomal recessive trait. It is due to deficiency of arylsulphatase B enzyme which results in accumulation of dermatan sulphate in tissues and its excretion in urine. It is characterized by growth retardation, enlargement of the skull with a long anteroposterior dimension and corneal opacities. Presence of normal intelligence as well as metachromatic inclusions in leukocytes distinguishes it from other mucopolysaccharidosis (Fig 3,a). The oral findings include short or stubby, malformed, peg-shaped, poorly formed and calcified teeth with delayed eruption. Gingival hyperplasia and hypertrophy of the maxillary alveolar ridges are often mentioned as the main oral manifestations of the Maroteaux-lamy syndrome (Fig 3,b). Also, the anterior teeth may present an open-bite relationship in association with macroglossia. (Alpoz et al., 2006; Guimaraes et al.,2010).



(a) MPS VI (usually with normal intellectual development).



(b) Gingival hyperplasia and hypertrophy of the maxillary alveolar ridges.

Fig. 3. Maroteaux-lamy syndrome.

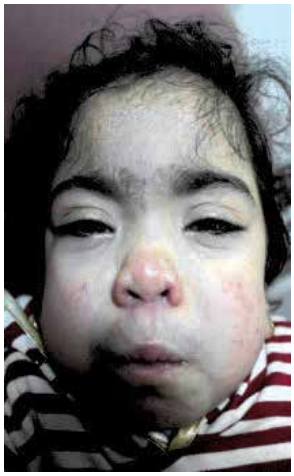
3.1.3 Scheie and Hurler /Sheie syndrome

Scheie syndrome (Mucoploysaccharidosis IS, OMIM #607016, Gene Map locus 4p16.3) represents the mildest form of mucoplysaccharidosis. An intermediate phenotype lying in between these two variants of mucopolysaccharidosis I is the Hurler /Sheie compound

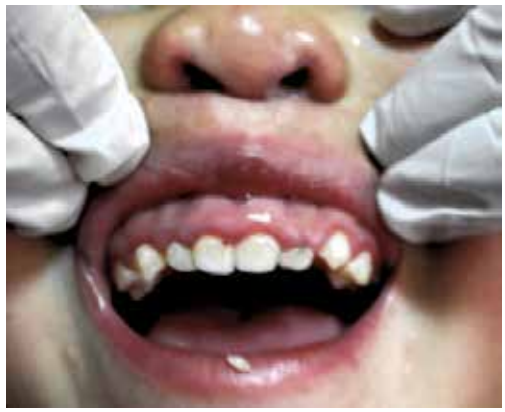
syndrome (Mucoploysaccharidosis I H/S, OMIM #607015, Gene Map locus 4p16.3) (Kelly, 1976).

3.1.4 Hunter syndrome

Hunter syndrome (Mucopolysaccharidosis II, OMIM #309900, Gene Map locus Xq28) is an X-linked recessive disorder causing a deficiency in the enzyme, iduronate-2-sulfatase (I2S) and accumulation of dermatan sulfate and heparan sulfate in various tissues and organs. It has similar but less severe manifestations than Hurler syndrome. It can be distinguished clinically from Hurler syndrome by mode of inheritance and absence of corneal clouding. Conductive and sensorineural deafness are frequent. Nodular or pebble like skin rash occur, especially over the scapulae (Kelly, 1976). Hunter syndrome presents the same oral manifestations as Hurler's (Fig 4) (Gardner, 1971).



(a) MPSII, coarse facial features (prominent forehead, flat nasal bridge).



(b) Mild gingival enlargement.

Fig. 4. Hunter syndrome.

3.1.5 Sly syndrome

Sly syndrome or Mucopolysaccharidosis type VII (OMIM #253220, Gene Map locus 7q21) is a lysosomal storage disorder, transmitted as an autosomal recessive trait and caused by beta-glucoronidase deficiency. It is characterized by mental retardation, short stature and macrocephaly. The oral features include mainly thickening of the alveolar ridges and rarely gingival hyperplasia (Bittencourt et al., 2000).

3.2 Mucolipidosis

3.2.1 I cell disease

I cell disease (Mucolipidosis II) (OMIM #252500, Gene Map locus 12q23.3) is an autosomal recessive disorder caused by a deficiency of the enzyme N-acetyleglucosamine-1-phosphotransferase which leads to the accumulation of mucopolysaccharides and mucolipids macromolecules. Gingival enlargement is one of the most striking features of

this syndrome and the patient's lower face has a fish-like profile. It is referred to as I cell disease based on the histopathologic features because the macromolecules that accumulate inside the cell form characteristic cytoplasmic inclusions (Mahfouz et al., 2010).

3.3 Miscellaneous lysosomal storage

3.3.1 Aspartylglucosaminuria

Aspartylglucosaminuria or AGU (OMIM #208400, Gene Map locus 4q33-4q35) is an autosomal recessive lysosomal storage disorder caused by deficiency of aspartylglucosaminidase leading to the accumulation of glycoasparagines in lysosomes. The main symptom is progressive mental retardation where the patients are only able to learn new skills and abilities up to the age of 16 years. They then undergo gradual somatic and mental deterioration. The facial features coarsen with age with characteristic sagging of the facial skin. Dysmorphic orofacial features include macroglossia, malocclusions, limited mouth opening as well as thick lips. Edematous buccal mucosa (leukoedema) and gingival fibromatosis are common in AGU patients. The gingival overgrowths were diagnosed histologically as fibroepithelial hyperplasia (Arvio et al., 1999).

3.3.2 Alpha Mannosidosis

Alpha Mannosidosis (OMIM #248500, Gene Map locus 19q13-19q12), is a rare lysosomal storage disorder, transmitted as an autosomal recessive trait, and is due to deficient activity of alpha mannosidase, resulting in an abnormal accumulation of mannose-containing residues. It is characterized by growth and mental retardation, coarse facial features and muscular hypotonia. The oral findings include macroglossia, widely spaced teeth and firm hyperplastic nodules of the gingiva which upon histologic examination reveals infiltration with foamy histiocytes. Blood smears show cytoplasmic vacuolization of lymphocytes and monocytes (Ischigami et al., 1995).

3.3.3 Niemann-Pick disease

Niemann-Pick disease (OMIM #257200, Gene Map locus 18q11-18q12 type C, 11p15), an autosomal recessive disorder caused by deficiency of a specific enzyme activity 'acid sphingomyelinase' with subsequent accumulation of sphingolipids in cells, throughout the body. Oral findings include thick lips, macroglossia and widely spaced teeth. Although gingival enlargement is not considered a constant feature, a case was presented with generalized grade III gingival enlargement, which recurred even after excision and thorough maintenance implying that there is a link between the disease and the gingival enlargement. Gingival biopsy upon histologic examination revealed infiltration with foamy histiocytes. Blood smear showed cytoplasmic vacuolization of lymphocytes and monocytes (Kaisare, 2007).

3.3.4 Anderson Fabry disease

Anderson Fabry disease or Angiokeratoma Corporis Diffusum (OMIM #301500, Gene Map locus Xq21-Xq22) is an X-linked recessively inherited disease due to deficiency of the enzyme ceramide trihexosidase, that results in intracellular accumulation of the glycolipid ceramide trihexoside in vascular endothelial cells, pericytes, fibroblasts, macrophages, and other cells of the body. The disease is characterized by painful crises involving the

extremities and the abdomen as well as angiokeratomas of the skin that may also involve the oral mucous membrane, mainly the labial mucosa followed by the buccal mucosa and the gingiva. Gingival enlargement may be present secondary to dilantin therapy. Young et al. (1978) presented a case with Fabry disease where granulomatous gingivitis has been described. Histologically, angiokeratoma of the gingiva shows ceramide inclusions not only in the connective tissue, but also in the oral epithelial cells.

3.3.5 Menkes Kinky hair disease

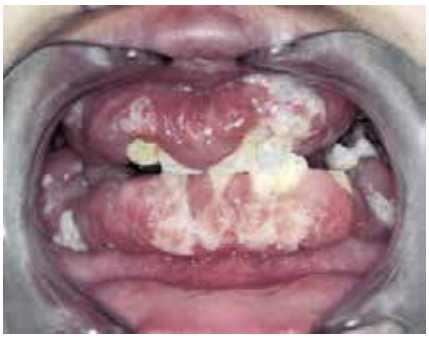
Menkes Kinky hair disease or Menkes Steely hair syndrome (OMIM #309400, Gene Map locus Xq13) is a rare X-linked recessive neurodegenerative disorder caused by a defect of copper transport and metabolism. It is characterized by brittle, sparse and twisted hair, and generalized depigmentation of the hair. The oral findings include delayed dentition and gingival hyperplasia (McKusick, 2011).

3.3.6 Ligneous periodontitis

Ligneous periodontitis, Plasminogen deficiency or Ligneous conjunctivitis (OMIM #217090, Gene Map locus 6q22) is an autosomal recessive disorder in PLG gene. Plasminogen deficiency is characterized by gingival swelling involving both the maxillary and mandibular arches, pinkish waxy painless masses that have no tendency to bleed with palpation and hyperplastic gingival papillae concealing most of the teeth. Areas of the gingiva covered with tough yellowish white membrane, thin pseudomembrane, that could be wiped away, overlay the tough part of the membrane (Fig.5,a). Other disease manifestations include; ligneous conjunctivitis (Fig.5,b), Corneal involvement that may lead to blindness in 30% of cases. Other system involvement such as laryngeal and tracheobronchial involvement resulting in voice change and obstructive pulmonary disease have been described. Characteristic histopathologic manifestations shown in (Fig.5,c) are epithelial hyperplasia and fibrin deposition underneath the epithelium and around the blood vessels. The dermis shows edema and perivascular mixed cellular infiltrate; mostly plasma cells, polymorphonuclear leukocytes, few lymphocytes, and mast cells. Amorphous hyaline-like eosinophilic material of the pseudomembranes, which resembles amyloid but negative for Congo red stain, that contains fibrin (El-Darouti et al., 2009).

