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Periodontitis  
A Useful Reference

*Edited by Pachiappan Arjunan*





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# **PERIODONTITIS - A USEFUL REFERENCE**

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Edited by **Pachiappan Arjunan**

## **Periodontitis - A Useful Reference**

<http://dx.doi.org/10.5772/65842>

Edited by Pachiappan Arjunan

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First published in Croatia, 2017 by INTECH d.o.o.

eBook (PDF) Published by IN TECH d.o.o.

Place and year of publication of eBook (PDF): Rijeka, 2019.

IntechOpen is the global imprint of IN TECH d.o.o.

Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from [orders@intechopen.com](mailto:orders@intechopen.com)

Periodontitis - A Useful Reference

Edited by Pachiappan Arjunan

p. cm.

Print ISBN 978-953-51-3605-7

Online ISBN 978-953-51-3606-4

eBook (PDF) ISBN 978-953-51-4610-0

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# Meet the editor



Dr. Pachiappan Arjunan is an assistant professor in the Department of Periodontics at the Dental College of Georgia, Augusta University (formerly Georgia Regents University). Dr. Arjunan earned his PhD degree from the National University of Singapore (NUS) and completed a postdoctoral training from the National Eye Institute, NIH. Dr. Arjunan leads his group of postdoctoral fellows, dental graduates, and predoctoral students at the Periodontal Molecular Immunology Laboratory in AU. Dr. Arjunan is actively involved in teaching dental predoctoral students at the Dental College of Georgia. He has received best teacher trainee award, research fellowship awards, and travel grant awards and has participated as a session chair (IADR/AADR), as a judge, as well as a session moderator in different scientific meetings. He is an active member in the departmental, college, and university committees. Dr. Arjunan, an expert in genomics and bioinformatics, has presented his scientific invention in both national and international conferences as an invited speaker. He has published more than 20 papers in reputed journals and book chapters as well. He contributes as an editor in InTechOpen, as one of the honorable consulting editors of *Eye and Brain* journal, and as an editorial board member of *EC Ophthalmology*, *Open Access Journal of Dental Sciences*, *POJ Dental and Oral Care*, and *SciFed Journal of Ocular Biology*. In addition, he serves as an external grant reviewer, a permanent reviewer of *JoVE*, and a reviewer for more than 15 renowned journals.





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## Preface

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In recent times, periodontitis has become a global health burden. An understanding of periodontal diseases and associated systemic diseases has exponentially increased, and the study of oral-systemic health connections has been rapidly advancing among scientists, clinicians, and the public as well. A robust knowledge in the pathophysiology of periodontal disease is mandatory to arrive at an unambiguous treatment planning and prevention of the disease.

This comprehensive book *Periodontitis - A Useful Reference* has been compiled by a team of experts with the main objective of providing an overview of the basic pathology of “periodontitis” and its implication on oral health and general systemic health. This book encompasses seven individual chapters under four sections. The essential subjects that are emphasized through the chapters in this book include key components of immunopathology, autoimmunity, host-bacteria interaction, and tissue or cellular changes that occur during periodontitis and therapies. The traditional clinical management using basic and reconstructive surgery and advancement in therapies is reviewed. Further, the overall impact of dental plaque in management of the disease itself and various considerations of the periodontal status of patients before and post-orthodontic treatment are highlighted in the subsequent chapters. The knowledge of remodeling process occurring in periodontal tissues during orthodontic treatments is clinically vital for the appropriate choice of mechanical stress application, to regulate the duration of treatment therefore avoiding adverse consequences. These important concepts have been well interrogated and presented in the second section. Thus, this compilation is anticipated to be a useful reference for the dental clinicians, scientists, and patients as well.

**In Brief:** An introductory chapter inaugurates the topic with a gist of the subject.

Chapter 2 gives insight into the implication of dental plaque, the microbiota, and the pathogenesis of periodontal disease. The current approaches in the management of periodontitis are reviewed.

Chapter 3 recapitulates the link between human oral microbiome, various cells of the immune system, and the host-pathogen interaction. This book chapter illustrates how the dysbiotic keystone pathogen *Porphyromonas gingivalis* interacts with other bacterial community in the oral cavity and promotes chronic inflammation and tissue destruction of periodontitis. A fundamental knowledge in the cellular response provoked by *P. gingivalis* infection is highlighted.

The following chapter (4) provides information on the remodeling process taking place in the periodontal tissues during various orthodontic tooth movements. The biological substances that are significant biomarkers of the changes during the orthodontic treatment are

discussed in this chapter. The importance of each biomarker involved has been enlisted. It will be a beneficial reference for the clinicians to decide on proper choice of orthodontic treatment to prevent adverse effects on the tooth-supporting structures.

The next chapter (5) presents the best available knowledge on the association of periodontal status in adult orthodontic treatment. The main objective of this discussion is to make the clinicians aware of the parameters to consider prior, during, and after the orthodontic treatment in adult dental patients. The authors have illustrated the bidirectional relationship between periodontal health and orthodontic treatment and the potential ways to standardize it. The authors have not only focused the pre-orthodontic parameters but also posttreatment aspects. Henceforth, this will be very useful information for both the clinicians to bring about effective orthodontic results and patients for a desirable treatment outcome.

This chapter (6) sheds light on the current literature concerning the role of microbiota in rheumatological conditions such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and systemic lupus. Here, the suggestive underlying mechanisms that link these systemic conditions to periodontal inflammation are reviewed. This chapter describes the role played by the oral microorganisms on host immunology and physiology, the understanding of which is very essential for the management of autoimmune disorders.

This chapter (7) summarizes the role of innate and specific immune response involved in the pathogenesis of periodontitis. The subdivisions demonstrate the role of cells and inflammatory mediators contributing to the inflammatory process of the ligamentous supporting structure and in bone resorption.

The concluding chapter (8) highlights the application and advancement in the usage of bioactive membranes in guided tissue regeneration (GTR) that aid in periodontal tissue regeneration. Here, the authors focus on a new biomaterial, namely, polycaprolactone, specifically Jagged1-coated PCL membrane. The added merits of coated PCL as they prevent the epithelium downgrowth in the lesion and promote formation of alveolar bone for a successful guided tissue regeneration are described in this section.

We are grateful to everyone who has supported and encouraged us in completing this project.

**Dr. Pachiappan Arjunan**

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# Introduction

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# Introductory Chapter: Periodontitis - A Useful Reference

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Pachiappan Arjunan, Radhika Swaminathan,  
Brunner Mark and Christopher W. Cutler

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.71386>

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## 1. Etiology

The oral cavity is a unique part of the human body and is considered the window of general health. Gingivitis is primarily caused by bacterial colonization called dental plaque, and it is one of the common oral diseases that are preventable [1]. Gingivitis can be resolved with proper oral hygiene and professional intervention. Periodontitis occurs if inflammation of the gingiva is not intervened and progresses, which leads to an active destruction of the periodontal supporting tissues including the alveolar bone. Periodontitis can occur either as a generalized or localized form. It can be chronic or aggressive in nature. The World Health Organization (WHO) estimates nearly 15–20% of the adult population aged between 35 and 44 years suffers from an advanced form of periodontitis [2]. The Center for Disease Control (CDC) reported that approximately 50% of the general population in the USA is affected with some types of periodontitis [2]. Periodontal inflammation establishes as a sequela of a complex interaction of mixed oral pathogens (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, and *Streptococcus gordonii*) in the endogenous plaque or the biofilm, and their toxic by-products exert pathogenicity on host tissues. This disruption develops a shift in the bacterial ecosystem [3] from good health to disease. The ultimate result is bone deterioration and tooth loss in vulnerable patients.

There is an extensive list of local and systemic risk factors associated with the periodontal disease [4]. These risk factors include smoking, diabetes, medications, hormonal imbalance in women, immunocompromised status, etc. Most commonly, periodontal disease occurs when plaque [5] is allowed to build-up along tooth and under the gingiva. Genetic and environmental factors also contribute to the progression of periodontitis. In addition to the exacerbated inflammatory responses and other risk factors, another important consideration is adverse

effects from undesirable tooth movement. Improper application of forces on the healthy teeth such as parafunctional habits like bruxism (teeth grinding), malocclusion, or even during orthodontic therapies itself induces and provokes gingival recession, clinical attachment loss, and extensive bone resorption. Periodontal disease most often occurs without overt symptoms and is rather painless. As far as periodontal disease is concerned, frequent professional maintenance and excellent home care are the best prevention before initiation of the disease.

## **2. Oral and general health**

The mouth serves as a diagnostic mirror for many systemic diseases. The dentist should be aware of all the associated systemic conditions that manifests in the oral cavity [6]. This assists both the clinicians and patients in early diagnosis, prevention, and better treatment results. In recent developments, a study on the pathological link between periodontal health and systemic ailments [7] invited more interest and follow-up among researchers and public health organizations. There are significant findings supporting the association between periodontal disease and systemic conditions [8] namely cardiovascular disease [9], cerebrovascular diseases, peripheral arterial disease, respiratory diseases, mental disorders, type 2 diabetes mellitus [10], digestive diseases, endocrine disorders, obesity, adverse pregnancy outcomes [11], renal disorders, and rheumatoid arthritis (RA). The risk associated with a bacteremia from a dental treatment prior to any invasive cardiac surgeries, organ transplants, and prosthesis implantations is being investigated. On the other hand, the optimization of dental health before high-risk surgeries and in patients with pre-existing systemic disorders like cardiovascular defects should be recognized and reinforced among patients and dental professionals. In recent days, there are numerous emerging clinical studies substantiating the links of chronic inflammation and predisposition to various cancers. The effect of chronic periodontitis on head and neck squamous cell carcinoma (OSCC) is of utmost priority in the world of research [12]. OSCC lesions have been reported to harbor very high levels of oral microbial colonies relative to contiguous healthy mucosa [13]. Hence, the importance of primary oral hygiene measures should be emphasized soon after the primary teeth erupts.

## **3. Role of immune system**

Many of the latest studies enlighten a possible bidirectional link between the mechanism of periodontitis and metabolic diseases where both conditions could exacerbate each other. Various clinical studies determined any derangements in the equilibrium of immune system because of chronic inflammation of the periodontium that has a greater impact on the general body health of patients with this disease relative to those who are free of gum disease. It has also been postulated that periodontal inflammation can modify the host susceptibility to acquire other diseases. Experimental animal studies have shown elevated levels of inflammatory markers in periodontitis. A proper knowledge of immune pathogenesis during chronic periodontitis can be a reliable source for clinicians to apply and modify new therapeutic approaches. The



innate immune response is the first line of defense against invasion of pathogens. The blood leukocytes, neutrophils, and monocytes possess arsenal of receptors that enable the detection of invading pathogens and production of reactive oxygen species, cytokines, and chemokines. There occurs a disorder in the immune homeostasis, which leads to further increase in bacterial population and release of additional inflammatory mediators [14]. These inflammatory mediators elicit exaggerated immune response in the body, which subsequently favors the pathogens like *P. gingivalis*.

#### **4. Host response to periodontitis**

The interaction between host response and the dysbiotic microbial community is significant for causing periodontitis. The keystone pathogen, especially *P. gingivalis*, is known to influence the host directly by tissue damage and indirectly by stimulating and modifying the immune system to favor their survival and multiplication. In normal healthy status, the gingival epithelium [15] acts as the physical barrier against mechanical stress, exogenous substances, and pathogenic bacteria. When inflammation of the periodontium exists, *P. gingivalis* employs unique strategies to survive, sustain, and persist in the oral cavity, particularly in the antigen-presenting dendritic cells [16–18]. Hajishengallis discovered *P. gingivalis* surviving in the macrophages [19]. *P. gingivalis* causes inhibition of IL-8 production by the epithelial cells and impairs the protective functions of polymorphonuclear neutrophils (PMNs). During periodontal inflammation, the epithelial surface becomes ulcerated by proteolytic activity from the by-products of bacterial colonies, resulting in exposure of the connective tissues and blood capillaries to the bacterial biofilm, which allows further periodontal destruction and progression of the overall disease process. More studies are needed to specify how these pathogenic bacteria induced by biofilm affect the host immune homeostasis. Investigations should also focus on how bacterial dissemination to distant sites in the human system causes different conditions such as cardiovascular diseases, diabetes, rheumatoid arthritis (RA), macular degeneration [20], Parkinson, and Alzheimer's disease [21].

#### **5. Treatment planning, outcome, and impact**

A comprehensive periodontal diagnosis allows opportunity for appropriate treatment. A thorough assessment of the local cause (plaque), systemic factors, functional (presence of caries, occlusal status), and esthetic need (orthodontic therapy) induces an effective treatment plan. The chief goal of periodontal therapies is to control the prevailing infection and prevent further damage to the periodontium. Therapeutic control is accomplished with the least invasive methods and surgical treatments as needed. Nonsurgical strategies range from improvements in basic oral hygiene practices to professional scaling and root planning with antimicrobial medications. When treating severe periodontal destruction, surgical and regenerative therapies such as reconstructive tissue therapy guided tissue regeneration (GTR) with the aid of bioactive substitutes and bone grafts are indicated. A combined interdisciplinary

approach with orthodontic management and periodontal therapy is most effective in adults with severe periodontitis to restore the disordered function, compromised esthetics, and overall well-being. On the other hand, it is vital to identify, eradicate, or modify the causal factors and risk factors associated with periodontitis.

It is appropriate to advance our knowledge of oral pathogens, their impact on host immune response, and the cellular level modifications, which occur during normal health, disease, and periodontal management. The collection of chapters in this book will be a valuable guide in this regard.

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## Biofilm and Periodontitis

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# Impact of Dental Plaque Biofilms in Periodontal Disease: Management and Future Therapy

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Veronica Lazar, Lia-Mara Ditu, Carmen Curutiu, Irina Gheorghe, Alina Holban, Marcela Popa and Carmen Chifiriuc

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69959>

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## Abstract

Oral cavity represents an ideal environment for the microbial cell growth, persistence, and dental plaque establishment. The presence of different microniches leads to the occurrence of different biofilm communities, formed on teeth surface, above gingival crevice or at subgingival level, on tongue, mucosa and dental prosthetics too. The healthy state is regulated by host immune system and interactions between microbial community members, maintaining the predominance of “good” microorganisms. When the complexity and volume of biofilms from the gingival crevice increase, chronic pathological conditions such as gingivitis and periodontitis can occur, predisposing to a wide range of complications. Bacteria growing in biofilms exhibit a different behavior compared with their counterpart, respectively planktonic or free cells. There have been described numerous mechanisms of differences in antibiotic susceptibility of biofilm embedded cells. Resistance to antibiotics, mediated by genetic factors or, phenotypical, due to biofilm formation, called also tolerance, is the most important cause of therapy failure of biofilm-associated infections, including periodontitis; the mechanisms of tolerance are different, the metabolic low rate and cell’s dormancy being the major ones. The recent progress in science and technology has made possible a wide range of novel approaches and advanced therapies, aiming the efficient management of periodontal disease.