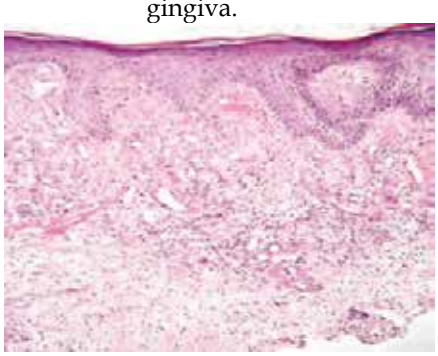
3.3.7 Cowden syndrome

Cowden syndrome or Multiple Hamartomas (OMIM #158350, Gene Map locus 10q23.31) is an autosomal dominant inherited disorder. In 80% of cases it is due to mutation in the PTEN tumor suppressor gene. Others may have mutations in certain subunits of succinate dehydrogenase, mitochondrial enzyme (Ni et al., 2008). Recently, methylation of the KILLIN gene has also been reported in patients with similar clinical features. Oral manifestations include cobblestone-like papules of the gingiva and buccal mucosa. However, the disease is characterized by learning disabilities, autism, and/or mental retardation, macrocephaly and multiple hamartomatous lesions, especially of the skin, mucous membranes, breast and thyroid. Verrucous skin lesions of the face and limbs, and multiple facial trichilemmomas are common findings. Hamartomatous polyps of the gastrointestinal tract, mucocutaneous lesions, and increased risk of developing neoplasms have been reported (Tan et al., 2011).

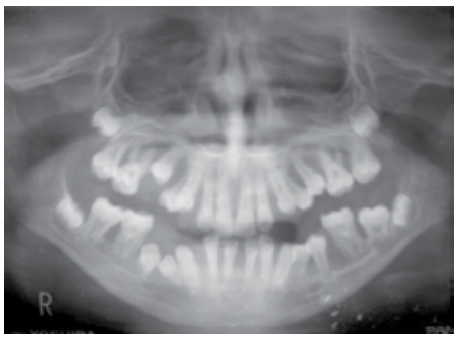


(a) Yellowish white pseudo-membrane covering most of the hypertrophic gingiva.

(b) Yellowish white pseudo-membrane affecting the tarsal conjunctiva.



(c) Fibrin deposition around dermal blood vessels associated with perivascular and interstitial mixed infiltrate.



(d) Panoramic radiograph showing floating teeth with severe alveolar bone loss.

Fig. 5. Clinical and histopathological characteristics in Ligneous periodontitis and Ligneous conjunctivitis.

4. Vascular disorders

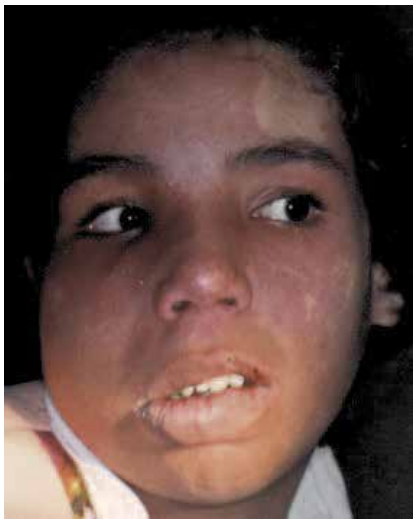
4.1 Sturge Weber syndrome

Sturge Weber syndrome or encephalofacial angiomatosis (OMIM #185300) is almost always a sporadic disease. However, there have been reports of cases with autosomal recessive and dominant inheritance. It has four main features; unilateral cutaneous nevi along trigeminal nerve sensory distribution (Fig.6,a), unilateral vascular hyperplasia of oral mucosa and gingiva, neurological manifestations and ocular complications (Pereira de Godoy et al., 2010; Zhou et al., 2010). Sturge-Weber syndrome is characterized by an intracranial vascular anomaly and calcification, leptomeningeal angiomatosis, most often involving the occipital and posterior parietal lobes (Fig.6b).

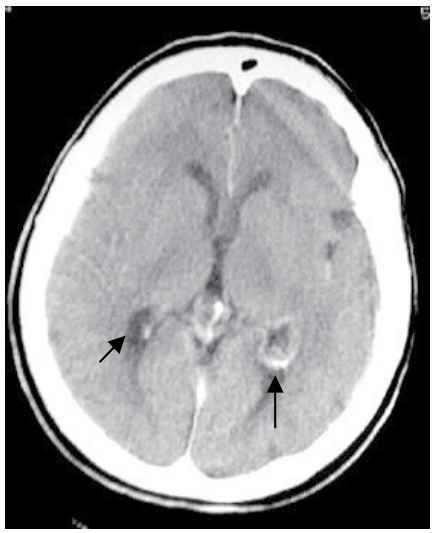
4.2 Klippel-Trenaunay syndrome

Klippel-Trenaunay syndrome or Angioosteohypertrophy syndrome (OMIM #%149000, Gene Map locus 8q22.3) has a paradominant inheritance (Happle, 1993), It is characterized by a triad of features, namely, vascular nevi, venous varicosities, and hyperplasia of hard and soft tissues in the affected area (Fig.7). Despite its rarity, Klippel-Trenaunay Syndrome

should be considered in the differential diagnosis of gingival enlargement. (Anand & Roshna, 2006). Gingival capillary hemangiomas, gingival fibroma, Gingival fibromatosis, gingival hyperplasia. Other oral manifestations include high arched palate, unilateral hypertrophy, or increase in size of periodontal tissues, tongue capillary hemangiomas, unilateral macroglossia, increase in size of fungi-form papillae, unilateral increase in lips size, teeth malformation, diastema formation, premature eruption of teeth on affected side, delayed exfoliation of primary teeth, early mineralization of roots on affected side, accelerated growth of teeth, anterior open bite, cross bite and floor of mouth capillary hemangiomas (Fakir et al., 2009; McKusick, 2011).



(a) Congenital large port wine stain involving the right side of the face and scalp extending to the left side.



(b) CT scan showing intracranial calcification.

Fig. 6. Sturge Weber syndrome.

Fig. 7. Unilateral limb oedema, hemihypertrophy , nevi & prominent nodules in Klippel-Trenaunay syndrome.

5. Disorders associated with characteristic dental abnormalities

5.1 Wilson syndrome

Wilson syndrome or Hepatolenticular degeneration (OMIM #277900, Gene Map locus 13q14.3-q21.1) is an autosomal recessive disorder due to mutation in ATP7B gene caused by low ceruloplasmin. It is characterized by multiple small red papules of the lips, gingival enlargement, early onset periodontitis, and repeated oral candidiasis. Enamel hypoplasia is the characteristic dental feature. The basal ganglia and liver undergo changes that express themselves in neurological manifestations and signs of cirrhosis (Huster et al., 2007). Histopathologic examination reveals granulomatous inflammation, thick irregular clumps of tortuous, red-staining abnormal elastic fibers. In a study, the lip papules may resemble elastosis perforans serpiginosa (Tovaru et al., 2010).

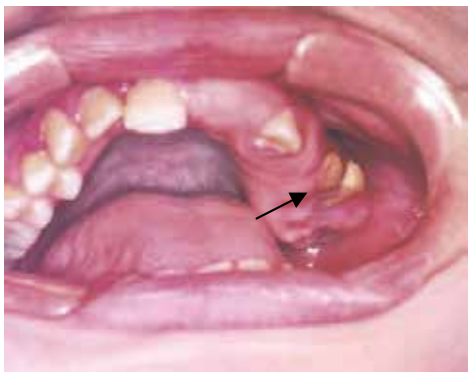
5.2 Goltz syndrome

Goltz syndrome, Focal Dermal hypoplasia or Goltz Gorlin syndrome (OMIM #305600, Gene Map locusXp11.23.) is an X-linked dominant mode of inheritance in 90% of the cases caused by PORCN gene mutation. It is characterized by atrophy and linear pigmentation of the skin, herniation of fat through the dermal defects, multiple papillomas of the mucous membranes or skin. Digital anomalies e.g. syndactyly, polydactyly, camptodactyly, and absence deformities. Partial anodontia is the characteristic dental feature. Other oral manifestations include lip papillomas, gingival enlargement and hypoplastic teeth.

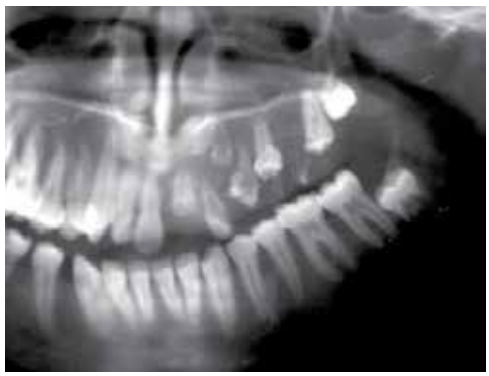
Characteristic histopathologic features showed deposits of fat cells or adipose tissue in the dermis (Maas et al., 2009; McKusick, 2011).

5.3 Odontodysplasia

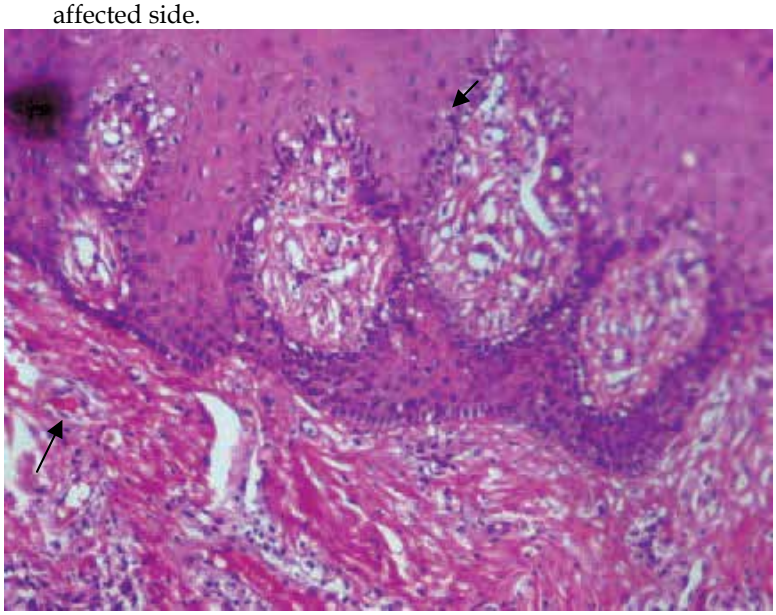
Odontodysplasia is an uncommon condition that can affect both primary and permanent dentitions. Both enamel and dentine are defected. Clinically, the teeth are mutilated in shape, pitted and yellowish to brownish in colour with excessive wear. Radiographically, enamel & dentine show lack of contrast, with decreased radiopacity rendering the tooth a ghost like appearance. The pulp chambers are wide and with open apices (Fig.8 a & b)



(a) Affected teeth are mutilated in shape, pitted and yellowish to brownish in color (arrow) with gingival enlargement in the affected side.



(b) Panoramic radiograph showing ghost like appearance of the affected teeth.



(c) Gingival biopsy showing odontogenic tissue in the epithelium and intramesenchymal calcifications.

Fig. 8. Regional maxillary odontodysplasia.

(Hamdan et al., 2004). It commonly presents as regional odontodysplasia where one or few teeth may be involved. One or more quadrants may be involved but generalized involvement is extremely rare (Shah and Gupta, 1998). Gingival enlargement is frequently reported with regional type. It may present as an isolated or associating epidermal nevus/ Schimmelpenning-Feuerstein-Mims syndrome (OMIM #%163200) (McKusick, 2011; Murakami et al., 1999). The exact etiology of odontodysplasia is still unknown. Genetic predisposition has been proposed but the presence of local irritating factors during tooth development has been more advocated. Gingival biopsy examination showed odontogenic tissue in the epithelium and intramesenchymal calcifications (Fig.8c).

6. Conclusion

Gingival enlargement is an important feature in many genetic disorders. It can be one of the main diagnostic features in some of these disorders e.g. ligneous periodontitis. In others gingival enlargement coupled with other clinical features direct the physician to further investigations. Accordingly, metabolic analysis, enzymatic essay, molecular analysis to detect the candidate genes and histopathological studies may be requested. Histopatological findings are considered of diagnostic value in a limited number of cases. They may become pathonemonic when coupled with clinical examination e.g. hyaline material in hyalinosis, fat deposits in Focal Dermal hypoplasia, odondogenic cells in odontodysplasia ……etc.

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13

Gene Polymorphisms in Gingivitis

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1. Introduction

Periodontal diseases (gingivitis and periodontitis) are inflammatory processes that involve the supporting tissues of the teeth. According to a currently used classification (Armitage, 1999), gingival diseases can be divided into four major groups: (1) dental plaque-induced gingival diseases, (2) gingival diseases modified by systemic factors, (c) gingival diseases modified by medication, and (4) gingival diseases modified by malnutrition. Plaque-induced gingivitis is the most common form of the periodontal diseases, affecting a significant proportion of the world population. The periodontal tissues react to the presence of bacteria with an inflammatory immune response. In the initial phase, components of the non-specific immune system play a main role while an efficient response of the tissues to the infection depends on a selective but not very specific recognition system. It is mainly based on the detection of chemical structures that are present on the surface of many different microorganisms, but do not occur on the surface of cells of the body. After recognition of these structures, the effector mechanisms of cells (e.g. macrophages, neutrophil granulocytes, etc.) are triggered and humoral systems (such as complement) are activated. The mechanisms of non-specific immunity neutralize and eliminate harmful products generated by pathogenic microorganisms and subsequently help regenerate the damaged tissues (Shapira et al., 2005). In the healthy periodontium, pathogenic bacteria are removed either mechanically (tongue movements, chewing of food, secretion of saliva, separation of epithelial cells) and also by production of gingival crevicular fluid. This fluid plays an important role in the defense mechanisms as it contains a number of proteins and cells whose composition depends on the inflammatory stage. A protection barrier between the plaque and the epithelium formed by neutrophil leukocytes in the sulcus area impedes bacterial penetration into the deeper tissue structures (Ohlrich et al., 2009). If bacteria penetrate subepithelially, due to a chemotactic action of the substances released by the bacteria and activated by cells of the affected tissue, the migration process of further cells

into the junctional epithelium and gingival sulcus increases. Mediators of leukocytes, together with the activated complement components, kinin system and products of arachidonic acid further amplify the inflammatory response leading to a widening of intercellular spaces between the cells of the junctional epithelium, which allows increased diffusion of bacterial products into the gingival tissues. In the early phase, lymphocytes begin to accumulate, at first T lymphocytes and activated macrophages predominate in the inflammatory infiltrate, in the late chronic (or established) phase a change to B lymphocytes and plasma cells is apparent (Seymour et al., 1988). Proliferation of the junctional epithelium spreads apically and laterally. If the process goes on untreated, the periodontal ligament and alveolar bone are destructed and a periodontal pocket forms. However, it must be noted that the pathological process does not always proceed at the same intensity; there are phases of acute exacerbation and chronic stagnation alternate.

In the 1960s experimental gingivitis studies (Loe et al., 1965) demonstrated that gingivitis is the response of the body to the build-up of dental plaque. These studies also showed a substantial variation in this response in different individuals; some people taking longer to manifest disease compared to others. Thus, while it has been known for many years that plaque is the etiological factor, the role of factors contributing to an individual's susceptibility is still not fully understood. Not all individuals with gingivitis will progress to periodontitis, and not all individuals with periodontitis will progress to tooth loss (Ohlrich et al., 2009). Therefore, periodontal diseases, including gingivitis, can be considered as a multifactorial condition influenced by the interaction between microbes and the host genome. Today, the role of external factors (e.g. microorganisms, smoking, stress etc.) is clearer than that of the genes. There are many scientific papers searching for the role of genes and their variants (polymorphisms) in the host response in aggressive and chronic periodontitis and their progression. In contrast, only a few studies examining the role of IL-1, IL-6, IL-10, TNF-α and MMP-9 genetic variability in gingivitis have been published to date. This review will analyze and summarize the literature on putative genetic risk factors predisposing to gingivitis. In addition, new data (still unpublished) on the role of interleukin-18 (IL-18) gene polymorphisms at positions -607C/A (rs1946518), -137G/C (rs187238) and -133C/G (rs360721) and their haplotypes in relation to gingivitis and microbial pathogens in Czech children are presented.

2. Genetic predisposition to gingivitis

For a detailed understanding of the interactions between bacteria and the inflammatory and immune responses of the individual, it is necessary to understand the pathogenesis of gingival disease. It is evident that an individual susceptibility to the presence of microbial pathogens plays an important role in the initiation and development of this disease.

At the end of the last century, the existence of a phenomenon called "*individual susceptibility*" was documented by many studies which proved familial aggregation of the disease and certain ethnic differences in the frequency of the occurrence of the individual forms of periodontal diseases. We assume that the genetic background of an individual plays a role in modulating susceptibility to a disease. Inheritance of susceptibility to periodontal disease varies enormously from nearly 100% in "Mendelian forms of periodontitis" to a minor rate of inheritance and a significant effect of external factors, as e.g. in plaque-induced gingivitis. Although the presence of some bacteria in plaque is a triggering factor, the organism response to this situation is significantly affected by the "genetic background" of the

individual (Hassell & Harris, 1995, Michalowicz, 1994, Takashiba & Naruishi, 2006, Yoshie et al., 2007, Laine et al., 2010). The phase of genetic analyses of the periodontal diseases started in the 1990s; it is usually referred to as the phase of looking for responsible – candidate – genes. Given the important role of inflammation and remodelling in periodontal tissues, the most frequently analyzed candidate genes are still the genes for selected receptors of the immune system, genes for proinflammatory cytokines, chemokines and growth factors, genes for matrix metalloproteinases and other enzymes.

2.1 Methodological aspects of complex disease studies

Research into the genetics of complex diseases represents one of the biggest challenges for scientists. From a methodological point of view, it is necessary to consider the etiopathogenetic character of complex diseases which differs from monogenic diseases in a number of aspects.

The term complex diseases (traditionally referred to as multifactorially conditioned) indicate diseases, the origin and development of which is affected by a "complex" of genetic and external factors. They do not exhibit classical Mendelian (dominant or recessive) inheritance as a result of a change in one locus (so-called diseases caused by one big reason). The genetic component of complex diseases also exhibits familial aggregation (i.e. non-random occurrence in families) but without an explicit type of transmission. Phenotype manifestation is a result of complex, often non-linear interactions, between the involved genes and also between the genes and external factors. Characteristic traits for complex diseases include: 1) incomplete penetration of a pathological phenotype [pathological phenotype in some persons who inherited "unfavorable" genotype does not manifest], 2) the existence of phenocopies [the same phenotype can be present also in persons who are not carriers of the given genotype], 3) genetic heterogeneity [allelic – there can be more "pathogenic" mutations or polymorphisms in the individual genes, loci – clinical manifestation can develop as a consequence of changes in the genes at various loci], 4) polygenic inheritance [predisposition to the disease increases only with the concurrence of a certain set of alleles, which if alone are not significantly pathogenic], 5) high population frequency of the alleles responsible for the development of the pathological phenotype, 6) co-operation of other mechanisms [e.g. imprinting, mitochondrial inheritance, etc.] (Lander & Schork, 1994).

Therefore, study of these diseases present numerous problems that must be solved – besides already mentioned an unclear inheritance model, there is also an unknown number of the genes involved and an ambiguous definition of the clinical phenotype. The concrete disorder which is relevant in the context of the given disease and "closer" to the genetic background than the resulting composed phenotype, is referred to as *intermediary phenotype*. There are principally two approaches for defining pathological phenotype (or disease) – the first approach is based on the *alternative concept of health and disease* (i.e. presence or absence of disease), the other approach corresponds to a so-called *gradation model*, disease and is perceived as a continuous function of a trait. In complex diseases it is rather a continuous transition of physiological values (phenotypes) to pathological ones. "Normality"of certain values and thus their implicitly presumed "unharmfulness" is reflected by so-called reference intervals used in clinical medicine. However, use of statistical methods allows researchers to study the contribution of the genetic variability to the observed phenotype variability while applying both the approaches – alternative concept and continual model.

Genetic variability, means that for the given gene more variants (i.e. alleles) with different frequency exist in population, counts for phenotype variability of traits. Based on population frequencies, these variants can be split into mutations and polymorphisms. The term *mutation* is usually used to indicate changes where frequency of a less frequent allele in population is lower than 1%. The term *polymorphism* conventionally indicates the existence of several (at least two) alleles; population frequency of the least frequent one is minimally 1%. The term *genetic architecture* of the given phenotype that characterizes how many genes and which of them participate in the genetic determination of a relevant disease, has not been determined for most of complex diseases. It is supposed that population frequency of a certain allele reflects its effect which can be either markedly pathogenic (in this case the relevant alleles are under selection pressure and are eliminated from the population – therefore they have a low population frequency) or in contrast low or neutral (alleles do not subject to selection and thus they are common in population). It has been assumed that these "neutral" variants may be the basis for the genetic determination of common complex civilization diseases (a so-called common disease/common variant hypothesis). According to this hypothesis, the frequency of certain genetic variants determining the susceptibility to a certain trait (frequency of which could have been originally relatively low) increased over the time due to selection neutrality or a certain advantage in the given environment which has disappeared in the present (for example high blood pressure, hypercholesterolemia, etc.). On the contrary, an alternative, less probable hypothesis (a so-called common disease/rare variant hypothesis) assumes that common complex diseases, manifestation of which markedly depends on the environment, are common due to universal exposition to the environment and not because of sharing the same genetic variants.