**Keywords:** dental plaque biofilm, periodontitis, host defense mechanisms, resistance mechanisms, therapeutic approaches

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## 1. Introduction

Oral cavity represents an ideal environment (e.g., appropriate temperature and nutrients) for the microbial cell growth, survival and persistence, and subsequent dental plaque

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biofilm establishment. The exact number of species from the oral plaque is not known, because some of them are not cultivable, but it is estimated to be between 700 and 1000 species, reaching densities of  $10^8$  bacterial cells/mg, much of them being uncultivable [1]. However, bacteria are the most numerous group in the oral microbiota, accompanied by a diverse collection of archaea, fungi, protozoa, and viruses. The oral microorganisms are generally commensal species, maintaining relationships with the host based on mutual benefits. They do not produce disease, but instead impede pathogenic species to adhere to mucosal surfaces [2, 3].

Dental plaque biofilm represents a polymicrobial community that remains relatively stable in health, consisting in species belonging to *Streptococcus*, *Actinomyces*, *Veillonella*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Treponema*, *Neisseria*, *Haemophilus*, *Eubacteria*, *Lactobacterium*, *Campytophaga*, *Eikenella*, *Leptotrichia*, *Peptostreptococcus*, *Staphylococcus*, and *Propionibacterium* genera.

The dental plaque biofilm formation follows many stages and begins at 1 h after washing, when the tooth surfaces are covered by an organic “pellicle” composed from salivary glycoproteins, carbohydrates and immunoglobulins, which are adsorbed on the hydroxyapatite surface through electrostatic interactions between calcium ions and phosphate groups with the oppositely charged groups of the macromolecules from the saliva. In a second stage, bacteria adhere to the pellicle and between them through the interaction between specialized structures or adhesins (glycocalyx, capsule, and fimbriae) with complementary receptors. The first colonizers are gram-positive cocci (*Str. mutans*, *Str. mitis*, *Str. sanguis* or *Str. oralis*, *Rothia dentocariosa*, or *Staphylococcus epidermidis*), gram-positive rods, actinobacteria (*Actinomyces israelis* and *A. viscosus*) and few gram-negative cocci [4]. The attached species secrete exopolymers such as glucans that contribute to the development of biofilm matrix and allow association of other species. Although initially the oral cavity offers an aerobic condition, oxygen is rapidly consumed by the aerobic bacteria (e.g., *Neisseria* spp.) or facultative anaerobic (e.g., *Streptococcus* and *Actinomyces* spp.), which are first colonizers creating appropriate conditions for the survival of obligate anaerobe species. When biofilm reaches maturity, the oral cavity becomes colonized predominantly by anaerobic bacteria [5].

In the oral cavity, the presence of different microenvironments leads to the occurrence of different biofilm communities, like those formed on the surface of teeth above the gingival crevice (the supragingival plaque) or at the subgingival level (the subgingival plaque), on the tongue, on the mucosal surfaces, or biofilms developed on dental prosthetics and fillings. Some microbial species are better adapted to some location. For example, based on their oxygen requirements, species could be classified as obligate aerobes, obligate anaerobes (as *Veillonella* and *Fusobacterium*), facultative anaerobes (as most streptococci and *Actinomyces*), and microaerophilic species that prefer low concentrations of  $O_2$  (from 2 to 10%) and capnophilic (species that grow best at high  $CO_2$  concentrations, from 5 to 10%, as *Neisseria*) [3].

When the complexity and volume of biofilms located in the gingival crevice increase, pathological conditions such as periodontitis or chronic gingivitis can occur. Literature of the



last decades has shown that almost all forms of the periodontal disease are consequences of the chronic, nonspecific or specific bacterial infections. If in the healthy individuals, the oral biofilms are comprised mainly of gram-positive facultative anaerobes (*Streptococcus anginosus* and *A. naeslundii*), in the above mentioned pathologic conditions, the percentage of gram-negative anaerobic bacteria increases and may include *Aggregatibacter* (previously *Actinobacillus*) *actinomycetemcomitans*, *Porphyromonas gingivalis*, *P. intermedia*, *Bacteroides forsythus*, *Campylobacter rectus*, *Eikenella sp.*, *Peptostreptococcus micros*, *Streptococcus intermedius*, *Prevotella sp.*, *Fusobacterium sp.*, *Capnocytophaga sp.*, *Veillonella sp.*, *Treponema* and other on-cultivable spirochetes, and the bacterial counts associated with the disease are up to 10(5) times larger than those of the same species found in healthy individuals [6, 7].

Perhaps, the three best-studied periodontal pathogens are *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and, more recently, *Bacteroides forsythus*, all three carrying pathogenicity islands and having the ability to secrete a number of virulence factors, including invasion of gingival epithelial cells and an abundant array of extracellular proteases. The last ones are responsible for the increase in vascular permeability and in the flow of gingival crevicular fluid (GCF), thus providing a rich source of nutrients for the subgingival plaque community.

*Porphyromonas gingivalis* is one of the most important periodontal pathogens, exhibiting the ability to adhere and invade epithelial tissue of the oral cavity *in vitro*. *Aggregatibacter* (*A.*) *actinomycetemcomitans* is associated with periodontal disease in preteen ages. *Fusobacterium nucleatum* is an important periodontal agent, especially in the rapid and progressive periodontal disease forms. *Prevotella intermedia* is black-pigmented, while *Bacteroides* (*B.*) *forseythus* an unpigmented gram-negative bacterium; *B. forseythus* has several virulence factors, including the production of trypsin-like proteases and polysaccharides, the ability to penetrate the host cell, or inducing of apoptosis. *Capnocytophaga* species are involved in the onset of the juvenile periodontal disease and in the periodontal disease of adults. These bacteria produce pro-inflammatory lipopolysaccharides and extracellular proteases that could destroy sIgA immunoglobulins. Prevalence of *Peptostreptococcus micros* in advanced periodontitis in adults has been reported as 58–63%. It was also positively associated with dental implant failure. Spirochetes were observed in a greater proportion in patients with periodontal disease than in healthy individuals [8]. Two important spirochetes species, i.e., *Treponema vincentii* and *T. denticola*, are also involved in periodontal disease. Both produce pro-inflammatory lipopolysaccharides and unusual metabolic products, such as indole, hydrogen sulfide and ammonia that are potentially toxic to the host cells.

Besides the microbial component, genetic, physiological, and behavioral factors are also involved in the pathogenesis of periodontal disease. Some people may be genetically susceptible to periodontal disease, but the genetic background involved is not clear. The hormonal changes associated with teen age and pregnancy could contribute to gingival enlargement. Smoking is among the factors that increase the probability to develop a periodontal disease. In smokers, reduced gingival blood flow, impaired wound healing, and increased production of inflammation-mediating cytokines were observed comparing with healthy persons. Smoking seems to increase the severity of periodontal disease, but also the response of the gingival

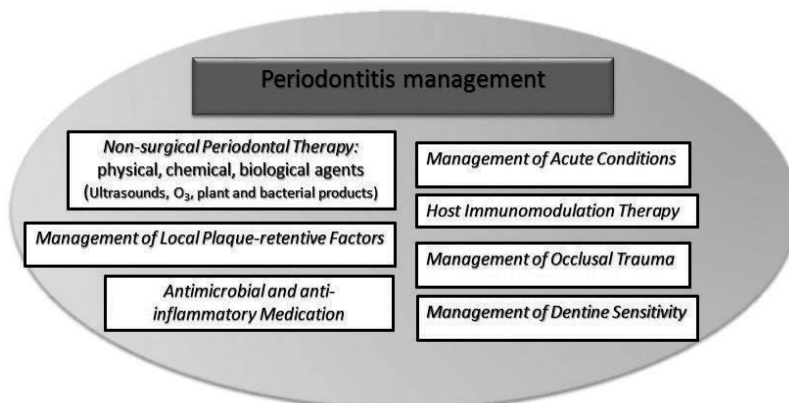
tissues to periodontal therapy is reduced, fact that contributes to a greater incidence of refractory disease and to the risk to lose teeth. Regarding age, the researches indicate that older people have the highest rates of periodontal disease. Other factors which may contribute to evolution of periodontitis are diet, stress, obesity, and some other underlying diseases such as diabetes, cardiovascular disease, osteoporosis, and rheumatoid arthritis. Certain medications could also be inappropriate for the evolution of periodontal disease. Also, a bad oral hygiene, tooth decay and tooth positioned incorrectly may also increase the risk of periodontal disease [9].

The management and therapy of periodontal diseases may be diverse and is usually adjusted depending on particularities of each case/patient (**Figure 1**). Since periodontal disease occurs when a bacterial biofilm (dental plaque) adheres to the boundary between the teeth and gingiva, causing chronic inflammation and progressively destroying the periodontal tissue that supports the teeth, the periodontal treatment involves scaling and root planning, which mechanically removes the causative bacteria biofilm together with the necrotic cementum from the surface of the tooth root. Appropriate application of this therapy eliminates periodontal tissue inflammation and stops the process of destruction of the same tissue. However, removing the cause of the disease does not regenerate the lost periodontal tissue to its original state [10].

The main approaches considered in the current therapeutic procedures include the following:

(1) *Nonsurgical periodontal therapy* aims in motivating and instructing the patient in adequate self-care, followed by periodical re-evaluation of the oral hygiene status. The primary goal of nonsurgical periodontal therapy is to control microbial periodontal infection by removing bacterial biofilm, calculus and toxins from the involved periodontal root surfaces [11].

A new nonsurgical therapy is the ozone-therapy; the disinfection power of ozone over other antiseptics makes the use of ozone in dentistry a very good alternative and/or an additional disinfectant to standard antiseptics. Due to safety concerns, initially only dissolved ozone in water and ozonated oils were recommended, but a new device used for the gas application



**Figure 1.** Periodontitis management—innovative strategies for reversing the chronic infectious and inflammatory condition.

with a suction feature allows now its safe intra-oral use, with better diffusion even in the dental hard tissues, for its healing and tissue regeneration properties, being indicated in all stages of gingival and periodontal diseases [12].

(2) *Management of local plaque-retentive factors* which refer to mal-positioned teeth, overhanging restorations, crown and bridgework, partial dentures and fixed and removable orthodontic appliances that can increase the risk of periodontal disease and can also prevent successful treatment and resolution of associated pockets. Local irritation and plaque retention caused by untreated carious lesions, subgingival and approximate overhanging crown margins can affect the attachment loss at patients with chronic periodontitis [13].

(3) *Antimicrobial medication* may refer to: full mouth disinfection (consisting in the instrumentation of all periodontal pockets in two steps within 24 h in combination with the adjunctive use of chlorhexidine mouthwash and gel to disinfect any bacterial reservoirs in the oral cavity), local antimicrobials (i.e., disinfectants such as chlorhexidine and locally delivered antibiotics or antiplaque mouthwashes) which have bacteriostatic and bactericidal activity and can inhibit the development of gingivitis, but despite this proved effect, they have a much reduced effect on established plaque and cannot prevent the progression of periodontitis [14], and systemic antibiotics (which are prescribed as an adjunct to root surface instrumentation) have been proposed to act by suppressing the bacterial species responsible for biofilm growth, leading to a less pathogenic oral environment [15].

(4) *Management of acute conditions* should be made as much as possible by local treatment, avoiding the use of systemic antibiotics if there is no significant sign of infection. The main acute conditions that may be associated with periodontal disease refer to periodontitis associated with endodontic lesions (which is a combined perio-endo lesion characterized by clinical attachment loss but also a tooth with a necrotic, or partially necrotic, pulp), periodontal abscess (occasionally occurring in patients with periodontitis, characterized by localized pain and swelling due to nondraining infection of a periodontal pocket), and necrotizing ulcerative gingivitis and periodontitis (characterized by marginal gingival ulceration with loss of the interdental papillae and a gray sloughing on the surface of the ulcers) [9].

(5) *Management of occlusal trauma* have been linked with periodontal disease for many years, but the role of occlusion in the etiology and pathogenesis of inflammatory periodontitis is still not completely understood [16].

(6) *Management of dentine sensitivity* is a condition some patients may experience following root surface instrumentation, especially those with sensitive teeth prior to treatment. Identification and treatment of the causative factors of dentine sensitivity help to prevent the condition from occurring or recurring. There are various treatment modalities available which can be used at home or may be professionally applied, such as toothpastes, mouthwashes, or chewing gums, and they act by either occluding the dentinal tubules or blocking the neural transmission [17].

(7) *Host modulation therapy* aims to modulate the destructive aspects of the host's immune-inflammatory response to the microbial biofilm by utilizing the anti-inflammatory drugs or oral products (i.e., sub-inhibitory doses of tetracycline). This approach has led to the emergence of a new field of "Perioceutics" which is based on the use of pharmaco-therapeutic

agents including antimicrobial drugs, as well as host modulatory therapy for the management of periodontitis. These host-modulating agents could be successfully used as adjunct components to the balance between periodontal health and disease progression in the direction of a healing response [18].

(8) *Dental prophylaxis* refers to various approaches including plaque elimination by regular tooth brushing, periodical professional removing of mineralized dental plaque or tartar, oral examination and evaluation of periodontal disease progression [19].

## 2. Oral microbiota: host interactions

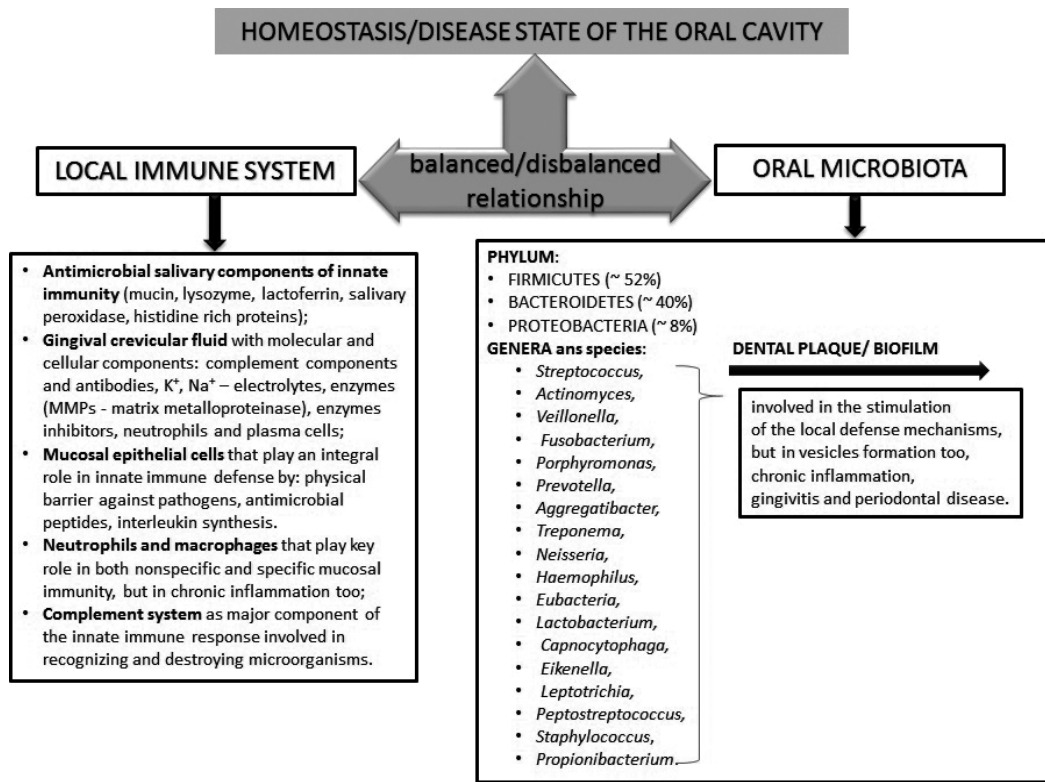
In 2001, Joshua Lederberg introduced the term microbiome signifying “the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease” [20]. These complex communities of microbes and their genes play a fundamental role in controlling the host physiology (metabolism, nutrition, immune system development, regulation of gastrointestinal and cardiovascular systems, etc.) [21] and also support the innate and adaptive host defenses in excluding exogenous (and often pathogenic) microorganisms [22]. The healthy microbiome in any individual patient has relatively lower taxonomic diversity, remaining relatively constant over time, this natural balance being termed “microbial homeostasis,” but its exact composition differs significantly across individuals [23].

The healthy state is highly regulated by the host immune system, and interactions between the microbial community members and with the host maintain a community dominated by “good” microbes, usually gram-positive *Actinobacteria* or streptococci (**Figure 2**) [24].

### 2.1. Host defense mechanisms

Host defenses play an important role in maintaining the homeostasis of the oral cavity. The alliance between the immune system and oral microbiota is responsible for the maintenance of tolerance to microbial antigens, the host monitoring and responding permanently to the colonizing microorganisms. Any changes in this symbiotic relationship induced by antibiotics, diet, and elimination of normal microbiota constitutive species increase the risk for autoimmune and inflammatory disorders [21].

Regarding the prenatal development of cellular components associated with the oral mucosa associated immune system, it was observed that the initial organization of Peyer’s patches can be immunohistologically detected at 11 weeks of gestation [25]. Epithelial cells positive for the secretory component of the sIgA and immunocytes positive for IgM can be detected in salivary gland tissue by 19–20 weeks and continue to predominate during gestation. After birth, immunocytes secreting IgA begin to dominate, but no IgA can be detected in saliva at birth. sIgA was detected in the neonates’ saliva as early as 3 days after birth, and its concentration increased more rapidly during the first 6 months after birth in infants exclusively breast fed [26]. Salivary IgA in young infants has the molecular characteristics of secretory IgA and predominates in saliva. Both IgA subclasses are present in the proportions characteristic of



**Figure 2.** The microbiota plays a fundamental role on the induction, training and function of the host immune system, the interactions between mucosal surfaces and microbiota accomplishing a key role in host defense, health and disease.

adult in 1- to 2-month-old infants, although the appearance of IgA2 is delayed in some subjects [27]. The infant apparently can activate mucosal immune responses quite early in life. For example, salivary antibody specific to organisms that originally colonize the oral cavity (e.g., *S. mitis*, *S. salivarius*) can be detected by 1–2 months of age. Most of these antibodies are sIgA, although some IgM antibodies can also be initially detected. Salivary sIgA1 and sIgA2 specific to *S. mitis* and *S. salivarius* components increase qualitatively and quantitatively during the first few years of life [28]. Salivary IgA specific to components of streptococci that require hard surfaces for colonization (e.g., *S. sanguis* and *mutans* streptococci) generally appear after tooth eruption [29]. The maternal placental-derived IgG with specificity toward oral microbiota is replaced by the *de novo* synthesis stimulated by the teething process. The collective contributions in the oral cavity of innate and antibody-based immune elements from the saliva, gingival crevicular fluid (and milk if breast feeding) may be considered together with diet, infectious dose, salivary receptors, and tooth integuments, as factors that can determine the outcome of initial colonization events on erupting tooth surfaces [25].

Saliva plays an important role in maintaining the oral homeostasis, through the flushing effects and its antimicrobial constituents like mucin, lysozyme, lactoferrin, salivary peroxidase, and histidine-rich proteins, which are all components of innate immunity [25]. Moreover, local concentrations of these proteins near the mucosal surfaces, periodontal sulcus (gingival crevicular

fluid), and oral wounds reinforced by immune and/or inflammatory reactions of the oral mucosa are primarily responsible for innate immunity [30]. *Lysozyme* is a hydrolytic enzyme that cleaves the carbohydrate components of the cell wall peptidoglycan, resulting in cell lysis. This enzyme is active against both gram-negative and gram-positive microorganisms; its targets include *Veilonella* species and *Actinobacillus actinomycetecomitans* [30]. *Lactoferrin* is an iron-binding glycoprotein that links to free iron in the saliva, causing bactericidal or bacteriostatic effects on various microorganisms requiring iron for their survival. Lactoferrin also provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions [31–33]. The *lactoperoxidase-thiocyanate system* in saliva has been shown to be bactericidal on some strains of *Lactobacillus* and *Streptococcus* by preventing the accumulation of lysine and glutamic acid, both of which being essential for bacterial growth [34, 35]. The *histatins*, a family of *histidine-rich peptides*, have antimicrobial activity against some strains of *Streptococcus mutans* and inhibit hemagglutination activity of the periodontopathogenic *P. gingivallis*. Also, they neutralize the lipopolysaccharides of gram-negative bacteria and exhibit fungicidal activity against several *Candida* species, *Aspergillus fumigatus*, some strains of *Saccharomyces cerevisiae*, and *Cryptococcus neoformans* [36, 37]. Some defense proteins, like chaperones HSP70/HSPAs (70 kDa heat shock proteins), are also involved in both innate and acquired immunity [30]. In addition, saliva contains abundant CD14 amounts from salivary glands in a soluble form, although LPS-binding protein was below detectable levels, suggesting that saliva CD14 is important for the maintenance of oral health [38].

Gingival crevicular fluid (GCF) is a fluid coming from the junctional epithelium of the gingiva that carries all key molecular (complement components and antibodies, K, Na–electrolytes, enzymes and enzymes inhibitors) and cellular (neutrophils and plasma cells) components of the immune response that are necessary to prevent tissue invasion by subgingival plaque bacteria [39, 40]. Composition of the GCF is the result of the interaction between bacterial biofilm adherent to the tooth surfaces and the cells of the periodontal tissues [41]. The GCF induces permanent changes in microbiota composition, playing an important role in the introduction of immune cells, and being a source of nutrients for resident microorganisms. A number of enzymes can be detected in GCF, including collagenases and elastases which are derived from phagocytic cells and are responsible for the destruction of gingival tissues [42]. Therefore, GCF components might serve as potential diagnostic or prognostic markers for the progression of periodontitis [23, 39]. Although the presence of cytokines was highlighted in GCF, there is no clear evidence of their involvement in the disease. However, interleukin-1 (IL-1) alpha and IL-1 beta are known to increase the binding of PMNs and monocytes to endothelial cells, stimulate the production of prostaglandin E2, release of lysosomal enzymes and bone resorption. On the other hand, interferon (IFN) alpha present in GCF has a protective role in periodontal disease because of its ability to inhibit bone resorption activity of IL-1 beta [43].

Mucosal epithelial cells play an integral role in innate immune defense by sensing signals from the external environment, generating various molecules to affect growth, development, function of other cells and maintaining the balance between health and disease [44]. Mucosal epithelial cells produce antimicrobial peptides that include the  $\beta$ -defensin family, cathelicidin (LL-37), calprotectin, and adrenomedullin. These epithelial antimicrobial peptides are important for wound healing and cell proliferation or exert chemotactic effect to immune cells [45]. It is now recognized that the antimicrobial peptide hBD-2 found in the supra-basal layer of epithelium, stimulates antigen-presenting dendritic cells that signal the adaptive immune system, in

addition to its antimicrobial activity [44, 46]. Also, it has been proposed that LL-37, the only antimicrobial peptide from the cathelicidin family, detected in gingival epithelium may be the product of neutrophils migrated through gingival epithelium rather than epithelial cells themselves [47]. LL37 is active against both gram-negative and gram-positive bacteria including established periodontopathogens, like *P. gingivalis* and *A. actinomycetemcomitans* [48].

Calprotectin is constitutively produced by neutrophils, monocytes, macrophages, and epithelial cells, its levels being positively correlated with the severity of periodontitis in GCF [49]. The antimicrobial activity of calprotectin is provided by its ability to bind calcium, zinc, copper, and manganese ions. These ions are essential for usual microbial functioning; thus, calprotectin is a growth inhibitory type of host defense [50].

Oral mucosal cells such as epithelial cells are thought to act as a physical barrier against the invasion of pathogenic organisms, but they have also the ability to produce inflammatory cytokines and express adhesion molecules. Gingival tissue of clinically healthy human also expresses low levels of a wide range of toll-like receptors (TLRs), including TLR1-TLR9 that mediate the response to a broad range of microorganisms [51, 52]. However, oral epithelial cells are refractory to many bacterial components, although they express toll-like receptors/MyD88 and acquire responsiveness after priming with IFN-gamma. When the cells are stimulated with lipopolysaccharides and neutrophil protease (PR3) after IFN-gamma priming, the cells produce interleukin 8 (IL-8), which is critical to Th1 and Th2 responses. PR3 itself is able to activate the cells through G protein-coupled protease-activated receptor-2 on the cell surface.

Also, gingival fibroblasts are well equipped to respond to bacterial components and may contribute to the IL-8 levels observed in clinically healthy tissue [53]. Studies in germ-free mice show that there are low levels of innate immunity mediators present in the periodontal tissue [54, 55], indicating that a basic level of cytokine expression is genetically programmed, without bacterial challenge. Any changes of dental plaque composition modify cytokine expression [54, 56]. As an example, an *in vitro* study is showing that the TLR response can be manipulated by *P. gingivalis* toward two types of lipopolysaccharides: PgLPS1690 (type I) and PgLPS1435/1449 (type II). Type I is a TLR4 agonist, thus activating the immune system, while type II is a TLR4 antagonist inhibiting the immune response to *P. gingivalis* [57]. The expression of these two types of LPS is regulated by the concentration of iron from the hemin found in the GCF [58]. During inflammatory process, *P. gingivalis* type II LPS expression increases which reduces the TLR4 response. It was proposed that this could facilitate survival and multiplication of the entire microbial community [59]. *P. gingivalis* can block gingival epithelial cells IL-8 production *in vitro*, by secreting a serine phosphatase that inhibits the synthesis of IL-8 [60]. This process can delay the recruitment of neutrophils preventing the proper formation of the neutrophil wall, facilitating initial microbial colonization of the periodontium [61]. Other bacteria such as *T. denticola* are also able to manipulate the interleukin response of the host by yet not understood mechanism(s) [62].

### 2.1.1. Neutrophils

The primary function of both nonspecific and specific mucosal immunity is to protect the teeth, jaws, gingiva, and oral mucosa against infection. In healthy individuals, periodontal tissue

contains a wall of neutrophils, between the plaque and the epithelial surface, and cellular infiltrate located in juxtaposition to the colonized tooth surface, closest to the dental plaque biofilm [63]. Expression of mediators such as interleukin 8 (IL-8), intercellular adhesion molecule (ICAM), E-selectin and  $\beta$  defensin molecules 1, 2 and 3 are required to form this neutrophil defense wall [64–66]. E-selectin is required for neutrophil migration from the highly vascularized gingival tissue, IL-8 is a key neutrophil chemo-attractant produced by epithelial cells, and ICAM facilitates adhesion of neutrophils to the tissue allowing formation of this wall [67, 68].

### 2.1.2. Complement system

The complement system is a major component of the innate immune response involved in recognizing and destroying microorganisms, with complex roles in homeostasis and disease. Activated complement fragments are abundantly found in the GCF of periodontitis patients, whereas they are absent or present in lower concentrations in healthy individuals [69, 70]. To be a successful pathogen in humans (and any other mammal), a microorganism needs to be able to avoid complement-mediated detection and killing. *In vitro* studies have shown that periodontal bacteria, such as *P. gingivalis*, *T. denticola* and *Prevotella intermedia* could interact with the complement system in complex ways that either inhibit or activate specific complement components [71]. One of the best-studied species from the oral cavity is *P. gingivalis* that produces membrane bound and soluble arginine-specific cysteine proteinases called “gingipains” that can destroy complement factors (C3 and C5) and thus render the bacteria resistant to the bacteriolytic activity of complement system [72, 73].

## 2.2. Oral microbiome dysbiosis and periodontitis

Recent discussions on the definition of general health have led to the proposal that human health is the ability of the individual to adapt to physiological changes, a condition known as *allostasis* [74]. The *allostasis* in the oral cavity is a complex phenomenon, since the relationship between the oral microbiome and its host is dynamic and any physiological or hormonal changes of the host can affect the balance of the species within these communities [75]. The idea that the accumulation of dental plaque is responsible for oral disease, but without discriminating between the different virulence levels of bacteria, has led to the “Non-Specific Plaque Hypothesis” [76]. The two most common oral diseases, caries and periodontal disease, are highly abundant among the population of industrialized countries, having a major impact on the populations’ well-being and healthcare providers [77].