In complex diseases, the genetic background analysis is further complicated by the presence of interactions of the individual genetic variants (so-called "gene-gene interactions") and their complex impact on the clinical manifestation. It is currently believed that these interactions can be described by a so-called multiplicative model which considers the existence of two (and more) risk loci while only a certain combination increases a risk of the given disease development.

In addition, when studying complex diseases, external factors play an important role by significantly modifying an innate disposition of an individual to some disease and must be taken into consideration. Although there are no doubts about the role environmental factors play in the origin and development of complex diseases, finding the method for their exact definition and quantification still remains a challenge.

2.2 Types of genetic studies

If inheritance is confirmed to play a substantial role in the diseases with characteristics of a complex trait, the next step is mapping and identification of the genes involved. Therefore, genetics of complex diseases has to find answers for these principal questions: a) what genes participate in the development of the given disease, b) which variants play the most important role. A number of methods exist for localization and identification of the genes predisposing to complex diseases, each of them having some advantages and disadvantages. Methodologically there are two basic approaches: *linkage* or *association studies*; in which one or more candidate genes can be studied (a so-called "*candidate gene approach*") or it is possible to work with the whole genome (a so-called "*genome-wide approach*").

Both these approaches differ but principally; while the association approach compares the occurrence of a relevant genetic variant among phenotypically different persons (so-called "case-control" studies), the genome-wide approach investigates transmission of the genetic and phenotypic trait in affected families. Linkage studies can be further split into parametric and non-parametric ("allele sharing methods"), association studies are either retrospective, prospective or in a form of a "transmission disequilibrium test", TDT.

2.2.1 Linkage analysis

Standard linkage analysis (so-called parametric) is a method effective for mapping monogenic diseases, but is rarely usable for study of complex diseases. For the linkage analysis it is necessary to know in advance (or estimate) the inheritance type and then to calculate recombinant and non-recombinant offspring. Then it is possible to: a) find out whether any genetic locus exists which recombines with a disease with frequency θ lower than 50%, this is the frequency expected in the loci that are not in linkage and b) estimate the value θ, which gives the highest lod score (a so-called θmax). It means that this approach is based on the assumption of some inheritance type, which, when applied to the analysis of complex diseases (or traits), encounters a lot of problems.

Methods of linkage analysis (i.e. non-parametric do not consider the model, do not work with the number of loci, external effects and probability of incomplete penetration) were developed. These methods are based only on the assumption that two affected relatives share the alleles predisposing to the disease origin. One of the variants of this method, the *linkage analysis of affected sibpair*, monitors equally affected, so-called concordant sibpairs (eliminating thus the decision whether the affected individual is a non-penetrant carrier of the alleles predisposing to the disease or whether he/she did not inherit these alleles) and the siblings are tested whether they inherited a certain section of the chromosome (marker) more frequently than it can be expected under random segregation. "Maximum likelihood odds ratio" is used to detect whether the allele-sharing deviates significantly from 50% (corresponding to chance), similarly as in the linkage analysis based on models, lod score is used to determine whether the decrease in recombination frequency below 50% is significant. The method of linkage analysis of the affected sibpairs is not burdened by possible false assumptions about the number of the loci involved and how the alleles in these loci interact. However, it is less sensitive and less accurate (Nussbaum et al., 2004). Another interesting method is the *linkage analysis of highly discordant* (differently affected) *sibpairs*. Similarly, this method does not require any assumption about the number of participating loci or the inheritance type. Presumably, these siblings will not share the same alleles at the loci involved in the given trait. DNA analysis is conducted using the polymorphic markers distributed along the whole genome. A decrease in the level of allele-sharing detected in some marker indicates that the given marker is in linkage to the locus whose alleles participate in the studied physiological variable.

2.2.2 Association analysis

Association retrospective studies do not deal with the transmission of a complex trait in families, but compare allele frequency of a certain gene among the groups of affected and control (non-affected) unrelated persons. These studies analyze the alleles of genes whose products are pathophysiologically involved in the given disease (so-called candidate genes). If a certain allele of the given locus in the affected persons occurs in a higher frequency than

in controls, then the association with a disease is considered. The strength of association is expressed as "odds ratio" calculated from the frequency of the relevant allele in patients and controls (OR=ad/bc, where a = number of patients with the given allele, c = number of controls with the given allele, b = number of patients without the given allele and d = number of controls without the given allele). If the frequency of the studied allele in patients is in accordance with that in controls the odds ratio equals 1. Direct association studies deal with concrete substitutions in which a direct, causal effect on a phenotype is presumed. On the contrary, so-called indirect association studies are based on the assumption that the alleles in the vicinity of the causal allele segregate jointly (in a so-called haplotype block) and markers in the area are in complete linkage disequilibrium (LD) with causal substitution. It means that the vicinity of the variant is more important than the effect of the substitution itself.

These association methods are an important tool for seeking the genes involved in the origin of complex diseases and are also used for the analysis of genetic determination of gingivitis. Their advantage is that they can quite easily be conducted (we need only samples from groups of affected individuals and controls). There is no need to work out any laborious pedigree studies and collect samples from relatives. On the other hand, the results obtained must be carefully interpreted because an increase in likelihood ratio for some allele of a certain locus is not evidence that this allele or the whole locus are actually involved in the disease pathogenesis. Firstly, the studied allele can be in linkage disequilibrium with another allele, which really participates in the pathogenesis of the given disease (as explained above). Second, more serious limitation of the association studies is their sensitivity to the consequences of population stratification. If the population is split into several subpopulations (e.g. based on nationalities) and unions between members of different subpopulations are only rare, it can happen that the disease occurring more frequently in one of the subgroups will be falsely associated with some of the alleles that are more frequent in this subpopulation than in the population as a whole. The origin of these "false" associations can be minimized, but not fully eliminated, by a careful selection of the controls.

Prospective association study is based on the defined population sample (cohort), with the determined presence of the studied risk factor, the development of the relevant disease (or detected phenotype) is monitored over a given time horizon. Subsequently, frequencies of alleles (or genotypes, haplotypes, etc.) are compared among symptomless individuals and patients with disease. The advantage of this approach is that it is not necessary to make selection from the control group; the main disadvantage being time and financial costs.

Transmission disequilibrium test, TDT is based on the analysis of genotypes of the affected persons (probands) and their parents who are heterozygotes for the studied polymorphisms (at least one parent must fulfill this condition). The method tests whether the allele of a certain genetic marker from the heterozygous parent is transmitted to the affected offspring so frequently as it could be expected under random Mendelian segregation (when each parental allele is transmitted with the likelihood of 50 %). Transmission with a higher frequency indicates an association of the relevant allele with the given disease. The advantage of this method is the fact that it does not require the analysis of siblings (affected or non-affected by the disease) and that it is not influenced by population stratification.

2.2.3 Study of haplotypes

The term *haplotype* denotes a combination of alleles of the individual polymorphisms and mutations in the gene, or a longer chromosome segment, or a particular nucleotide sequence of one of the pairs of homologous chromosomes that is inherited as a whole. While studying the candidate genes in association studies, attention has been devoted to a haplotype analysis. It is due to the fact that protein production encoded by a relevant candidate gene is a result of summary effects of all variants in the given gene which can affect transcription (or splicing, translation, RNA stability, posttranslational modifications, etc.) and which are difficult to reveal by the analysis of the individual variants. In addition, it has been suggested that genetic variability in a population is unambiguously structured in haplotypes; the haplotype analysis thus reduces the numbers of the analyzed variants, which are not mutually independent, increasing thus statistical power of the test.

A variety of methods exist for haplotype detection but none of them is quite trivial. In linkage studies, haplotypes can be derived if maternal and paternal genotypes are known, suitable programs are for example: GENEHUNTER (Nyholt, 2002), Simwalk2 (Sobel & Lange, 1996) or Merlin (Abecasis et al., 2002). In other cases, haplotypes can be either derived statistically or established through molecular haplotyping. The method for the haplotype inference from genotype data is used most frequently, namely in large association studies. Available statistical programs are based on some of three algorithms: (a) maximum likelihood [SNPHAP, THESIAS (Tregouet et al., 2004), PLEM (Qin et al., 2002)], (b) parsimons [e.g. HAINFREX (Clark et al., 1998)] or (c) Bayesian [HAPLOTYPER (Niu et al., 2002), PHASE (Stephens et al., 2001, Stephens & Donnelly, 2003)]. Besides the algorithm itself, these programs differ in a number of other parameters and initial presumptions such as set size, requirements for maintenance of Hardy-Weinberg equilibrium, requirements for completeness of genotype data, etc. A number of surveys and comparisons of available software packages have been published (Niu, 2004, Salem et al., 2005).

2.2.4 "High-capacity" techniques

High-capacity "high-throughput" methods have been intensively developed for a rapid and simultaneous assay of a large number of markers in the scope of the whole genome or its part in vast sets. Other interesting approaches are, for example, "multiplexing" (for a parallel detection of a large number of markers – e.g. SNPs in one reaction) or "DNA pooling" (using mixed DNA samples collected from hundreds to thousands of individuals in which allele frequencies are determined). The high-capacity techniques often use the methods of DNA microarrays and techniques based on PCR and their modifications (Shi, 2002, Tsuchihashi & Dracopolli, 2002, Liu B et al., 2004, Brenan et al., 2009).

2.3 Interpretation of results from genetic studies

The aim of genetic research into complex, multifactorially conditioned diseases, such as gingivitis, is to find, by means of proof of positive linkage or association of a particular parameter with the disease, risk or protective variants increasing or on the contrary decreasing the susceptibility to the origin or development of the studied disease. However, the issue of establishing the causality of any relationship found is more complicated; none of the methods given above is able to confirm it directly.

Today association studies are preferred for the analysis of complex diseases (despite the disadvantage mentioned above with the problematic selection of the control group). This is

given by the character of these diseases (first of all polygenic type of inheritance, participation of the alleles with predominantly "smaller" effect, late clinical manifestation that restricts the availability of the analysis of affected relatives) and mainly by an important role of the external environment which can be studied with linkage studies only with difficulties.