The factors responsible for the transition from periodontal health to either gingivitis or periodontitis are the acquisition of certain species/combinations of species (**Table 1**) and less than optimal host response, which in extreme cases removes the local environment by causing loss of dentition to protect the host from life-threatening bacterial infections [78]. Although the periodontal disease microbiomes are more diverse in terms of community structure, that structure is quite similar across different patients [79]. Gingivitis is associated with an increased microbial load ( $10^4$  to  $10^6$  organisms) and a corresponding increase in the percentage of gram-negative organisms (15–50%) [80]. An interesting finding is that the microbiota of older subjects, with no prior history of gingivitis, had up to 45% gram-negative species including *Fusobacterium*



<b>Normal</b>	<b>Gingivitis</b>	<b>Periodontitis</b>
<i>Streptococcus oralis</i>	<i>Streptococcus oralis</i>	<i>Porphyromonas gingivalis</i>
<i>Streptococcus sanguis</i>	<i>Streptococcus sanguis</i>	<i>Aggregatibacter actinomycetemcomitans</i>
<i>Streptococcus mutans</i>	<i>Capnocytophaga ochracea</i>	<i>Treponema denticola</i> other spirochetes
<i>Streptococcus anginosus</i>	<i>Capnocytophaga gingivalis</i>	<i>Bacteroides intermedius</i> <i>B. pneumosintes</i>
<i>Actinomyces odontolyticus</i> subsp. <i>nucleatum</i>	<i>Actinomyces iraelii</i> , <i>Act. naeshundii</i>	<i>Fusobacterium nucleatum</i>
<i>Eubacterium nodatum</i>	<i>Actinomyces odontolyticus</i>	<i>Selenomonas sputigena</i>
<i>Campylobacter gracilis</i>	<i>Eubacterium brachy</i>	<i>Lactobacillus sp.</i>

**Table 1.** The specific composition of subgingival bacterial plaque in gingivitis and periodontitis.

*nucleatum*, *P. gingivalis*, *P. intermedia*, *Campylobacter rectus*, *Eikenella corrodens*, *Leptotrichia*, and *Selenomonas* species, demonstrating that an individual is more likely to carry gram-negative bacteria and periodontal pathogens in healthy sites with increasing age [59, 81]. It is likely that the presence of these periodontal pathogens in healthy sites alters the host response, rendering these sites more susceptible to active disease in the future.

The microbiota associated with periodontal disease seems to display a significant enrichment in specific metabolic pathways, compatible with an oxygen poor environment, and the availability of amino acids and lipids as major carbon and nitrogen sources [82]. The unbalanced microbiota is rich in lipid-degradation pathways, as well as other known virulence-related activities, such as lipopolysaccharide (LPS) biosynthesis with local inflammatory effect [83].

The presence of pathogens within this community can lead to the clinical manifestations of periodontal disease, which in turn can lead to additional changes in the community due to the increased availability of nutrients released by the damaged tissue.

Although the bacteria rarely invade the tissues and cause acute infections (e.g., *Prevotella intermedia* invades both epithelial cells and macrophages), they could release substances that penetrate the gum and directly causes the tissue destruction by the enzymes and endotoxins action or indirectly through the induction and maintenance of the chronic inflammatory process, leading to the progressive destruction of collagen in the connective tissue that hold teeth in the gum [84, 85]. The presence of *P. gingivalis* and high colonization by *A. actinomycetemcomitans*, *T. denticola* and *P. intermedia* plays an important role in severe periodontitis.

The presence of *P. gingivalis* in the dental plaque biofilm results in the inhibition of components of the innate host defense system. This is caused by the lack of IL-8 that normally guides leukocytes to the site of bacterial colonization. In addition, E-selectin that facilitates leukocyte

exit from the vasculature into surrounding tissue is absent. The local inhibition of these inflammatory mediators results in the lack of sufficient leukocytes to properly control dental plaque growth, proposed to be one of the major factors in the development of periodontitis. Also, it has been demonstrated that individuals co-infected with *T. denticola* and *P. intermedia* were more likely to have periodontitis than were those infected with a single pathogen [86].

Gram-negative bacteria in particular are known to release large amounts of cell wall material as outer membrane vesicles containing lipopolysaccharide, lipid and protein that are believed to represent a normal mechanism of membrane turnover. In addition, the release of membrane vesicles and cell wall fragments serves to protect bacteria in the biofilm by acting as decoys that bind and activate innate host defense components (i.e., alternative pathways of activation of complement system) and that would otherwise bind to the surface of viable bacteria and kill them; in the same time, the total amount of LPS and the consecutive inflammatory effect are increasing [59].

Lipopolysaccharide has been reported to pass through an intact epithelial cell barrier and concentrate around blood vessels in the *lamina propria*, interacting with nearly all cell types present in the periodontium, inducing a large increase in the numbers of leukocytes, especially neutrophils, in the sulcus or pocket, causing ulcerations [87]. At an early stage, the infiltrate is dominated by B and T (Th1 and Th2) lymphocytes. Subsequently, the lesion becomes dominated by B cells, and less T cells, macrophages and neutrophils, all of which becoming activated. As the disease worsens, periodontal pockets deepen, the components of the extracellular matrix of the gingiva and periodontal ligament are destroyed, and alveolar bone is desorbed [88]. On the other hand, bacterial LPS can subsequently interact with macrophage or dendritic cell receptors, including CD14 and TLRs, to stimulate the production of inflammatory cytokines, especially IL1, and other proinflammatory mediators [89].

The collagen and other components of the perivascular extracellular matrix are destroyed, by the release of lysosomal enzymes by phagocytes and the production of cytokines that stimulate the release of metalloproteinases (including collagenase) by connective tissue cells or cytokines that activate bone resorption [90]. Four distinct pathways may be involved with this destruction: plasminogen dependent, phagocytic, osteoclastic and matrix metalloproteinase (MMP) pathway which is the most prevalent, as revealed by the larger amounts of collagenase and gelatinase (MMP1, MMP2, MMP9, and MMP13) found in the crevicular gingival fluid of patients with periodontitis [91–93]. Cleavage of collagen I by MMP13 seems to be the initial step of the entire bone resorption process [94, 95], and subsequently, denatured collagen fragments are also degraded by gelatinases, MMP2 and MMP9 [96].

Several species, in the subgingival plaque constitution, produce volatile fatty acids (butyrate, propionate, and isobutirat) and peptide N-formyl-methionyl-leucyl-phenylalanine, as sulfide ions, hydrogen sulfide, and methyl mercaptan with cytotoxic effect on endothelial cells and gingival fibroblasts [97, 98].

The bacterial products and epithelial response activate perivascular mast cells to release histamine that activates vascular endothelial cells to release IL-8 within the vessels to assist in localizing neutrophils. B and T lymphocytes are activated by antigens or unspecific mitogens to

proliferate and give rise to clones of effector cells; B cells are driven to differentiate into clones of antibody producing plasma cells [99, 100]. Bacterial material released in the periodontium provides thus a major form of communication between dental plaque and the host [78].

Bacteria could activate myeloid cells (e.g., monocytes or neutrophils) to elicit IL-1, and this cytokine then activates a nonmyeloid cell (e.g., fibroblasts, endothelial, or epithelial cells) to secrete additional inflammatory mediators, interleukins 6, 10 and 12, tumor necrosis factor alpha, prostaglandin E2, interferon gamma, and a series of chemotactic substances: monocyte chemoattractant protein, macrophage inflammatory protein, and RANTES (regulated on activation, normal T-cell expressed, and secreted) [88, 101].

After the trend of 1950s and early 1960s, when periodontal treatment was based on the non-specific plaque hypothesis, criteria for defining periodontal pathogens have been developed.

In periodontal disease, the precise identification of certain organisms (e.g., a particular clone (JP2) of *Aggregatibacter actinomycetemcomitans*) is required to identify the risk factors for localized aggressive periodontitis in young adults. Taking into account the difficulties of periodonto-pathogen cultivation, new techniques have been developed in order to detect bacterial species associated with periodontal disease: PCR-based methods in single or multiplexed approaches, sequencing 16S rRNA gene fragments or a housekeeping gene, next-generation sequencing [102]. Now, the Human Oral Microbiome Database project is undergoing, which aims to catalog all bacterial species found in the oral cavity. However, the molecular techniques are limited to the detection of a selected number of pathogens, so other important disease factors arising from the deregulation of the local host response could be missed [103].

### **3. Mechanisms of dental plaque resistance to antimicrobials and strategies to fight them**

Dental plaque displays properties that are typical for biofilms, being structurally and functionally organized polyspecific communities embedded in an extracellular matrix of exopolymers on mucosal and dental surfaces [104], functionally organized and benefiting of increased metabolic efficiency, pathogenic synergism and enhanced virulence, greater resistance to host stress factors and tolerance to all kind of antimicrobials [105].

Resistance to antibiotics (genetic or phenotypical, due to biofilm formation) is the most important cause of nonefficient therapy of biofilm-associated infections from the oral cavity, and it is multifactorial.

One of the main reasons for the antibiotics ineffectiveness against the periodontal pathogen bacteria is that they grow in biofilms, becoming increasingly powerful, aggressive, and difficult to destroy. The antibiotics effective doses and the minimum concentration to eradicate biofilms are very difficult to achieve *in vivo*, especially in the local treatments. Biofilm penetration by biocides or antibiotics is typically strongly hindered. To increase the efficiency of new treatment strategies against bacterial and fungal infections, factors that lead to biofilm growth

inhibition, biofilm disruption, or biofilm eradication are being sought. These factors could include antibiotics, e.g., chlorhexidine, triclosan, povidone-iodine active against *P. gingivalis* and *F. nucleatum* biofilms [106]—azithromycin and other macrolides could block quorum sensing mechanism and the alginate polymer formation [107].

The widespread use of antibiotics has evolutionary and ecological consequences, leading to the recruitment of more genes into the *resistome* and *mobilome*, with adverse consequences for human welfare [108], so new approaches are urgently needed to help regain control over infectious diseases, including periodontal disease.

There are theories which support that horizontal gene transfer of resistance determinants can occur in the oral biofilm [109–112]. This strongly suggests that exchange of mobile genetic elements between commensals and pathogenic bacteria can contribute to the emergence of drug resistance in the oral cavity. Oral microbiota exhibits different resistance mechanisms, presumably due to the complex microbial interactions and the genetic fluidity in oral biofilms. The possibility of conjugation among oral bacteria using an erythromycin-resistant (Erm) shuttle plasmid, from *T. denticola* to *S. gordonii*, was revealed [112].

Amoxicillin and penicillin resistance have been described in *Veillonella* sp., *Fusobacterium*, and *Prevotella denticola* isolated from root canals [113, 114]. High levels of penicillin resistance have been demonstrated in the  $\alpha$ -hemolytic streptococci (*Streptococcus mutans*, *S. salivarius*, *S. oralis*, and *S. mitis*) and represent a cause for concern.

Generally, the  $\alpha$ -hemolytic streptococci are very highly resistant to cephalosporins; *Enterococcus* sp. isolated from root canal exudates patients with periodontal lesions revealed high-level of cephalosporins resistance [115]. In contrast, Kuriyama et al. found that the genera *Porphyromonas* and *Fusobacterium* showed susceptibility to all cephalosporins, while *Prevotella* species were highly resistant [114].

Mechanisms of metronidazole resistance include mutations in the enzymes responsible for reduction of the antibiotic to its active form, mutations resulting in decreased entry of the antibiotic into the cell and mutations in transporters causing the efflux of the antibiotic. These mechanisms have been demonstrated in different species, e.g., *Lactobacillus* sp., *Gemella morbillorum*, *Actinomyces israelii*, *Clostridium butyricum*, *Eikenella corrodens*, and *A. actinomycetemcomitans* [116]. Four genes, *nimA*, *nimB*, *nimC*, and *nimD*, chromosomal or plasmid located, able to confer moderate to high-level metronidazole resistance have been revealed in *Bacteroides* sp. [117].

Mechanisms of tetracycline resistance include efflux proteins, production of ribosome protection proteins, and enzymatic modification of the antibiotic. Tetracycline resistance is encoded by *tet* genes. Antibiotic profiling of  $\alpha$ -hemolytic streptococci isolated from the oropharynx of healthy Greek children showed a high percent of tetracycline resistant isolates [118], the majority of isolates being represented by *S. mitis*. Okamoto et al. [119] studied the prevalence of *tetQ* gene in genus *Porphyromonas* and *Prevotella* sp. They have been demonstrated that *tet(M)* represented the most common gene, which encodes a ribosomal protection protein, carried on Tn916/Tn1545-like conjugative transposons in *Streptococcus* sp., *Granulicatella* sp., *Veillonella* sp. and *Neisseria* sp., from the oral cavity [120].

Resistance to erythromycin is commonly conferred by the acquisition of *erm* gene, antibiotic inactivation by an enzyme encoded by *mph*, and efflux of macrolides by an ATP-binding transporter encoded by *msrA* expressed by *S. aureus* isolates [116]. Low-level macrolide resistance in the oral microbiota may also be associated with the expression of genes from the *mef* family, encoding another efflux pump, and recently have been found on a conjugative transposon Tn1207.3 in *Streptococcus pyogenes* [121]. There have been described [122] erythromycin-resistant  $\alpha$ -hemolytic streptococci, such as *S. oralis*, *S. salivarius* and *S. sanguis* in healthy Greek children. *P. intermedia* isolates carried *erm*(F) alone or *tetQ* alone, but in other oral anaerobes, macrolide resistance often occurred in conjunction with tetracycline resistance. In *Gemella* sp. and commensal viridans streptococci isolates from oral cavity, macrolide resistance was associated with the *aphA-3* gene [123].

There are studies which support that the resistance to chlorhexidine resistance in *Streptococcus mutans* and *S. sobrinus* isolates from dental plaque is plasmid-mediated [124, 125].

Bacteria growing in biofilms often exhibit differing phenotypes compared with their counterpart, respectively planktonic or free cells. There have been described numerous mechanisms for the differences in antibiotic susceptibilities in biofilms relative to planktonic state growth cells. Among these, there have been demonstrated by several studies that oxygen limitation [126], antibiotic penetration into the biofilm [127], the presence of persister cells [128], biofilm-associated cells that grow significantly more slowly than planktonic cells and, as a result, take up antimicrobial agents more slowly [83], and also the maturity of the biofilm might also be important contributors to increased resistance. Results of previous studies have demonstrated also other important mechanisms responsible for resistance to antibiotics [129]: biofilm growth is associated with an increased number of mutations, leading to generation of antibiotic-resistant phenotypes of bacteria, and genes involved in antibiotic resistance are correlated with biofilm phenotype [130]; the production of the exopolysaccharide matrix contributes to an increased cell survival by slowing down antimicrobial diffusion speed; and the differences in metabolic activity among bacteria. It has been revealed that slow-growing and nongrowing bacteria contribute to increased biofilm resistance to antibiotics [131]. The up-regulation of efflux pump proteins and activation of quorum sensing systems reduces and neutralizes incoming antibiotics.

Altered gene expression represents another difference between bacteria grown in biofilms compared with planktonic cells. There are numerous genes that are either positively or negatively regulated by the complex regulatory networks, efflux pumps when the bacteria are growing as a biofilm compared with planktonic cultures [132, 133].

Numerous plants are used in traditional medicine against various diseases. Furthermore, plant extracts have pronounced antimicrobial activities when used at sub-inhibitory concentrations, which are usually very low concentrations with minimal or no effect against host cells. Using sub-inhibitory concentrations of an antimicrobial compound, namely concentrations which do not interfere with bacteria growth, but only with their behavior, leads to reduced risk of developing resistance to that compound. The most recent strategies propose the targeting of communication control, as QS signaling, since quorum sensing is not an essential process, and QS mutants in general have not displayed growth

defects, but this signaling controls virulence and biofilms. The quorum sensing mechanism involves the production, release and detection of chemical signaling molecules, which permits communication between microbial cells and gene expression regulation in a cell-density-dependent manner. Granted, interfering with the regulation of virulence factor production developing resistance mechanisms against quorum-inhibiting therapies, may be a difficult proposition for bacteria, which could help promote long-term efficacy of anti-QS therapies [134].

Recent studies revealed that numerous plant-derived compounds and essential oils (EOs) exhibit increased antimicrobial properties, by interfering with QS controlled phenotypes such as adherence, biofilm, formation, motility and pigment production, affecting also antibiotic susceptibility, or revealing microbiostatic properties [135–137]. Not only plant extracts, but also propolis extracts have proved to possess a broad spectrum activity against various gram-positive and gram-negative bacteria: *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., *Bacillus* spp., *Enterobacteria* (*Klebsiella pneumoniae*, *Escherichia coli*), *Pseudomonas aeruginosa*, and *Helicobacter pylori* [138, 139]. The antibacterial activity of propolis is mainly correlated with caffeic acids, flavonoids, phenolic esters, and aromatic compounds [140].

Relatively few research works on propolis ability to inhibit biofilm formation have been published. Duarte et al. have shown that propolis inhibits the growth of oral microorganisms and the activity of bacteria-derived glucosyltransferases (GTFs), responsible for glucan synthesis which favors bacterial adhesion and plays an essential role in the development of pathogenic dental plaque [141]. Bulman et al. showed that propolis contains compounds that inhibit signaling mediated by N-acyl-homoserin-lactone in *Pseudomonas aeruginosa* PAO1 [142]. Our studies have shown that the 30% Romanian propolis tincture presented antibacterial activity toward *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*, and antibiofilm activity against *S. aureus* [139]. Associated with the use of some antibiotics, the efficacy and duration of propolis extract action is more pronounced, and these organisms do not develop antibiotics resistance, as demonstrated for dexpanthenol associated with propolis against *P. aeruginosa* and *S. aureus* strains isolated from infected wounds.

These data confirm that natural extracts have anti-QS, antiseptic, and antivirulence properties and can easily inhibit biofilm formation as well as disrupt the mature biofilm structure. Thus, plant and/or bee extracts in combination with other antimicrobial strategies could provide an effective microbicidal tool for the treatment of various bacterial and yeast infections. However, due to difficulties in cultivating anaerobic periodontal pathogens, there are only few studies concerning the efficiency of vegetal extracts against periodontal pathogenic strains. Chifiriuc et al. (2009) demonstrated that usnic acid selectively inhibited the biofilm development by Gram-positive bacteria and the expression of hemolytic properties of strains isolated from the dental plaque [143].

It has been shown that mouthwash with essential oils (EO) might be a reliable alternative to chlorhexidine (CHX) for controlling gingival inflammation, dental plaque development, bacteremia with anaerobic bacteria in patients with mild-to-moderate gingivitis and oral malodor [144, 145]. Moreover, the diluted EO displayed no detectable detrimental effects on human gingival and periodontal ligament fibroblasts, while diluted CHX reduced both cell migration and long-term survival [146]. The regular long-term use of the EO-based mouth

rinse improved the efficacy of a 0.05% cetylpyridinium chloride- and fluoride-containing mouth rinse [144]. Linalool and  $\alpha$ -terpineol exhibited strong antimicrobial activity against periodontopathic and cariogenic bacteria [147]. *Salvadora persica* root stick extracts and its active component benzyl isothiocyanate are very efficient against oral pathogens involved in periodontal disease as well as against other gram-negative bacteria [148]. The adjunctive use of EO has been shown to promote significant clinical attachment level gain and probing pocket depth reduction in deep residual pockets [149]. A gel containing 10% *Lippiasidoides* (LS) was evaluated and has been shown to reduce plaque, bleeding, and gingival index within the experimental period of 21 days [150].

These scientific data suggest that the antibiofilm compounds should be used in various combinations, in order to develop innovative early combinatory strategies [151] which may potentially strongly support classical treatments and cause an increase of their effectiveness in case of chronic infections, such the periodontal ones.

Other strategies in the improvement of the biological activity used in the management of periodontal diseases are based on the encapsulation of therapeutic drugs in appropriate shuttles. Drug delivery by liposomes with different encapsulated bioactive molecules (such as bacteriocins, enzymes, antiseptics, antibiotics, and vegetal compounds) can assure a controlled delivery of some antimicrobial substances at infection's situs, with a great efficiency in dental caries and every other biofilm-associated infection [152]. Encapsulation technologies, which may shield substances such as nisin from degradation by digestive enzymes and effectively deliver the encapsulated contents at the same time, represent new direction in the field of preventive medicine [153].

## 4. Future perspectives for the therapeutic management of periodontitis

Despite numerous current approaches, periodontitis still remains one of the most common disease, causing moderate-to-very-severe health damage and complications in a high number of individuals. Perspectives for future and advanced therapies aiming for efficient management of periodontal conditions flourished in the last decade, and the recent progress in science and technology made possible a wide range of novel approaches to be successfully applied.

### 4.1. Periodontal tissue regenerative therapy

This approach was formulated in 1993 by Langer, who proposed tissue engineering as a possible technique for regenerating lost periodontal tissues. This rapidly emerging research field represents the interface between materials science and biocompatibility and integrates cells, natural, or synthetic scaffolds, and specific signals to obtain new tissues [154].

Tissue engineering applied for bone and periodontal regeneration combines three key elements to enhance regeneration: use of progenitor cells, design of scaffolds or supporting matrixes, and selection of suitable signaling molecules. Cell sources of progenitor cells may be represented by periodontal ligament-derived cells, periodontal ligament-derived

mesenchymal stromal cells, periosteal cells, gingival epithelium and fibroblast cells but also bone marrow-derived mesenchymal stem cells [154].

Scaffolds and supporting matrices are required to offer a three-dimensional (3D) support and assist periodontal tissue regeneration. These structures have important roles, such as: (1) suitable framework, which maintains the shape of the defect; (2) physical support for the healing area, so that there is no collapse of the surrounding tissue into the wound site; (3) 3D substratum for cellular adhesion, migration, proliferation and production of extracellular matrix; (4) selective barrier to restrict cellular migration; and (5) delivery vehicle for growth factors and differentiation molecules [154]. Biomaterials utilized for efficient scaffolds are very diverse, and they may be included in various categories, such as: ceramics (i.e., hydroxyapatite, beta tricalcium phosphate), polymers (i.e., synthetic: polyglycolic acid, polylactic acid and polycaprolactone; natural: collagen fibrin, albumin, hyaluronic acid, cellulose, chitosan, polyhydroxyalkanoates, alginate, agarose, polyamino acids, etc.), and synthetic polyesters (i.e., polyglycolic acid, polylactic acid and polylactic-co-glycolic acid) [155].

In order to increase efficiency, regenerative therapy relies on the incorporation of various bioactive molecules into scaffolding materials, to improve cellular development and tissue healing. The incorporation of specific bioactive molecules within the scaffold is aiming to ensure a sustained and controlled release of bioactive molecules for longer periods of time. These bioactive molecules can be incorporated directly into the scaffolding material or with along with a delivery vector, which ensures its stability and controlled release.

The most utilized bioactive molecules to be integrated in scaffolds designed for periodontal tissue regenerative therapy are as follows: platelet-derived growth factor (potent mediator of periodontal tissue regeneration, currently approved for the treatment of periodontal defects—commercially available as Gem-21 (Osteohealth, Shirley, NY)); fibroblast growth factor (which has a profound effect on periodontal soft tissue and bone healing and also stimulates angiogenesis) [156]; bone morphogenetic proteins (disulfide-linked homodimer that promotes periodontal healing) [157]; insulin-like growth factor (which has mitogenic effects on periodontal ligament fibroblastic cells and can stimulate the synthesis of DNA in periodontal ligament fibroblasts) [158]; transforming growth factor beta (act as bone coupling factor linking bone resorption to bone formation) [159]; and periodontal ligament-derived growth factor (it is a highly specific autocrine chemotactic agent for human periodontal ligament cells, which is 1000-fold more potent than many known growth factors, and has no chemotactic effect on gingival fibroblast or epithelial cells, thereby promising its utility for biological therapeutic regime needed for cell-specific periodontal regeneration) [159].

Recent progress in tissue engineering has allowed the delivery of such molecular factors by various means. Gene therapy and nano-delivery represent the most investigated approaches for the delivery of bio-active molecules, useful for regenerative medicine applied for periodontal disease.

## 4.2. Gene therapy

Gene therapy presents certain advantages when compared to other therapies. Because both cell transplantation and laboratory cell culturing are not needed, gene therapy may be safer



and more cost-effective than cell-based therapies [154, 160]. Platelet-derived growth factor and bone morphogenetic proteins are the most utilized for delivery in periodontal regenerative therapy. Plasmid and circular vector-based delivery of platelet-derived growth factor proved safety favorable characteristics for clinical use. Moreover, the expression of platelet-derived growth factor genes was prolonged for up to 10 days in gingival wounds, when administered through this approach. It seems that continuous exposure of cementoblasts to platelet-derived growth factor has inhibitory effect on cementum mineralization, possible via the upregulation of osteopontin and subsequent enhancement of multinucleated giant cells in cementum-engineered scaffolds [161].

The delivery of genes that encode the bone morphogenetic proteins stimulate the formation of periodontal tissue formation. Moreover, the expression of this gene promoted successful regeneration of alveolar bone defects around dental implants [161]. Ribonucleic acid mediated silencing, a novel approach, is based on the principle of RNA interference (RNAi), a novel mechanism of action whereby the expression of certain genes detrimental to the tissue regeneration process is silenced by RNAs. The first siRNA-based therapeutic tested in human clinical trials was the vascular endothelial growth factor (VEGF)-targeted RNA for the treatment of macular degeneration of the retina. Tumor necrosis factor- $\alpha$ -targeted siRNA can suppress osteolysis induced by metal particles in a murine calvaria model, opening the way to the application of RNAi in orthopedic and dental implant therapy [162]. The use of RNA-based therapeutics for tissue regeneration is still in its early stages. Nevertheless, RNAi promises to be an effective therapeutic tool and may be successful in periodontal regeneration [154].

## 5. Conclusions

Dental plaque is a model of polyspecific biofilm, very studied mainly due to its accessibility, but also to its implications in dental caries, periodontitis, and periodontal disease—this one being an irreversible affection once launched, very spread in the world. It is of great interest for the field of dentistry and for medicine too, due to its complications, more or less severe, local and at distance too, infectious and noninfectious. A lot of scientific knowledge is accumulated, and therapeutically progresses are done in this field, but the topic still needs improvements and remains a challenge.

Studies have shown that almost all forms of the periodontal disease are consequences of the dental plaque biofilms and of chronic, nonspecific or specific bacterial infections and chronic inflammation too. Along with dental plaque, the occurrence of periodontal diseases is influenced by numerous other factors, such as the virulence and resistance mechanisms of involved microbial species but also host-related factors. Understanding the complex organization of dental plaque biofilms, the interactions between commensal and pathogenic species in this community but also the relation with the host is vital for elucidating the mechanisms of periodontal diseases and drawing novel therapeutic perspectives.

Although traditional preventive and therapeutic approaches relying on adequate hygiene, mechanical removal of the dental plaque, surgery, and antibiotic treatment are still widely

utilized, recent strategies propose the utilization of numerous modern techniques to specifically target particular aspects of periodontal disease. Their implementation depends on the extensive knowledge regarding intimate biological parameters of various periodontal conditions and could be more effective in both the prevention and therapy of such diseases.

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# **Periodontal Tissue Alteration During Health, Disease and Treatment**

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# Cellular Response Mechanisms in *Porphyromonas gingivalis* Infection

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69019>

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## Abstract

The pathogenicity of the periodontal biofilm is highly dependent on a few key species, of which *Porphyromonas gingivalis* is considered to be one of the most important pathogens. *P. gingivalis* expresses a broad range of virulence factors, of these cysteine proteases (gingipains) are of special importance both for the bacterial survival/proliferation and for the pathological outcome. Several cell types, for example, epithelial cells, endothelial cells, dendritic cells, osteoblasts, and fibroblasts, reside in the periodontium and are part of the innate host response, as well as platelets, neutrophils, lymphocytes, and monocytes/macrophages. These cells recognize and respond to *P. gingivalis* and its components through pattern recognition receptors (PRRs), for example, Toll-like receptors and protease-activated receptors. Ligation of PRRs induces downstream-signaling pathways modifying the activity of transcription factors that regulates the expression of genes linked to inflammation. This is followed by the release of inflammatory mediators, for example, cytokines and reactive oxygen species. Periodontal disease is today considered to play a significant role in various systemic conditions such as cardiovascular disease (CVD). The mechanisms by which *P. gingivalis* and its virulence factors interact with host immune cells and contribute to the pathogenesis of periodontitis and CVD are far from completely understood.

**Keywords:** host-microbe interaction, immune cells, pathogen recognition receptors, intracellular signaling, inflammatory responses, *Porphyromonas gingivalis*, gingipains, LPS, cardiovascular disease, treatment

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## 1. Introduction

Evidence suggests that it is the early host-inflammatory and immune responses to the oral microbiota that changes the subgingival environment and favors the emergence of periodontal

opportunistic pathogens during the development of periodontitis. Substances released from the dental biofilm, such as lipopolysaccharides, proteolytic enzymes, and other virulence factors, activate the innate immune system and initiate an inflammatory response, which disrupts the host-microbe homeostasis. The activation of immune cells leads to a release of an array of inflammatory mediators, for example, cytokines, chemokines, proteases, reactive oxygen species (ROS), and eicosanoids, which struggle against the bacterial burden. However, the complexity of the microbial biofilm of the subgingival dental plaque and the failure of the acute inflammation to resolve lead to an accumulation of mediators of the innate and adaptive immune systems that collectively promote chronic inflammation and tissue destruction. How host cells discriminate commensal from pathogenic microbial species and why this ability seems to differ between individuals is currently unknown. The variation in individual susceptibility to develop periodontal disease appears to be determined by the magnitude of the inflammatory response to a dysbiotic microbial community and whether only the innate or also the adaptive immune pathways are activated.