Facts about etiopathogenesis of multifactorially conditioned diseases, noted above, suggest that genetic predisposition to gingivitis is affected by a number of loci that may interact with each other in a complex fashion. It means that the genetic variant if studied separately is not necessarily associated with the given phenotype or given disease. This may be the reason why a number of inconsistent results of SNPs analyses from different studies exist. There is a clear trend towards more complex–haplotype or whole-genome studies that can analyze simultaneously thousands to tens of thousands of markers for the given phenotype. This strategy evades one of the disadvantages of candidate-gene association studies as it does not work with an assumption of relevant gene-disease association. On the other hand, it is necessary to analyze a large number of gene variants in sets containing relatively few individuals, which leads to an increased probability of false positive results. The methods commonly used for multiple comparisons correction (e.g. Bonferroni correction, Holm's method, etc.) are more conservative in the situation where high LD between variables are present.

Although as mentioned above molecular-genetic methods which are able to analyze rapidly a huge number of markers are available, "ideal" statistical programs for the evaluation of the acquired data are still missing (eg. how to minimize the likelihood of false positive results at a high number of the analyzed variants in a small number of persons, evaluation of interaction effects between the particular genes and gene variants and environment). Of more recent methods, so-called multilocus methods can be mentioned (Hoh et al., 2001, Hoh & Ott, 2003). They have been used for a relatively short time and it is too early to evaluate them.

3. Strategy of the recovery of published data

A comprehensive literature search on the PubMed database up to January 2011 was conducted using the keyword gingivitis in combination with the words gene mutation or polymorphism. The studies selected for the review (a) were written in English, (b) had a case-control design including patients with gingivitis (G), and (c) reported genotype distribution.

In the present review, the most common variant of the polymorphic locus is nominated as a normal (N) variant (allele). The less frequent allele is designed as a rare (R) variant (allele). Table 1 gives the frequencies of the carriage rate of the R-alleles (frequency of N/R and RR genotypes) among patients and controls. In addition, we showed in this table whether or not the authors of the cited papers have reported statistically significant differences between cases and controls.

4. Candidate genes in relation to gingivitis

Considering the significant role of inflammation in gingivitis, of all the candidate genes, mainly the proinflammatory (IL-1, IL-6, TNF-α and LT-α) and antiinflammatory (IL-10) cytokine genes and genes for matrix metalloproteinases (MMP-9) have been analyzed (Table

1). This review presents all studies investigating association of genetic polymorphisms in relation to plaque-induced gingivitis in the "case-control study" design.

Ethnicity of subjects	Gene	SNP	Controls		Patients with gingivitis		Associated with gingivitis	Reference
			n	R-allele carriage	n	R-allele carriage		
Caucasian	IL-1β	+3953C/T	45	27%	20	45%	-	Galbraith et al., 1999
	TNF-α	-308G/A		24%		35%	-	
Caucasian	IL-10	-1082G/A	86	59%	174	75%	+[1]	Dashash et al., 2005
Caucasian	IL-10	-1082G/A	84	64%	164	77%	- (+[2])	Dashash et al., 2006
		-819C/T		39%		43%	- (+[2])	
		-592C/A		39%		43%	- (+[2])	
Caucasian	IL-1RN	86 repeat	48	35%	98	43%	+[3]	Dashash et al., 2007
Caucasian	IL-6	-174G/C	183	61%	272	68%	+[4]	Izakovicova Holla et al., 2008
		-572G/C		11%		12%	- (+[5])	
		-597A/G		64%		66%	- (+[5])	
Caucasian	IL-18	-607A/C	151	62%	147	64%	-	Vokurka et al., 2009
	MMP-9	-1562C/T		17%		29%	+[6]	

- = association not found, + = association found

+[1] An association with gingivitis was found for R-allele carriage

- (+[2]) Genotype GCC/GCC (-1082/-819/-592) was protective against gingivitis

-[3] R-allele (IL-1 RN*2) was protective against gingivitis, even after correction for plaque

+[4] An association with gingivitis was found for R-allele carriage and R/R genotype carriage even after correction for plaque levels

- (+[5]) Haplotype C(-174)/G(-572)/A(-597) was associated with gingivitis

+[6] An association with gingivitis was found for MMP-8 R-allele carriage (mainly in boys) and for combined genotype: carriage of MMP-9 C/T and IL-18 C/C genotypes

Table 1. Gene polymorphisms and carriage rate of the rare (R) – allele in case-control studies and association with susceptibility to gingivitis

4.1 Polymorphisms in the IL-1 gene cluster

Interleukin-1, one of the main proinflammatory cytokines, is an important mediator of the immune response. IL-1 production may be induced by a number of stimuli, including products of microorganisms or proinflammatory mediators produced by cells during the immune response. Pleiotropic effects of this interleukin include activation of the endothelial cells, which helps migration of neutrophils and monocytes/macrophages to an inflammatory site, proliferation of fibroblasts, activation of osteoclasts and release of enzymes participating in intercellular substance destruction, so-called matrix metalloproteinases. The role of IL-1 in periodontal disease pathogenesis has been confirmed both in animal models (Assuma et al., 1998) and in human medicine; the increased levels of this interleukin are found in tissues and sulcular fluid in patients with gingivitis or periodontitis (Ishihara et al., 1997, Rawlinson et al., 2000).

The genes encoding for IL-1α and IL-1β (proinflammatory) and IL-1/IL-1-receptor antagonist (IL-1RN, antiinflammatory) cytokine are assigned to chromosome 2q13-21. In all three genes several polymorphic loci have been described. Based on the number of published reports, the two single nucleotide polymorphisms (SNPs) connected with cytosine - thymine interchange (C/T), one within the promoter region of the gene for IL-1α (at position -889) and the other within the fifth exon of the IL-1β gene (at position +3953), appear to be the most studied genetic variant in periodontal diseases. The IL-1α -889 and IL-1β +3953 R-alleles have been shown to increase and the IL-1RN VNTR (a variable number of 86-bp tandem repeat in the second intron) R-alleles, to decrease gene transcription or the protein production levels (Shiroddria et al., 2000, Pociot et al., 1992, Andus et al., 1997) resulting in the R-allele carriers in a more pronounced IL-1 proinflammatory response (Laine et al., 2010).

Results of two case-control studies of IL-1 genes in patients with gingivitis are presented in Table 1. The SNP IL-1β +3954 (+3953) was firstly analyzed as a risk factor for gingivitis (Galbraith et al., 1999). Groups of twenty patients with gingivitis and 20 controls of unknown periodontal health status were compared and no differences in IL-1 +3954 allele or genotype frequencies were found. In contrast, a significant association of this variant with advanced adult periodontitis was confirmed (Galbraith et al., 1999). However, more recent studies have reported a significant association between IL-1RN gene polymorphism and gingivitis in 146 Caucasian children (Dashash et al., 2007). The IL-1 RN*2 allele (A2) was protective against the development of gingivitis. The same allele has been previously associated with an increased production of IL-1 RN (Hurme and Santtila, 1998) and a protective role against rheumatoid arthritis (Lee et al., 2004). Moreover, studies of IL-1 antagonists on periodontitis in animal models have shown that IL-1 Ra is able to inhibit the osteoclast-like cell formation mediated by A. actinomycetemcomitans Y4 capsular polysaccharide in mouse marrow cultures and also to inhibit the differentiation of osteoclasts induced by IL-1 (Nishihara et al., 1995).Taken altogether, the IL-1 gene cluster polymorphisms cannot be considered as general risk factors for gingivitis susceptibility. A very low number of case-control studies have been published so far. The decision whether and how the above given variants can modulate (increase or decrease) risk of gingivitis development will require other studies to be performed and be subject to a meta-analysis.

Another question is the effect of genetic variability on gingival inflammatory parameters. Lang and colleagues (2000) conducted a prospective longitudinal study that investigated the association between the IL-1 complex (IL-1α -889C/T and IL-1β+3954C/T) genotype and gingival inflammation assessed using the bleeding on probing (BOP). The results for 139 non-smoking subjects indicated that IL-1 positive genotype patients were found to have a significantly higher chance of presenting BOP and twice as likely to have increased BOP over a four-appointment recall (Shapira et al., 2005). Using the experimental gingivitis model, Jepsen et al. (2003) investigated the relationship of IL-1 genotype and the susceptibility to develop gingivitis in 10-positive and 10-negative volunteers with healthy gingiva. They did not find any association between parameters of gingival inflammation (such as BOP) and IL-1 genotype after 21 days of no plaque control. Also Goodson and colleagues (2000) found significant differences in BOP between similar groups of subjects (7 IL-1 positive vs. 13 IL-1 negative) in a 10-day experimental gingivitis trial. In contrast, Scapoli et al. (2005) did not find any significant association between IL-1α +4845 (-889), IL-1β (-3953) or combined genotype of both variants and the clinical parameters in the

experimental gingivitis trial in the overall population (N=96) of high and low responder. However, genotype distributions of IL-1RN and IL-1β -511 variants were statistically significantly different between both groups. Müller and Nusair (2007, 2010) described the effect of combined alleles 2 (R-alleles) of both IL-1α -889 and IL-1β +3954 on lower gingival bleeding tendency in plaque-induced gingivitis in a group of fifty subjects of 19-28 years of age. The present studies suggested a subtle influence of the IL-1 gene cluster polymorphisms on gingival inflammation. However, further well-designed larger studies are necessary to confirm these preliminary findings and the role of IL-1 variants in gingivitis susceptibility.

4.2 Polymorphisms in the IL-6 gene

Interleukin-6 is a pleiotropic cytokine produced by a variety of cells, such as gingival fibroblasts, endothelial cells, monocytes and T lymphocytes. It regulates differentiation and/or activation of macrophages and T cells, growth and differentiation of B cells and production of antibodies (Papanicolaou et al., 1998). IL-6 is known to stimulate protein production by hepatocytes during the acute phase of a systemic inflammation. IL-6 expression under the normal conditions is minute. However, it is significantly stimulated by proinflammatory products, such as endotoxin (LPS). Besides affecting inflammation, IL-6 activates osteoclasts thus disturbing the balance between bone formation and degradation (Ota et al., 1999). On the other hand, it can also induce production of the IL-1 antagonist receptor and soluble receptor for TNF-α and thus block effects of these proinflammatory mediators, which suggest possible IL-6 anti-inflammatory effects (Tilg et al., 1994).