## 2. *Porphyromonas gingivalis* in periodontitis

There are a number of bacterial species that are associated to periodontitis, based on their detection in periodontal pockets, their pathogenicity, and the immunological responses they evoke [1]. The red complex is a consortium of three periodontal bacterial species, *Treponema denticola*, *Tannerella forsythia*, and *Porphyromonas gingivalis*, which are linked to each other and to diseased sites [2]. The development and progression of periodontitis is believed to be due to a synergistic and dysbiotic polymicrobial community, and the oral biofilm (dental plaque) [3]. A biofilm is a highly structured, three-dimensional matrix with a simple circulatory system. The biofilm provides physical protection and a gradient of oxygen, allowing anaerobic species to grow in the deeper pocket, and aerobic species near the surface. Furthermore, metabolic by-products from one species can be used as nutrients by other species in the biofilm, the so-called cross-feeding [4]. The keystone species hypothesis suggests that some species, like *P. gingivalis*, exerts a disproportionately large effect in the biofilm. *P. gingivalis* can turn from a natural low-abundance microorganism residing in the oral cavity to an opportunistic pathogen that interferes with the host immune system and from a normal, symbiotic microbiota, and enables the transition and emergence into a dysbiotic bacterial society that drives the progress of periodontitis [2, 5]. *P. gingivalis* is a late colonizer usually found in a rather low number in the dental plaque, and interestingly, *P. gingivalis* is not able to induce periodontitis in germ-free mice, suggesting that *P. gingivalis* is dependent on the complex microbial community. Through synergistic interactions, the biofilm promotes colonization, nutrition acquisition and subvert, and evades host immune responses [4, 6].

*P. gingivalis* is a non-motile, proteolytic, and Gram-negative rod that expresses several virulence factors that are related to colonization of oral tissues, periodontal tissue destruction, and evasion of the host responses [7]. *P. gingivalis* exhibits genotypic and phenotypic diversity, which results

in differences in virulence and in the capacity of individual strains to colonize and induce destruction of periodontal tissues. Certain strains may therefore exhibit a higher pathogenic potential than others and may be linked to a more severe form of periodontitis [8–12]. The asaccharolytic bacterium *P. gingivalis* grows under anaerobic conditions and acquires metabolic energy by fermenting amino acids. *P. gingivalis* also uses micronutrients, such as metal ions for anabolic and catabolic purposes, as well as vitamin K. *P. gingivalis* expresses a broad range of virulence factors, all of which add to enhanced growth and survival in a hostile environment [7]. However, the virulence of *P. gingivalis* is affected by its surroundings, including other bacterial species in the biofilm and host-derived factors. By altering the gene expression of virulence factors, *P. gingivalis* can adjust to a more or less virulent phenotype depending on the environment [13].

Fimbriae are hair-like protrusions emanating from the outer cell surface that facilitate the adherence and colonization of the bacterium. Indeed, fimbriae are critical for mediating the initial bacterial interaction with the host tissue. *P. gingivalis* expresses major and minor fimbriae, encoded by the *fimA* and *mfa1* genes, respectively. Today, six *fimA* allele types are known (*fimA* I, Ib, II, III, IV, and V). These variants are more or less associated to periodontitis [14]. *P. gingivalis* isolated from periodontally healthy persons more often expresses type I, II, or V. Types Ib, II, and IV, on the other hand, are more associated to diseased periodontal pockets [9, 15]. Major fimbriae can attach and bind to host cells, extracellular matrix (ECM), as well as salivary proteins. Major fimbriae can also facilitate binding to other bacteria, both *P. gingivalis* itself and other species. Minor fimbriae have a role in biofilm formation [14, 16].

As a Gram-negative species, *P. gingivalis* possesses lipopolysaccharides (LPS). Intriguingly, the lipid A part of *P. gingivalis* LPS has a structure that is heterogeneous. The number of associated fatty acids coupled to the disaccharide core varies, resulting in penta- or tetra-acylated lipid A moieties that allows interaction with both Toll-like receptors (TLR) 2 and TLR4 [17]. It is the availability of hemin in the microenvironment that defines which lipid A form that *P. gingivalis* expresses, enabling the bacteria to determine how it interacts with the host to elicit various inflammatory responses [8, 18].

Gingipains are cysteine proteases which probably are the most vital virulence factor expressed by *P. gingivalis*. Gingipains are membrane-bound, as well as secreted from the bacterium, thus, *P. gingivalis* can exert all the various gingipain activities at distant sites. *P. gingivalis* possesses arginine-specific gingipains, Rgp (RgpA and RgpB), encoded by *rgpA* and *rgpB*, respectively, and the lysine-specific gingipain, Kgp, encoded by *kgp*. *P. gingivalis* expresses numerous proteolytic enzymes, but the gingipains are by far the most important ones, accounting for at least 85% of the total proteolytic activity. Furthermore, they are implicated and play key roles in adherence and colonization of the host, in nutrition acquisition by cleaving host proteins, in neutralization of host defense mechanisms, and in manipulation of the host inflammatory response. In summary, gingipains are vital for bacterial survival and proliferation *in vivo* [7]. In the process of adherence and colonization, *P. gingivalis* utilizes fimbrial adhesions, but nevertheless,

gingipains are also necessary in these steps. RgpA and Kgp contain hemagglutinin-adhesin domains, which are directly involved in conjugation with other bacterial species, thereby promoting the construction of the bacterial biofilm. These domains also enable binding to ECM, as well as interaction with host cells [19–21]. Rgp is also important for processing various *P. gingivalis*-derived proteins. For instance, Rgp is necessary for the modification of major fimbriae to the mature form [22]. Gingipains are also key mediators in dysregulation of the host immune response [23, 24].

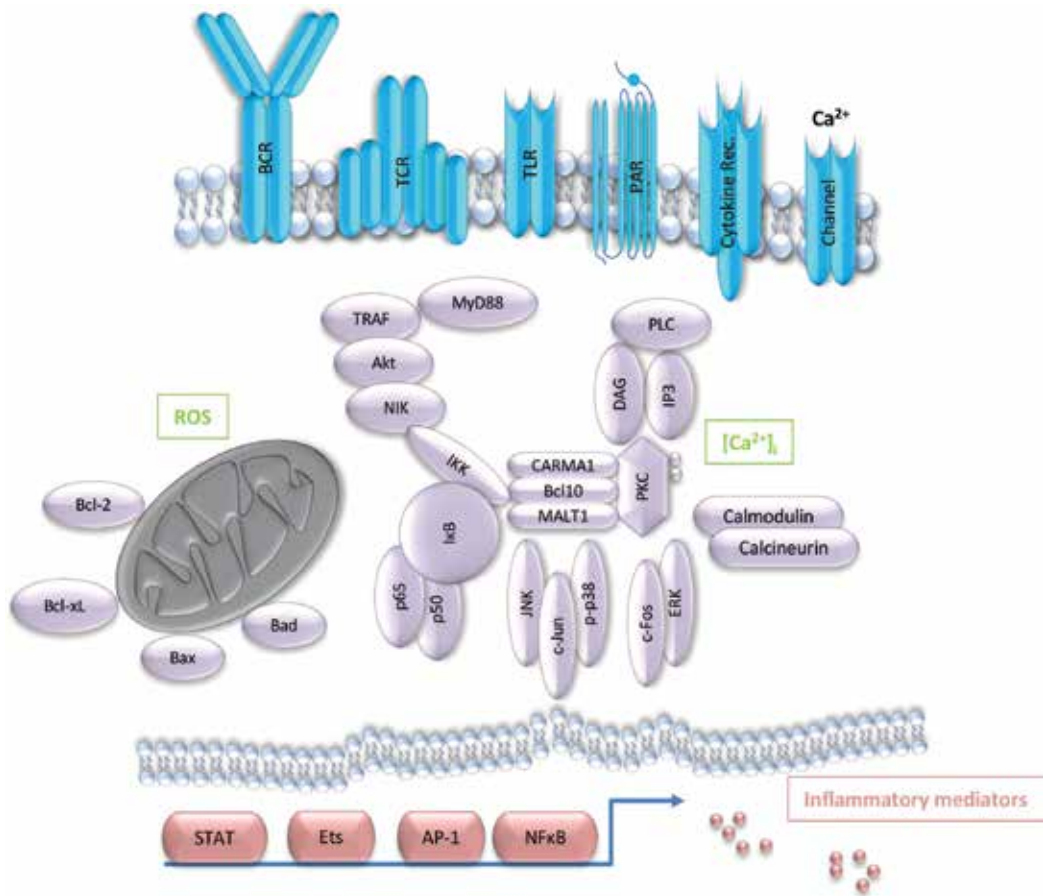
Some *P. gingivalis* strains possess a capsule. Encapsulated strains are more virulent since they have been shown to be more invasive and more resistant to phagocytosis [25–27]. *P. gingivalis* also releases outer membrane vesicles, small cargos that are shed from the outer bacterial membrane that are loaded with LPS, gingipains and other proteases, fimbriae, and capsule (encapsulated strains). The shedding of outer membrane vesicles occurs at a higher rate during colonization and biofilm formation, enabling immune modulation at sites distant from the actual site of infection [28].

### 3. Mechanisms of *P. gingivalis* interaction with host cells

*P. gingivalis*, as a keystone pathogen, has the ability to interfere with the host in such ways that the growth and survival of the entire biofilm is promoted and enhanced. It is vital for *P. gingivalis* in a hostile environment to be able to counteract, modify, and manipulate the host immune response in order to survive and evade the various host defense mechanisms. Although it is important to evade the host defense mechanisms, it is also of essential importance to induce inflammation to secure a constant delivery of nutrients to the biofilm through the formation of the nutrient-rich-inflammatory exudate that constitutes the gingival crevicular fluid. *P. gingivalis* has indeed evolved elaborated strategies to diminish as well as promote inflammation [5]. The complement system, which targets microbes, is itself a target for proteolysis by gingipains. In fact, *P. gingivalis* can both inhibit and stimulate the complement system [29]. Also, depending on the type of lipid A expressed, *P. gingivalis* can act as both a TLR4 agonist and an antagonist and regulate the TLR4-dependent immune responses [10, 18]. Realizing all the clever ways of escaping, it may not come as a surprise that *P. gingivalis*, as an additional function on the repertoire, also is resistant to oxidative killing by phagocytes and can survive phagocytosis by macrophages [26, 30]. Furthermore, *P. gingivalis* is able to activate the coagulation cascade and the kallikrein/kinin cascade, thereby enhancing inflammation [31–33]. *P. gingivalis* can invade host cells and replicate within the cell [34]. *P. gingivalis* is also able to protect itself from neutrophil-released reactive oxygen species, leaving the oxidative burst effortless and instead contributing to the destruction of the periodontium [13, 30].

The interactions between the host immune system and the oral microbial flora involve complex cellular and molecular mechanisms. Several cell types, for example, epithelial cells, dendritic cells, osteoblasts, and fibroblasts that reside in the periodontium, are part of the innate host response, as well as platelets, neutrophils, and monocytes/macrophages. Cells of the innate immune system recognize and respond to pathogens (e.g., LPS, fimbriae, DNA, and proteases) through pathogen recognition receptors (PRRs). Important PRRs are TLRs and protease-activated receptors

(PARs). Ligation of PRRs induces downstream signaling pathways that modify the activity of transcription factors that regulates the expression of genes linked to inflammation. Early cellular events leading to a phosphorylation cascade of mitogen-activated protein kinase (MAPK) signaling include the activation of Protein kinase C (PKC) by diacylglycerol and calcium. Signals transduced via MAPK pathways lead to the assembly and activation of the transcription factor AP-1. TLR activation results in the recruitment of an adaptor protein, which in many cases involves MyD88, followed by a signaling cascade that phosphorylates, polyubiquitylates, and degrades I $\kappa$ B. This allows the transcription factor NF $\kappa$ B to translocate to the nucleus and induces gene expression (**Figure 1**). AP-1, NF $\kappa$ B, and other transcription factors cooperatively regulate genes, such as inflammatory mediators and growth factors that are important in many biological processes [35, 36]. This is followed by the release of inflammatory mediators such as CXCL8 and interleukin (IL)-6. The chemokine CXCL8 attracts and recruits neutrophils to the site of infection and promotes monocyte adhesion to the vessel wall. The infiltrating neutrophils, as well as resident cells and macrophages, release cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1,



**Figure 1.** Overview of receptors and intracellular signaling pathways in response to virulence factors of *P. gingivalis*. See text for details.

and IL-6. These inflammatory mediators will eventually contribute to tissue destruction with alveolar bone loss and a sustained chronic inflammation. In addition, the innate immune system will in turn also activate the adaptive immune system with the involvement of lymphocytes [1, 2, 5].

How host-derived factors such as cytokines, hormones, and reactive oxygen species affect periodontal biofilm formation and bacterial virulence is poorly studied and thus not well understood. A recent study suggests that the host-inflammatory responses affect the physiology of bacteria, for example, by utilizing inflammatory mediators as transcription factors [37]. It thus seems quite reasonable that bacteria have evolved mechanisms to sense their environment and to respond to their surrounding by using inflammatory mediators as regulators to be able to adjust and adapt to a changing environment. Consequently, it is possible that early host-inflammatory and immune responses affect and modulate the composition and function of the oral biofilm and the progression of periodontitis.

TLRs are a family of receptors which are of high importance in the innate immune response in sensing pathogens and other danger-associated signals. LPS and fimbriae originate from *P. gingivalis* signals mainly through TLR2, which mediates the release of inflammatory mediators like CXCL8 [38–40]. *P. gingivalis*-mediated activation of TLR2 has been demonstrated to stimulate differentiation and formation of osteoclasts [40]. A study showed that TLR2<sup>-/-</sup> mice more rapidly cleared *P. gingivalis* infection, had a more efficient phagocytosis of *P. gingivalis*, and also resisted alveolar bone loss despite being repeatedly infected with *P. gingivalis* [41]. TLR2 expression has also been found to be upregulated by *P. gingivalis* [42]. During inflammation, the hemin concentration in the gingival crevicular fluid is high and the tetra-acylated lipid A form is expressed. The tetra-acylated lipid A is acting as a TLR4 antagonist, suppressing TLR4-mediated inflammatory events. The TLR4 antagonist also competitively blocks the binding of TLR4; hence, TLR4 is unable to respond to other bacterial species as well. In addition, since the outer membrane vesicles contain LPS, and can penetrate through the gingival tissue, *P. gingivalis* can dampen the TLR4 effects for the entire oral microbial community. When the hemin concentration is low, inflammation is promoted by expressing penta-acylated lipid A, which works as a TLR4 agonist [10, 18, 43].

PARs have been found to be activated by proteolytic cleaving by gingipains, leading to increased inflammatory response with the release of inflammatory chemokines [39, 44]. PAR2 activation has been demonstrated to induce alveolar bone loss in rats. Since PAR2 is expressed by the cells in the periodontium, *P. gingivalis* and its gingipains are able through PAR2 activation to significantly contribute to the release of several pro-inflammatory mediators that cause degradation of the periodontal tissue [45]. Furthermore, *P. gingivalis* per se has been demonstrated to upregulate the PAR2 expression in gingival fibroblasts [39].

A gradient of CXCL8 is normally established in the healthy periodontal tissue with the highest concentration at the border of the symbiotic dental plaque. This gradient establishes a “wall” of neutrophils, a continuous flow of migrating neutrophils that transit from the vasculature into the periodontium and the gingival crevice. *P. gingivalis* can interact with CXCL8 and this gradient in several ways [2]. In contact with gingival epithelial cells,

*P. gingivalis* expresses phosphoserine phosphatase SerB, which contributes to CXCL8 inhibition [46]. Gingipains are well known to cleave CXCL8, as well as other cytokines and chemokines, such as IL-6, IL-6 receptor, CXCL10, TNF- $\alpha$ , CD14, IL-4, and IL-12 [23, 24, 44, 47–52]. By targeting inflammatory mediators such as CXCL8, the resulting chemokine paralysis leads to inhibited neutrophil recruitment, thereby promoting the growth of the biofilm. Consequently, *P. gingivalis* undermines innate immunity [2]. Furthermore, CXCL8 is secreted in two different isoforms, as a 72 amino acid (CXCL8-72aa) variant from immune cells and as a 77 amino acid variant (CXCL8-77aa) from non-immune cells such as fibroblasts. CXCL8-72aa is a stronger chemoattractant than CXCL8-77aa, but after cleavage of CXCL8-77aa by gingipains, this is shifted so that the CXCL8-77a has a higher chemotactic potential. This could be a mechanism whereby *P. gingivalis*, by creating a gradient of gingipains across the periodontal tissue can suppress neutrophilic response in the periodontal pocket where the concentration of gingipains is the highest. At a more distant site, with lower concentrations of gingipains, the chemotactic function of CXCL8-77aa is increased, enhancing the inflammatory response and thereby promoting leaky vessels and a constant delivery of nutrients to the biofilm [47, 53].

## 4. Host cell responses in the oral cavity

### 4.1. Gingival epithelial cells

The first line of host defense in the gingiva consists of the epithelial cells forming a physical barrier against mechanical stress, exogenous substances, and pathogenic bacteria. This is achieved through different cell-cell junctions, including tight junction and gap junction. *P. gingivalis* uses different strategies to survive and persist in the oral cavity, and invasion of epithelium is one tactical approach in its lifestyle. The advantages of intracellular translocation of *P. gingivalis* into the cells include evasion from immune responses and antibiotics, and accessibility to disseminate to other sites, which collectively leads to persistence and proliferation [4]. The mechanism by which *P. gingivalis* enters epithelial cells is initiated by fimbriae that bind to  $\alpha 5\beta 1$ -integrin, followed by the formation of cellular pseudopodia and entry through early endosomes. Intracellular bacteria are then either sorted to late endosomes followed by lysosomes for degradation, or fused with autophagosomes and subsequently degraded in autolysosomes. However, a large number of bacteria are able to escape through recycling pathways for exocytosis and are able to infect new cells, which facilitate deeper penetration into the host tissue [54]. While in other cell types, such as endothelial and smooth muscle cells, *P. gingivalis* has been reported to reside and persist within autophagosomes, followed by the prevention of lysosomal fusion and formation of autolysosomes [55, 56]. Interestingly,  $\alpha 5\beta 1$ -integrin on epithelial cells has recently been shown to positively correlate with cells in S phase of the cell cycle, and *P. gingivalis* persistence may be associated with the ability to preferentially target dividing cells [57]. The virulence of intracellular *P. gingivalis* is associated with its ability to degrade paxillin and focal adhesion kinase, and may explain the significant periodontal tissue degradation and lack of wound healing and tissue regeneration processes in periodontitis [58, 59].

Epithelial cells also participate in innate immune responses by secreting a variety of cytokines and chemokines, such as TNF, IL-6, and CXCL8 [60]. *P. gingivalis* suppresses cytokine and chemokine accumulation below basal levels *in vitro*. These effects are most probably due to the potent enzymatic action of proteinases. Indeed, leukocytes are manipulated by *P. gingivalis* to express a limited repertoire of inflammatory mediators, while suppressing CXCL8 release, which is termed “local chemokine paralysis” [61]. Interestingly, *P. gingivalis* significantly increased TGF- $\beta$ 1 expression from gingival epithelial cells. TGF- $\beta$ 1 functions as a growth factor with anti-inflammatory characteristics. Besides TGF- $\beta$ 1, *P. gingivalis* was observed to induce the expression of a wide array of different growth factors, including Insulin-like growth factor (IGF), Platelet-derived growth factor (PDGF), endothelial growth factor (EGF), and Hepatocyte growth factor (HGF). We have previously shown that *P. gingivalis* induces high levels of HGF in clinical samples from patients with periodontitis. However, the activity of HGF was significantly reduced in patients compared to healthy controls [62].

## 4.2. Gingival fibroblasts

Gingival and periodontal ligament fibroblasts are the main cell types found in the connective tissue of the periodontium, and they are exposed to pathogens once the epithelial barrier is breached [2, 63]. Fibroblasts provide a structural tissue framework (stroma) and define the microanatomy of the tissue with the key function to regulate and maintain integrity of the connective tissue. Homeostasis of connective tissues is maintained through the production of ECM and by modifying existing ECM by secreting matrix metalloproteinases (MMPs) that cleave and degrade ECM components [64]. The ability of fibroblasts to secrete as well as respond to growth factors and cytokines/chemokines allows reciprocal communication with adjacent cells that facilitates homeostasis of the tissue. Considering the functions of fibroblasts makes it easy to realize that fibroblasts play a vital role in tissue development, differentiation, and repair. Fibroblasts are also of importance in tissue destruction by the release of MMPs and pro-inflammatory cytokines and chemokines [63–65]. PAR1 and TLR2 have been shown to be important in the interaction between gingival fibroblasts and *P. gingivalis*. Gingival fibroblasts can sense *P. gingivalis* through PAR1 and TLR2, and the activation of these receptors leads to the secretion of CXCL8 and IL-6, suggesting that fibroblasts could make a substantial contribution to the inflammatory process seen in periodontitis [38, 39, 66]. Furthermore, *P. gingivalis* is able to modify this response by cleaving fibroblast-derived cytokines through the proteolytic activity of the gingipains and thereby hampering the antimicrobial capacity of the fibroblasts [23, 24, 66].

## 4.3. Leukocytes

Periodontitis is characterized by interaction between a number of oral pathogens, such as *P. gingivalis*, and blood leukocytes. Neutrophils and monocytes are well equipped with PRRs, such as TLRs, nuclear-oligomerizing domains  $\frac{1}{2}$ , and PARs. This arsenal of receptors enables the detection of invading pathogens and production of reactive oxygen species, cytokines, and chemokines. We have shown that *P. gingivalis* is capable of inducing ROS in isolated neutrophils and in whole blood, and stimulating the release of inflammatory mediators, such as IL-1 $\beta$  and CXCL8 [67]. Both these cytokines are capable of priming neutrophils, endothelial



cells, and other vascular cells in an autocrine and paracrine manner. Studies have demonstrated that gingipains hydrolyze pro-inflammatory cytokines, but not growth factor/anti-inflammatory cytokines, which result in aberrant immune cell recruitment to the site of infection, ensuring a continued low-grade infection.

The critical balance of different T-cell subsets has previously been described to play an important role in the inflammatory process underlying periodontitis. The presence of specific antibodies for oral bacteria in patients with periodontitis indicates an involvement of adaptive immune responses [68], of which different T-cell subsets play a detrimental role in the pathogenesis of this inflammatory disease. The T-cell-associated cytokine profile in gingival tissue suggests an engagement of T-helper (Th) 1, Th2, and Th17 cells [69–71]. These T-cell subsets are associated with host-derived tissue destruction and bone loss, through, for example, Receptor activator of nuclear factor kappa-B ligand (RANKL) expression. Exaggerated pro-inflammatory responses from T-cells can be controlled by regulatory T-cells (Tregs) that display protective effects through the secretion of anti-inflammatory IL-10 and TGF- $\beta$ 1. Tregs have a central role in maintaining homeostasis by regulating other leukocyte functions and thereby avoiding extensive immune cell activation and its pathological consequences, for example, in periodontitis. Interestingly, we have previously shown that T-cell interaction with *P. gingivalis* leads to a gingipain-mediated inactivation of IL-2 [72], which may thus downregulate Tregs and support the process of periodontitis. Thus, the inhibition of gingipains and maintenance of a Treg-mediated beneficial homeostasis may be a successful strategy for the prevention and treatment of periodontitis.

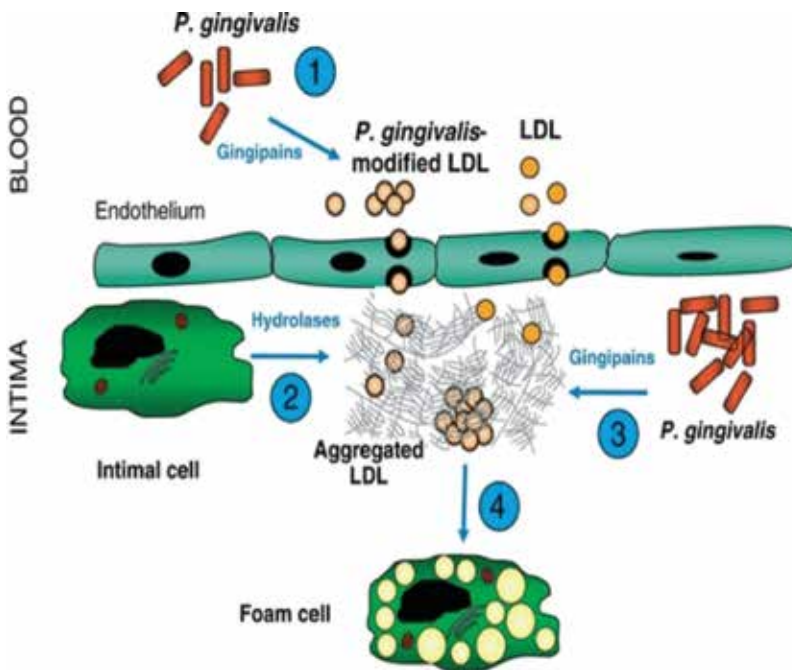
## 5. Periodontitis, systemic inflammation, and cardiovascular disease

Periodontal disease is today considered to play a significant role in various systemic conditions and, in the past decade, the enhanced prevalence of cardiovascular disease (CVD) among patients with periodontitis has received increased attention [73, 74]. Several periodontal bacteria and their agents have been identified in atherosclerotic plaques, for example, *P. gingivalis*, *Fusobacterium nucleatum*, *T. forsythia*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *T. denticola* [75–78]. The occurrence of periodontal bacteria in coronary artery plaques was found to be 5-fold greater in patients with severe periodontitis compared to those with medium periodontitis [79], and DNA from periodontal bacteria, including *P. gingivalis*, was identified in more than 70% of carotid plaque samples [80]. Furthermore, *P. gingivalis* has been shown to influence the development of abdominal aorta aneurysm, involving the activation of TLRs and MMPs [81]. Several animal experiments have demonstrated that oral and systemic infection with periodontal bacteria induces atherosclerosis [74]. Hokamura and Umemura [82] showed that the administration of *P. gingivalis* in a mouse model induces arterial intimal hyperplasia associated with upregulation of the calcium-binding protein S100A9.

When the periodontal disease develops, the gingival epithelium becomes ulcerated by proteolytic activity, for example, by *P. gingivalis*, leading to exposure of the underlying connective tissues and blood capillaries to the bacterial plaque biofilm. At medium periodontitis, the

ulcerative area in the oral cavity ranges between 8 and 20 cm<sup>2</sup>, which means that large amounts of periodontal bacteria and their toxins and metabolic products have a chance, during chewing and oral hygiene activities, to disseminate into the bloodstream and cause transient bacteremias and systemic inflammation [74]. By entering the circulation, the bacteria and/or their components (e.g., proteases, fimbriin, and LPS) activate platelets and neutrophils, induce ROS production, and trigger inflammatory processes in coronary vessels.

Studies using knockout mice orally infected by *P. gingivalis*, demonstrate that atherosclerosis, involving the accumulation of macrophages and inflammatory mediators (CD40, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) in atherosclerotic lesions, is highly dependent on TLR2 [41, 83]. In correlation, interaction between *P. gingivalis* and human blood cells, for example, platelets, neutrophils, monocytes, and T-cells, is mainly mediated by TLR2 and has dramatic inflammatory and immunomodulatory effects, including cellular aggregation, oxygen radical production, low-density lipoprotein (LDL) oxidation, and release and degradation of cytokines. Furthermore, *P. gingivalis* changes the expression of more than thousand genes in vascular smooth muscle cells [84]. For example, *P. gingivalis* upregulates genes involved in proliferation, for example, the TGF $\beta$ 1 pathway and production of matrix proteins, but downregulates pro-inflammatory genes, such as those involved in IL-1 $\beta$ , IL-6, and CXCL8 production. *P. gingivalis* also caused a dramatic increase in the expression of angiopoietin2 (ANGPT2), which is highly correlated with inflammation and atherosclerosis, whereas ANGPT1, inhibitor of inflammation, was downregulated [85, 86]. These effects are mediated via gingipain R, possibly through PAR signaling. Furthermore, the level of another angiogenic factor, vascular endothelial



**Figure 2.** A novel biochemical link between periodontitis and cardiovascular disease.

growth factor (VEGF), increases in patients with periodontitis, and periodontal treatment reduces its concentration [87]. These data indicate that *P. gingivalis* causes a shift from contractile smooth muscle cells to proliferating and matrix-producing smooth muscle cells, which contributes to the growth of the fibrous atherosclerotic plaque, and promotes vascular inflammation and angiogenesis.

*P. gingivalis* has also been shown to modify LDL and promote phenotypic shift of monocytes to foam cells [75, 77, 88]. Our group has previously found fragmentation of the dominating apoprotein of LDL, apo B-100, by *P. gingivalis* and its Rgps [88]. Consequently, our findings together with others suggest that *P. gingivalis* during translocation in circulating blood modifies LDL to an atherogenic form which may represent a link between periodontal disease and atherosclerosis (Figure 2).

## 6. Host cell responses in the circulation and vascular wall

Endothelial cells possess secretory and immunological properties and play therefore important roles in the cardiovascular system. The association of periodontitis with cardiovascular complications includes the induction of endothelial dysfunction, oxidative stress, and systemic inflammation [89]. Furthermore, patients with periodontitis have increased levels of pro-inflammatory mediators, including C-reactive protein (CRP), IL-6, and TNF that may induce endothelial dysfunction [90]. Endothelial dysfunction, which is the initial step in the development and progression of atherosclerosis, is mediated by endotoxins and gingipains of periodontal bacteria. These toxins lead to an impairment of normal endothelial function, including vessel permeability and immune cell adhesion and function [91, 92]. Furthermore, *P. gingivalis* and other periodontal pathogens induce the expression of endothelin-1, a potent vasoconstrictor released by endothelial cells [93, 94]. Endothelin-1 expression has shown a positive correlation to pro-inflammatory cytokines TNF, IL-6, and IL-1 $\beta$  [95], and a negative correlation to anti-inflammatory mediators, for example, angiopoietin-1 [96, 97].