The IL-6 gene is localized on chromosome 7 (7p21, Bawcock et al., 1988). The IL-6 5´-region contains numerous polymorphisms that directly influence the expression of the protein. The most frequently studied variants are three single-nucleotide variants (SNPs) at positions -174G/C, -572G/C and -597G/A together with AnTn polymorphism (Osiri et al., 1999, Terry et al., 2000). The first studies dealing with the "functionality"of the given variants underlined an important role of -174G/C polymorphism that involves a binding site for the transcription factor NF-IL-6; the presence of the C allele of this polymorphism leads to a lower basal and by lipopolysaccharide and IL-1 stimulated expression and reduction in plasma IL-6 levels compared to the common G allele (Ferrari et al., 2003). More recently, it has been shown that a number of other promoter polymorphisms, and mainly their mutual interactions, can significantly affect the expression of these genes (Müller-Steinhardt et al. 2007).

There is only one case-control study on the role of IL-6 polymorphisms in gingivitis (Table 1). Data on the relationship of 3 promoter polymorphisms in the IL-6 gene for the development of gingivitis was summarized in our previous study (Izakovicova Holla et al., 2008). The study included 455 children aged 11-13 years. Plaque-induced gingivitis was diagnosed in 272 of them and 183 children were healthy. Differences in allele frequencies or genotype distributions were not statistically significant for IL-6 -572G/C and -597G/A polymorphisms. However, frequency of IL-6 -174 R-allele was significantly higher in children with gingivitis and this allele was associated with gingivitis regardless of the amount of plaque. Boys who carried -174 C allele with the simultaneous presence of plaque were at the highest risk for the gingivitis development. Haplotype analysis proved a significant difference in haplotype frequencies between the healthy and diseased children, risks for the gingivitis development were nearly 1.5-times higher in children with CGA (-174C/-572G/-597A) haplotype and on the contrary reduced in children with GGG variant.

However, Scapoli et al. (2007) did not find any significant association between -174G/C and -597G/A variants and the clinical parameters in the experimental gingivitis trial in the overall population (N=96) of high and low responder. In addition, genotype distributions of both IL-6 polymorphisms did not differ between both groups statistically significantly.
We concluded that the IL-6 polymorphisms may be associated with plaque-induced gingivitis susceptibility, similarly as with CP susceptibility (Laine et al., 2010). However, other studies and meta-analysis are needed before the final conclusion is made.

4.3 Polymorphisms in the IL-10 gene

Interleukin-10 (IL-10) is an antiinflammatory cytokine synthesized by activated monocytes and T-lymphocytes in response to inflammation (Kobayashi et al., 2011). It inhibits production of several cytokines such as IL-1α, IL-1β, IL-6, TNF-α, and IL-10 itself. IL-10 production is partially genetically determined (Westendorp et al., 1997). The gene encoding for IL-10 is located on chromosome 1q31-q32, in a cluster with other interleukin genes, such as IL-19, IL-20 and IL-24. Several polymorphic sites in the IL-10 promoter region have been identified at positions -1082, -819, -627, -592, and -590. SNP at position -1082 lies within a putative Ets transcription factor binding site (Kube et al., 1995), variant at position -819 may affect an estrogen responsive element (Scarel-Caminaga et al., 2004) and polymorphism at -592 can have a negative regulatory function (Kube et al., 1995). The IL-10 -1082G/A, -819C/T and -592C/A variants showed strong linkage disequilibrium and form two common haplotypes. The R-allele of the -592 polymorphism has been associated with decreased synthesis of IL-10 *in vivo* and *in vitro* (Koss et al., 2000, Crawley et al., 1999). IL-10 plays a protective role in periodontal destruction due to inhibition of matrix metalloproteinases (MMPs) and receptor activator for nuclear factor-κB (RANK) system. Therefore, the individuals with decreased production of IL-10 can be less protected against bacterial pathogens.
In Table 1, results from two published studies investigating IL-10 gene polymorphisms in gingivitis susceptibility are presented. Dashash and colleagues in the first study (2005) analyzed the relationship between IL-10 -1082G/A variant and susceptibility to gingivitis among 260 Caucasian children (86 controls and 174 patients), aged 8 to 12 years from the UK. An increased risk of having gingivitis was found in allele A positive children, regardless of plaque or age. This allele was previously associated with low production of this cytokine (Turner et al., 1997). One year later, the same authors published haplotype analysis of three IL-10 gene variants at positions -1082, -819 and -592 in the same group of subjects and found that GCC haplotype was protective and ACC and ATA haplotypes were associated with gingivitis manifestation.
In conclusion, IL-10 alleles, genotypes and haplotypes have been associated with gingivitis susceptibility. However, both studies were published by the same authors using the same subjects and the results have not yet been replicated in another population. Therefore, the IL-10 gene may be one of the "candidate" genes for gingivitis but these positive associations must be confirmed in other ethnic groups.

4.4 Polymorphisms in the TNF genes

Tumor necrosis factor α (TNF-α) and tumor necrosis factor β (TNF-β, newly lymphotoxin α - LT-α) are key pleiotrophic proinflammatory cytokines. TNF-α is released primarily by monocytes and macrophages and triggers a cascade of other mediators of inflammation

(Beutler & Grau, 1993). LT-α which plays an important role in tumor cell destruction and viral infections is released mainly by T-lymphocytes.
Besides its local para- , juxta- and autocrine effects within the proinflammatory activities, TNF-α stimulates expression of some enzymes important in the remodelling of extracellular matrix; so-called matrix metalloproteinases - e.g. MMP-1, MMP-8 and MMP-13 (Panagakos & Kumar, 1995; Johansson et al., 1997) participating in degradation of periodontal ligaments (Birkedal-Hansen H, 1993). A great number of studies have found elevated levels of these cytokines in sulcular fluid and higher expression of TNF-α in the inflamed periodontal tissues in patients (Roberts et al., 1997). Besides the effects on the soft tissues of the periodontium, TNF-α is one of key cytokines that promotes bone resorption.
The genes for TNF-α and LT-α lie in the MHC class III region on the long arm of chromosome 6 (6p.21) between HLA-B and DR (Carroll et al., 1987). Many polymorphisms have been found in the TNF gene cluster, the G/A variant at position –308 in the promoter region of the TNF-α gene being the most profoundly studied. The less frequent allele of this polymorphism (-308A, R-allele) has been associated with increased production of TNF *in vitro* (Braun et al., 1996) and an increased risk of developing many diseases, including periodontitis (Galbraith et al., 1999). Only one study about the role of TNF-α polymorphism in gingivitis has been published so far (Table 1).
Galbraith and colleagues (1999) studied 20 subjects with plaque-associated gingivitis and 20 patients with adult periodontitis aged 35 and 65 years. Referent population consisted of 45 unrelated Caucasian subjects of unknown periodontal health status. For the TNF-α -308 variant, the frequencies of the R-allele were comparable between the reference subjects and patients with gingivitis and varied between 22.5-26.7%. The second, but not case-control study was afterwards published by Scapoli et al. (2007) on the group of 96 systemically and periodontally healthy individuals as a 21-day experimental gingivitis clinical trial. No relationship between the TNF-α -308 or LT-α +252 polymorphism and susceptibility to gingivitis was demonstrated.
To date there are very limited data analyzing possible effects of TNF gene variants in plaque-induced gingivitis; none, so far, has proven an association of the TNF gene(s) with this disease.

4.5 Polymorphisms in the MMP-9 gene

Enzymes denoted as matrix metalloproteinases (MMPs) are important mediators of tissue destruction in periodontal disease. Matrix metalloproteinases are secreted by various cells - polymorphonuclear leukocytes, macrophages, fibroblasts, epithelial and endothelial cells. (Birkedal-Hansen H, 1993). MMPs expression and activity are regulated by different mechanisms, including modulation of transcription, activation of latent pro-forms and inhibition by tissue inhibitors of metalloproteinases – TIMPs (Brew et al., 2000).
MMP-9 (also known as gelatinase B or 92-kDa type IV collagenase) is one of the MMPs active against denaturated collagen (gelatin) and collagen types IV, V and XI together with proteoglycans and elastin. The coding gene is located on chromosome 20q11.2-13.1 and several polymorphisms have been detected in the MMP-9 gene, some of them have been found to be "functional" (Zhang et al., 1999). The C to T substitution in position -1562 increases transcriptional activity of the MMP-9 in macrophages, as confirmed by *in vitro* studies (Zhang et al., 1999). In agreement with these findings from the *in vitro* experiments it was found that persons carrying the T allele have increased MMP-9 levels in plasma (Zhang

et al., 1999). Higher levels of the MMP-9 in patients with periodontitits in comparison to the healthy population were also observed (Ingman et al., 1996). However, in later studies no associations of MMP-9 functional polymorphisms with plasma MMP-9 levels in healthy were found (Demacq et al., 2008). No correlation between MMP-9 -1562C/T variant and salivary MMP-9 levels was found. However, significantly higher levels of MMP-9 were detected in patients with CP and correlated with clinical parameters of periodontal destruction (Isaza-Guzman et al., 2011).

Data on frequencies of polymorphism in the gene for MMP-9 (-1562C/T) together with polymorphism in the gene for IL-18 (–607A/C) in children with gingivitis have been published only in one study so far (Table 1). In the association case-control study in Czech adolescents aged 11-13 years, the authors demonstrated differences in frequencies of MMP-9 –1562C/T polymorphism between children with healthy gingiva and those with plaque-induced gingivitis. Boys with gingivitis carried the T allele (and genotypes with this allele) significantly more frequently compared to children with healthy gingiva (15.9% vs. 6.5%). Patients with combined genotype CT (of MMP-9 -1562C/T) and CC (of IL18 -607A/C) had 5.6 times higher risk of the gingivitis development compared to persons without this genotype (Vokurka et al., 2009).

Although the matrix metalloproteinase genes seem good candidates for their association with periodontal inflammation and destruction, investigations have not yet yielded sufficient data for conclusions about the role of these genes in plaque-induced gingivitis.

5. Interleukin-18 (IL-18) gene polymorphism in relation to gingivitis and microbial pathogens in Czech population

5.1 Introduction

Interleukin-18 (IL-18) belongs to possible factors that can play a role in the development of periodontal disease. It is a pro-inflammatory cytokine, a member of the IL-1 family that was originally described as INF-γ inducing factor that modulates both innate and adaptive immunity (Okamura et al., 1995). It is generally considered to be involved in the T-helper type 1 (Th1)-mediated immune response. IL-18 directly increases the production of TNF-α and subsequent release of IL-1β and IL-8 from monocytes. On the other hand, IL-18 might initiate Th2 responses with production of IgE via the stimulation of IL-4 and IL-13 synthesis in mast cells and basophils and eosinophil recruitment. The local production of IL-1β and IL-18 in the gingival tissue samples (Johnson & Serio, 2005) or gingival crevicular fluid (Orozco et al., 2006) increases with increasing inflammation and IL-18 was the predominant cytokine at both gingivitis and periodontitis sites. Among other functions, regulation of expression of MMPs and stimulation of MMP-9 active form can be mentioned (Delaleu et al., 2004).

The gene for human IL-18 is located at chromosome 11q22.2-22.3. It is composed of 6 exons (Kalina et al., 2000) and several polymorphisms have been identified in the *IL-18* gene (Zhang et al., 1999). Among them, -607A/C (rs1946518), -137C/G (rs187238) in promoter and -133C/G (rs4988359) in intron 1 have been studied in more detail with respect to transcriptional activity or IL-18 production by monocytes (Liang et al., 2008; Arimitsu et al., 2006). A change from C to A at position -607 disrupts a potential binding site for cAMP-responsive element binding protein, while allele C at -137C/G has been shown experimentally to disrupt a confirmed H4TF1-binding site (Giedraitis et al., 2001). The -

133C/G SNP in intron 1 is situated in an NF-1 binding site that is supposed to activate the transcription of a number of immune proteins, such as transforming growth factor (TGF)-β1, tumor necrosis factor (TNF) receptor 2, and IL-1β (Krohn et al., 1999).
There are only three studies of the IL-18 gene polymorphisms in chronic periodontitis (Folwaczny et al., 2005, Noack et al., 2008, 2009). Only one previous study on the potential association between IL-18 -607A/C variant and risk of gingivitis has been published so far by our group (Vokurka et al., 2009). With respect to the key role of interleukins in inflammatory response in gingivitis, the IL-18 gene is an obvious functional candidate for this disease. We analyzed the distributions of the IL-18 -607A/C, -137C/G and -133 C/G alleles, genotypes, and haplotypes in Czech children aged 11 to 13 years with plaque-induced gingivitis and with healthy gingiva.

5.2 Materials and methods

5.2.1 Subjects

A total of 572 Caucasian adolescents (311 boys and 261 girls) of exclusively Czech nationality, aged 11-13 years, selected from the ELSPAC (European Longitudinal Study of Pregnancy and Childhood) Brno group, which comprises over 5000 children and their families, was examined to assess gingival health in this case-control study. Children underwent a dental examination at the Clinic of Stomatology, St. Anne´s University Hospital and Faculty of Medicine, Masaryk University. Inclusion criteria consisted of a simple informed consent of the respective children and their families and of their willingness to participate. Thus, the randomness of the set is ensured; although a slight drift towards families with mothers with higher education can be expected. The study group comprised children with clinical evidence of plaque induced gingivitis (N=307, 197 boys and 110 girls). The healthy group (N=265, 114 boys and 151 girls) included children who had healthy gingiva with no clinical signs of inflammation (GI index = 0).
The clinical assessment was carried out by one investigator and the following parameters were assessed: DMFT (decayed/missing/filled teeth) score, gingival index (GI), plaque index (PI) and calculus index (CSI) as previously described (Izakovicova Holla et al., 2008). The radiograph examination was not performed as it was not part of routine care for these adolescents and would therefore have been deemed unethical. Phenotype status was assigned without knowledge of genotype by independent investigators.
The study was performed with the approval of the Committee for Ethics of the Medical Faculty, Masaryk University Brno and written informed consent was obtained from all parents (in case of children), in line with the Helsinki declaration before inclusion in the study.

5.2.2 Molecular assessment of periodontopathic bacteria

Microbial samples were taken from the subgroups of randomly selected subjects (N=207). Microbial samples were collected from gingival sulcus of each quadrant by inserting a sterile paper point into a base of the pocket for 20 seconds. Bacterial plaque samples from each individual were pooled in one tube. The detection of periodontal bacteria was performed using a commercially available microarray system (ParoCheck®; Greiner Bio-One GmbH, Frickenhausen, Germany), which allows the simultaneous detection of up to 20 different oral bacterial species based on species-specific highly conserved regions from the 16S rRNA gene. The ParoCheck® chip is a coated glass slide with a total of 86 DNA

measuring points, which can be evaluated by all commercially available microarray scanners. In this study, dental paper points soaked with gingival fluid were shaken out into deionized water and 1 µl aliquot was put in a 20- µl reaction mixture containing 0.2 µl Taq DNA polymerase and 18.8 µl Master Mix supplied with the Parocheck® kit (containing buffers, $MgCl_2$, dNTPs, DNase-free water and fluorophore-labelled primers). PCR was performed to amplify the target sequences and the cycling conditions used were as follows: 94°C for 1 min followed by 45 cycles at 95°C for 20 s, annealing at 60°C for 20 s, 72°C for 30 s and final elongation at 72°C for 1 min. Next, the labelled amplified products were hybridized to pathogen-specific oligomers according to the manufacturer's instructions. This step was first performed at 60°C in a steam-saturated atmosphere for 5 min. Next, 30 µl hybridization buffer was mixed with 5 µl PCR product at room temperature and incubated for 2 min at 95°C using a heating block. Then, 25 µl hybridization mix was transferred into each well of the chip and incubated for 10 min at 60°C. After washing and then drying using an air spray, the chip was read using a scanner (Axon 4100 A; Axon Instruments Inc., Union City, CA) and the software Paroreport 20. The bacterial counts were semi-quantitatively analyzed on a graduated scale ranging from 0 to 4 according to the dot intensity measured and were calibrated to serial dilutions of the relevant microorganisms.

5.2.3 Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by a standard method using the proteinase K digestion of cells. For detection of single nucleotide polymorphisms (SNP) in promoter at positions -607C/A and -137C/G allele-specific PCR according to Giedraitis et al. (Giedraitis et al., 2001) was slightly adopted. The original PCR-RFLP method according to Kruse et al. (Kruse et al., 2003) was used for the analysis of SNP in intron 1 at position -133C/G). The PCR products were then digested by the restriction enzyme SmaI. The primers, PCR conditions, and restriction enzymes used are listed in Table 2. The genotyping was performed by one investigator unaware of the phenotype. 10% subjects were genotyped twice for each polymorphism to verify correctness of the analysis.

SNP	Primers	Annealing temperature	Enzyme
-607A/C (rs1946518)	5´-TAACCTCATTCAGGACTTCC- 3´; 5´-CTTTGCTATCATTCCAGGAA- 3´; 5´-GTTGCAGAAAGTGTAAAAATTATTAA-3´; 5´- GTTGCAGAAAGTGTAAAAATTATAC- 3´	64°C, 57°C	-
-137C/G (rs187238)	5´-AGGAGGGCAAAATGCACTGG-3´; 5´- CCAATAGGACTGATTA-3´ 5´-CCCCAACTTTTACGGAAGAAAAG-3´; 5´CCCCAACTTTTACGGAAGAAAAC-3´	68°C, 62°C	-
-133C/G (rs4988359)	5´-GTATTCATAAGCTGAAACTCCCCG-3´; 5´-TGTTCTATGGCATTAGCCTTAC-3´	53°C	*SmaI*

Table 2. PCR-RFLP analysis

5.2.4 Statistical analyses

Comparisons were made between allelic and genotype frequencies in the cases and controls. The allele frequencies were calculated from the observed numbers of genotypes. The significance of differences in the allele frequencies among groups was determined by Fischer's exact test. χ^2 analysis was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and for comparison of differences in genotype combinations among groups. Variations in the quantity of subgingival bacteria corresponding to the particular genotypes were tested by Kruskal-Wallis ANOVA and/or Fisher´s exact test. Only the values of P less than 0.05 were considered significant. To examine the LD between all SNPs, pairwise LD coefficients [D´] and haplotype frequencies were calculated using the SNPAnalyzer program (http://snp.istech.info/istech/board/login_form.jsp). Contingency table analysis, odds ratio (OR), 95% confidence intervals and significance values were estimated with the use of the Statistica ver. 9.0 (Statsoft Inc., Tulsa, Oklahoma, USA) program package.

5.2.5 Results

The genotype and allele frequencies of the IL-18 gene polymorphisms in healthy children and those with gingivitis are presented in Table 3. The frequencies of -607A/C, -137C/G and -133C/G genotypes in the healthy controls and plaque-induced gingivitis group were in accord with those expected by the Hardy-Weinberg equilibrium (P>0.05). There were no significant differences in the allele and genotype frequencies between children with gingivitis and healthy controls (p = 0.35 and p = 0.90 for -607A/C, p=0.35 and p=0.89 for -137C/G, and p=0.17 and p=0.57 for -133C/G variant, respectively, Table 3).

		Genotypes of IL-18 -607A/C			Alleles		
	N	A/A (%)	A/C (%)	C/C (%)	A	C	P
Healthy gingiva	265	41 (15.5)	138 (52.1)	86 (32.4)	0.415	0.585	
Gingivitis	307	45 (14.7)	157 (51.1)	105 (34.2)	0.402	0.598	P = NS
		Genotypes of IL-18 -137C/G			Alleles		
	N	C/C (%)	C/G (%)	G/G (%)	C	G	P
Healthy gingiva	265	28 (10.6)	112 (42.3)	125 (47.2)	0.317	0.683	
Gingivitis	307	29 (9.4)	129 (42.0)	149 (48.5)	0.305	0.695	P=NS
		Genotypes of IL-18 -133C/G			Alleles		
	N	C/C (%)	C/G (%)	G/G (%)	C	G	P
Healthy gingiva	265	120 (45.3)	119 (44.9)	26 (9.8)	0.677	0.323	
Gingivitis	307	150 (48.9)	133 (43.3)	24 (7.8)	0.705	0.295	P=NS

P - statistical significance for the comparison of genotype or allele frequencies between the two groups by χ2-test (for genotypes) or Fisher's exact test (for alleles).

NS = non-significant differences

Table 3. Genotype and allele frequencies of the IL-18 gene polymorphisms in controls and patients with gingivitis

As haplotype analyses may be of a higher informative value for drawing associations between phenotypes and genetic variation than SNPs, we also assessed haplotype frequencies using the SNPAnalyzer 2 program. Of eight haplotype combinations found, only three had frequency higher than 10% because all three variants in the IL-18 gene were in tight linkage disequilibrium with each other to various degrees (D´= from 0.656 to 0.786 in controls and D´= from 0.726 to 0.790 in patients with plaque-induced gingivitis). We found no significant differences in frequency of the IL-18 haplotypes between children with gingivitis and control subjects (Table 4).

Haplotypes			Healthy gingiva	Gingivitis	OR (95%CI)
-607A/C	-137C/G	-133 C/G			
C	G	C	0.510	0.521	1.05 (0.83-1.32)
A	C	G	0.250	0.239	0.91 (0.69-1.19)
A	G	C	0.117	0.119	1.00 (0.70-1.46)
C	G	G	0.034	0.040	1.08 (0.55-2.10)
A	C	C	0.027	0.037	1.37 (0.66-2.86)
C	C	C	0.230	0.029	1.27 (0.62-2.59)
A	G	G	0.021	0.016	0.86 (0.38-1.92)
C	C	G	0.017	0.000	0.17 (0.02-1.47)
				P = NS	

NS = non-significant differences

Table 4. The frequencies of the IL-18 haplotypes in both groups

Possible links between genetic variants of IL-18 and microbiological colonization (occurrence of bacteria in gingival sulcus, including *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, T. denticola, P. micros,* F. *nucleatum, E. corrodens, A. viscosus, C. rectus, S.mitis, S. gordonii, P. nigrescens, S. constellatus, V. parvula, C. gingivalis, A. odontolyticus, C. concisus, E.nodatum, C. gracilis*) were assessed in the subgroups of subjects (N=215). Although we found a significant difference in the occurrence of periodontal bacteria between the children with gingivitis and healthy controls (P=0.00002 for *P. gingivalis*, P=0.0001 for *A. actinomycetemcomitans*, P=0.000001 for *P. intermedia*, P=0.000004 for *F. nucleatum*, P=0.0002 for *A. odontolyticus*, P=0.0009 for *P. micros*, P=0.007 for *C. gingivalis*, P=0.008 for *C. gracilis*, P=0.01 for *S. constellatus,* P=0.002 for *S. mitis*, and P=0.01 for *C. rectus*) (Table 5), no relationships between IL-18 variants and microbial pathogens were observed in any group (data not shown).

Bacteria	Healthy gingiva	Gingivitis	P level	OR (95% CI)
	occurrence of bacteria in gingival sulcus (%)			
P. gingivalis	2.94	25.2	0.00002	11.11 (2.58-47.72)
A. actinomycetemcomitans	35.3	64.0	0.0001	3.26 (1.78-5.98)
P. intermedia	19.1	61.9	0.000001	6.87 (3.43-13.75)
T. forsythia	1.5	5.0	NS	3.55 (0.43-29.48)
T. denticola	2.9	7.9	NS	2.84 (0.61-13.17)
P. micros	27.9	51.8	0.0009	2.77 (1.48-5.18)
F. nucleatum	22.1	55.4	0.000004	4.39 (2.26-8.52)
E. corrodens	77.9	82.7	NS	1.36 (0.66-2.79)
A.viscosus	50.0	58.3	NS	1.40 (0.78-2.50)
C. rectus	11.8	25.9	0.01	2.62 (1.14-6.01)
S. mitis	76.5	92.1	0.002	3.58 (1.56-8.23)
S. gordonii	80.9	86.3	NS	1.49 (0.69-3.24)
P. nigrescens	4.4	9.4	NS	2.24 (0.61-8.13)
S. constellatus	29.4	15.1	0.01	0.43 (0.21-0.86)
V. parvula	38.2	49.6	NS	1.59 (0.88-2.88)
C. gingivalis	16.2	33.1	0.007	2.56 (1.23-5.35)
A.odontolyticus	29.4	56.1	0.0002	3.07 (1.65-5.70)
C. concisus	0.0	3.6	NS	*
E. nodatum	0.0	1.4	NS	*
C. gracilis	1.5	11.5	0.008	8.72 (1.13-67.17)

NS = non-significant difference
*not applicable (small numbers)

Table 5. Occurrence of bacteria in children with healthy gingiva and patients with gingivitis

5.2.6 Discussion

Plaque-induced gingivitis affects most children and adults. It is characterized by inflammation of the gingiva without loss of connective tissue attachment, alveolar bone or teeth. Given the multifactorial nature of inflammation and the major role of IL-18 in modulating immune functions, the possible association of polymorphisms in the IL-18 gene with plaque-induced gingivitis has been investigated. Previously we demonstrated no significant association of IL-18 -607A/C variant within the IL-18 gene with gingivitis (Vokurka et al., 2009). In a much larger (twice as large) study, we screened three IL-18 SNPs (at positions -607A/C, -137C/G, and -133C/G) alone and in combination (i.e. haplotypes) in Czech adolescents with and without plaque-induced gingivitis. We again failed to find any association of the three SNPs alone with gingivitis. In addition, no differences in haplotype frequencies were found between children with healthy gingiva and gingivitis and no

significant relationships between microbial pathogens, IL-18 polymorphisms and gingivitis were determined. Only three other studies have analyzed IL-18 gene variant in periodontal diseases. Folwaczny et al. (2005) did not find an association between several IL-18 polymorphisms at positions -656, -607, -137, +113, +127 and codon 35/3 and periodontitis in Germany. The frequencies of the alleles and genotypes for -607A/C and -137C/G SNPs observed in their study were similar to our data both in healthy subjects and in children with gingivitis compared to patients with periodontitis. Indeed, some race/ethnicity-based differences exist in the relative frequencies of the IL-18 genotypes in non-Caucasian subjects (summary from Innate Immunity Programs for Genomic Applications, IIPGA database is available at http://www.innateimmunity.net). Two more recent studies (Noack et al. 2008, 2009) analyzed associations of IL-18 polymorphisms at positions -368G/T and -838C/A (plus two TLR4 variants) with aggressive and chronic periodontitis and failed to find an association between IL-18 polymorphisms and periodontal destruction. However, IL-18 production is regulated on different levels of transcription, translation and post-translation. This may be the reason why, although the IL-18 gene is a plausible "candidate" gene for periodontal inflammation, simple phenotype-genotype association may be difficult to find.
In conclusion, all to date published results reject the hypothesis that functionally relevant IL-18 gene variants have a major effect in periodontal disease – gingivitis, as well as chronic or aggressive periodontitis. Assuming that inflammation of gingival and periodontal destruction are multifactorial conditions, it would be interesting to study the impact of IL-18 variants in a more complex genetic background of bacterial recognition and host response in gingivitis.

6. General conclusions

Inflammatory diseases of periodontium are one of the most common disorders in populations and together with dental caries are a major cause of tooth loss. Based on severity, intensity and location of the inflammatory process, it is possible to distinguish between gingivitis, and periodontitis. Inflammation of periodontal tissues may be associated not only with oral health but also with general state (mainly diabetes mellitus, cardio-vascular diseases, pulmonary diseases, etc.), (Herzberg & Meyer, 1996, Taylor et al., 1996, Grossi & Genco, 1998, Persson GR & Persson RE, 2008, Williams et al., 2008).
The origin of periodontal disease depends on the interaction of numerous endogenous and exogenous factors. The presence of a microbial plaque on the tooth surfaces initiates inflammation of the periodontal tissues. Periodontal pathogens are mainly anaerobic bacteria whose pathogenicity and virulence correlates with their quantity, biochemical and physical conditions within the sulcus or periodontal pocket and individual host's responsiveness. The exact mechanisms of the disease development and progression have not been fully clarified yet. Gingivitis may, or may not, progress to periodontitis (Brown & Löe, 1993). Today the great majority of these diseases are considered a multifactorial problem (a so-called complex disease) initiated and maintained by bacteria but significantly affected by a response of the individual (Kornman et al., 2000). Unlike monogenic diseases, gene variants participating in the development of complex diseases are not rare, distinctly pathogenic mutations. These are mostly "common" variants of "normal" genes (it means polymorphisms), often the most frequent alleles in population whose original (assumed) evolution advantage was lost under the condition of the current civilization.

Molecular and genetic research has not devoted much attention to periodontal diseases Although these diseases do not lead to premature death, study of their etiopathogenesis is very important as the high prevalence of periodontal diseases in population has very important social and economic consequences. Furthermore, we are not able to prevent the onset of these diseases in all individuals as primary preventive intervention is possible only after a detailed clarification of etiopathogenesis (thus also genetic determination of these diseases), which makes secondary preventive interventions even more urgent. This involves earlier diagnosis, mainly in the asymptomatic or incipient stages, efficient therapy and, last but not least, possibilities of determination of the individual's risk for the development of more serious forms of the disease.

The association between plaque-induced gingivitis and genetic polymorphisms has been studied on only seven genes in several studies, therefore it is difficult to conclude whether the analyzed polymorphisms can be involved in etiopathogenesis of gingivitis. Furthermore, there are several reasons for discrepancies in the findings reported in these studies:

1. One of the main problems is very low "power of study" due to small sample sizes. Most associations refer to small odds ratios and relatively large confidence intervals which contributes to the risk for false positive or negative results.
2. So far the most common case-control association study design is suitable for a lot of potential methodological problems connected with different definitions of clinical disease phenotype, different selections and definitions of control subjects, diversity of ethnic backgrounds of study samples etc. (Ioannidis, 2003).
3. Because of the complexity of plaque-induced gingivitis and the large number of host derived and environmental/external factors involved in disease pathogenesis, it is logical to assume that multiple genetic variants (SNPs) on different genes may contribute to overall disease susceptibility. As such, a simple cause and effect relationship between a particular genetic allele and a disease is not possible. Therefore, rather than associations of the individual polymorphisms, combinations of polymorphisms in connection with other factors should be investigated by multivariate analyses.

In conclusion, research on genetic polymorphisms in plaque-induced gingivitis has had limited success in unravelling significant and reproducible genetic factors for susceptibility to these diseases. Taken together the data published so far on gene variants in gingivitis, we can conclude that there is an insufficient number of studies to draw relevant conclusions. Nevertheless, the preliminary evidence suggests that polymorphisms in the IL-10, IL-1RN, IL-6 and MMP-9 may be associated with plaque-induced susceptibility. Results of genetic research, if verified in larger study cohorts, it could also lead to new diagnostic possibilities and help improve therapy of this disease or find parameters determining the risk of progression to more serious forms of periodontal disease. These findings could thus be a stimulus for an individual-based treatment approach and its optimization. Further clinical and genetic studies verifying the importance of these findings and their usability for monitoring of this disease activity still will have to be conducted.

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Gingival diseases are a family of distinct pathological entities that involve the gingival tissues. These signs and symptoms of these diseases are so prevalent in populations around the world that they are often considered to be "normal" features. The diseases are now classified into two main groups namely: Plaque-Induced and Non-Plaque Induced Gingival Diseases. This book provides dentists, dental hygienists, dental therapists and students with a comprehensive review of gingival diseases, their aetiology and treatment.

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