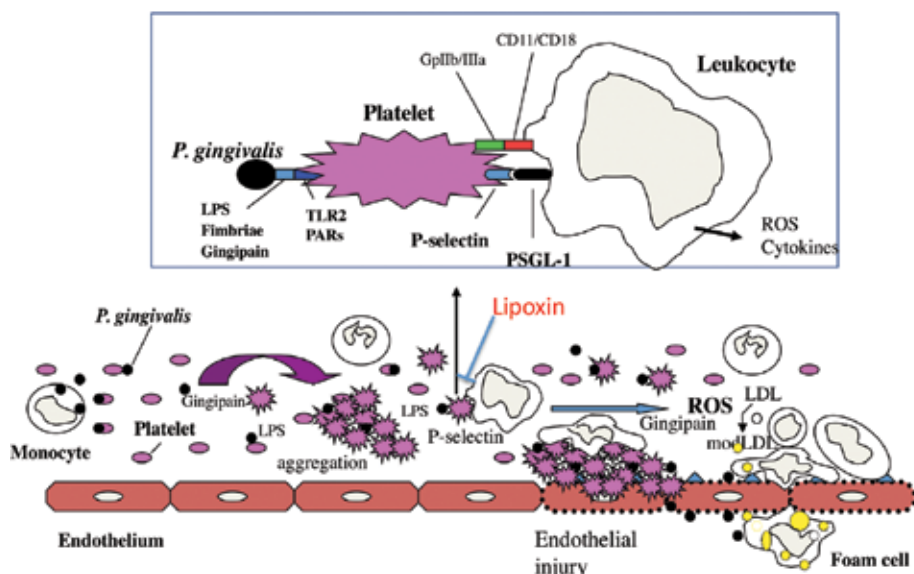
Platelets are key players in hemostasis and acute thrombosis and are initial actors in the development of atherosclerotic lesions often triggered by endothelial dysfunction [98]. However, they are also involved in the immune system and express a broad repertoire of immune cell features such as TLRs, the immunoglobulin  $\gamma$ -receptor Fc $\gamma$ RIIA, complement receptors, inflammatory mediators, as well as microbicidal activities, for example, thrombocidins [99, 100]. Furthermore, platelets bind to and encapsulate bacteria, release ROS and recruit and activate leukocytes and regulate inflammatory processes of the vessel wall [101]. These characteristics make it possible for platelets to recognize and respond to pathogens, such as *P. gingivalis*, and engage other immune cells for enhanced bacterial clearance and inflammatory response.

Several studies suggest that platelet-leukocyte interaction is an essential underlying inflammatory process in atherosclerosis, and patients with cardiovascular disease have an increased number of neutrophil-platelet aggregates in the blood circulation [102, 103]. In correlation, we have shown that *P. gingivalis* markedly induces the formation of large aggregates of

neutrophils and platelets, associated with ROS production and lipid peroxidation, in whole blood and that this effect is dependent on CD11b/CD18-fibrinogen-GpIIb/IIIa interaction, and Rac2 and Cdc42 activation [104, 105] (**Figure 3**). In addition, mice challenged with *P. gingivalis* were found to form platelet-neutrophil aggregates, whereas knockout TLR2<sup>-/-</sup> mice did not. Human platelets express TLRs (TLR 1, 2, 4, 6, and 9), which could be key molecules linking periodontal infection and CVD. For example, TLR2-mediated platelet activation involving the activation of GpIIb/IIIa and P-selectin contributes to the formation of platelet-leukocyte complexes and ROS production [99].

Platelets activation by TLR1/2 receptor ligands results in aggregation as well as secretion of inflammatory mediators such as RANTES, macrophage migration inhibitory factor (MIF), and plasminogen activator inhibitor-1 (PAI-1) [105]. Interestingly, these platelet-derived factors are degraded by gingipains from *P. gingivalis* [105]. Regulated on activation, normal T-cell expressed and secreted (RANTES) is induced by *P. gingivalis* and its lipopolysaccharides and is thus implicated in periodontitis, where elevated levels have been detected in the gingival crevicular fluid of patients with periodontitis [106]. It has been demonstrated that *P. gingivalis*, in addition to TLR2, also can trigger platelet activation via PAR receptors. Through the action of Rgp on PARs, *P. gingivalis* activates platelets by increasing intracellular-free calcium and induces aggregation [105]. In correlation, Lourbakos et al. and McNiol and Israels [107, 108] have demonstrated that gingipains activate PAR1 and PAR4 on platelets leading to aggregation and secretion. We have shown that *P. gingivalis* triggers platelet aggregation through

## Bacteria-platelet hypothesis of atherosclerosis



**Figure 3.** Model showing platelets as a linker between periodontal infection and innate immune response at the vessel wall.

gingipain interaction with PARs and sensitizes platelets for activation by epinephrine, which may explain the association between periodontitis, stress and CVD [109].

## 7. Preventive and treatment strategies

Periodontal pathogens reside in biofilms of subgingival dental plaque and form complex polymicrobial communities. The failure of the immune system to resolve bacterial biofilms results in an accumulation of inflammatory mediators that accelerates the disease state toward a chronic inflammatory condition. Bacterial biofilms are difficult to treat, and conventional methods, including mechanical removal and scaling and root planning (SRP), are still being used. These methods are less efficient and new preventive/treatment strategies are needed. A new approach includes the administration of adjunctive antibiotics systemically in combination with SRP. Different antibiotics have been applied, and a combination of metronidazole and amoxicillin was found to be effective at reducing pocket depth and clinical attachment gain compared to SRP alone, reviewed in [110]. Although antibiotic therapy is effective in modern medicine, microorganisms that are resistant to single or multiple antibiotics have emerged. The development of new families of antibiotics has significantly declined, which is associated with high costs and concerns for possible effects on the commensal microbiota and host health [111]. It is evident that new alternative strategies to traditional antibiotic therapy are needed. New approaches to combat bacterial infections include antibodies, vaccines, bacteriophages, probiotics, and antimicrobial peptides (host- and bacteria-derived) [111–114]. These strategies of promising candidates to traditional antibiotics deserve more consideration.

## 8. Concluding remarks

In summary, it is possible that *P. gingivalis* has a role in pathogenic oral biofilms to undermine important factors of innate immunity, by altering the functions of receptors and their intracellular signaling pathways and the levels of effector molecules, and thereby antagonizing an effective host response. These activities of key periodontal pathogens could contribute to an adaptation and maturation of dysbiotic biofilm communities and promote chronic inflammation and tissue destruction of periodontitis. Increased understanding of the interbacterial interactions that occur in the oral polymicrobial biofilm and its interplay with the host immune system is of uttermost importance for identifying novel targets for the prevention, diagnosis, and treatment of periodontitis and associated systemic disorders.

## Acknowledgements

This work was supported by research grants from the Swedish Research Council (2016-04874), the Swedish Heart-Lung Foundation (20130576), the Knowledge Foundation (20150244, 20150086), and the foundation of Magnus Bergvall (201500823).

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# **Biomarkers of Periodontal Tissue Remodeling during Orthodontic Tooth Movement in Mice and Men: Overview and Clinical Relevance**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69648>

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## **Abstract**

Tooth movement by orthodontic force application is dependent on remodeling in periodontal ligament and alveolar bone, involving the activation of complex cellular and molecular mechanisms correlated with several macro- and microscopic biological changes. The orthodontic process involves the activation of many complex cellular and molecular mechanisms mediated by the release of chemical substance cascades by many cells of the periodontium. Mainly during the early stage of application of orthodontic forces, an inflammatory process can occur in the periodontium as a physiological response to the tissue stress. Several potential biomarkers of the biological alterations after an orthodontic force application expressing bone resorption and formation, periodontal ligament changes, and vascular and neural responses, may be detected. The appropriate choice of the mechanical force to achieve the highest rate of tooth movement in the shortest time of treatment avoiding adverse consequences is a primary objective of a specialist. Thus, an insight into the biological phenomena occurring during the orthodontic therapies by evaluating these biomarkers may be quite relevant for the clinicians. In this chapter, two models of study, i.e., mice and men, were used to describe the clinical usefulness of some biomarkers in orthodontics.

**Keywords:** orthodontics, periodontal ligament, tooth movement, gingival crevicular fluid, biomarkers

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## **1. Introduction**

Tooth movement by orthodontic force application is dependent on remodeling in periodontal ligament (PDL) and alveolar bone, correlated with several macro- and microscopic biological changes.

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After more than 100 years, since the first article on the theory of tooth movement was published by Carl Sandstedt (1904–1905), the specialists have reasonably good understanding of the sequence of events involved in the orthodontic tooth movement at tissue and cellular levels [1]. Orthodontic movements represent a continual and balanced process characterized by bone deposition in tension sites and bone resorption in the pressure ones. The mechanical stress from the application of orthodontic forces induces the activation of many cellular and molecular mechanisms mediated by the release of chemical substance cascade allowing the transmission of signals from extracellular matrix. These alterations lead to a gradual remodeling of the mineralized (alveolar bone) and nonmineralized (periodontium) tooth supporting tissues during the orthodontic movement [2].

Mainly during the early stage of application of orthodontic forces, an inflammatory process can occur in the periodontium as a physiological response to the tissue stress. Several potential biomarkers of the biological alterations after an orthodontic force application may be detected, specifically expressing bone resorption and formation, periodontal ligament changes, and vascular and neural responses.

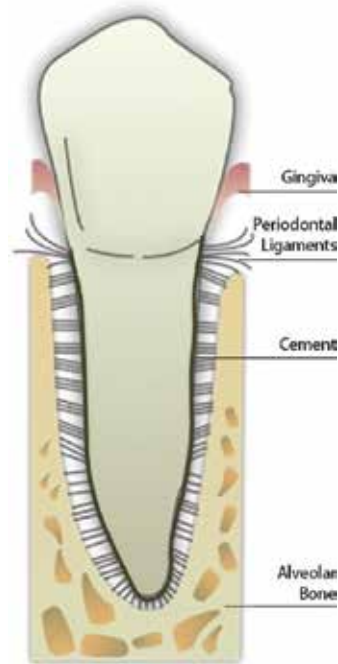
The appropriate choice of the mechanical force to induce the highest rate of tooth movement in the shortest period of treatment avoiding adverse consequences is a primary objective of a specialist. To identify the degree of remodeling occurring in the periodontal tissues during an orthodontic treatment by monitoring the levels of certain biochemical mediators may be a clinically useful procedure.

In this chapter, the importance of evaluating the levels of substances as valid biomarkers of periodontal effects of an orthodontic treatment is emphasized through a description of the specific role of each of them. Only studies on mice and rats have been considered as animal models, whereas in order to monitor the expression of these biomarkers noninvasively in humans, changes in the composition of gingival crevicular fluid (GCF) during orthodontic and orthopedic tooth movement have been selected as first choice. Substances involved in bone remodeling are produced by periodontal ligament cells in sufficient quantities to diffuse into the gingival crevicular fluid. Thus, by means of two different study models “mice and men,” the clinical usefulness of some biomarkers in orthodontics is properly analyzed.

## **2. Anatomy and function of periodontal ligament and alveolar bone**

“Periodontium” comes from the Greek “what is around the tooth.” It includes all structures that anchor the tooth to the alveolar bone surrounding forming a strong support structure [3]. Its components are gingiva, periodontal ligament, cement, and alveolar bone (**Figure 1**).

The periodontal ligament is the structure interposed between the tooth root and the alveolar bone, with an area of 0.25–0.5 mm. It is a connective tissue whose main component is represented by a set of elastic collagen fibers, parallel to each other, inserted on one side in the cementum and on the other in the lamina dura of the alveolar bone (Sharpey’s fibers). The oblique direction of these supporting fibers on the tooth surface provides the tooth with enough elasticity to distribute the masticatory forces over a large surface of the alveolar process, allowing the



**Figure 1.** The tooth and its supporting tissues: gingiva, periodontal ligament, cement, and alveolar bone.

tooth to oppose a greater resistance to forces exerted during masticatory function. The other component of the periodontal ligament is the cellular element consisting of undifferentiated mesenchymal cells and their lines of differentiation in fibroblasts and osteoblasts, together with the neural elements, and vascular tissue fluid from the circulatory system.

The alveolar bone or cortex alveolaris is the portion of the jaws functionally dependent on the teeth. The alveolar bone forms the socket where the tooth is located. Under physiological conditions, the alveolar bone is located approximately 2 mm apical to the cement-enamel junction (CEJ). In general, the bone is a mineralized connective tissue, which consists of organic material. Bone is a very dynamic tissue remodeling continuously throughout life. Histologically, it is a particular type of connective tissue of support, consisting of cells dispersed in an abundant extracellular matrix, formed by fibers, and amorphous substance of glycoproteic origin. The bone tissue is composed of two associated phases: organic and mineral inorganic. The organic phase is constituted for 90% of collagen type I. The mineral phase is constituted by calcium and phosphorus combined in a little crystallized hydroxyapatite, and other ions which usually are found in the surface layers.

Osteoblasts and osteoclasts constitute the bone cellular components. The former synthesize and secrete the organic matrix. Osteoblastic cells, after the deposition of the matrix that is subsequently mineralized, remain embedded in it turning into osteocytes, which remain in mutual connection via cytoplasmic extensions, and also in vasa connection through a series of small channels. The osteoclasts, instead, are multinucleated cells formed by fusion of precursor cells

consisting of hematopoietic stem cells from the family of mononuclear phagocytes. They are the mediators of the continuous resorption of bone. Osteoclasts occupy small depressions on the bone's surface, called Howship's lacunae, caused by erosion of the bone by specific enzymes.

The characteristic trabecular structure is constituted by external component of the compact bone and the inner cancellous bone, providing a great resistance to mechanical stress [4].

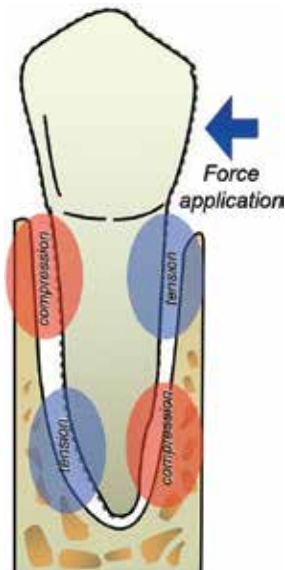
### **3. Periodontal and bone responses to physiologic masticatory activity**

Orthodontic therapy applies light and continuous forces to the teeth and to the related facial structures. The term biomechanics in orthodontics refers to the complex reactions in response to a specific orthodontic force application [5]. Each tooth is attached to the surrounding alveolar bone by the periodontal ligament (PDL), a robust structure collagen support around the tooth root with cellular components and tissue fluids. These last two elements play a key role in normal physiological masticatory system. The collagen of the ligament, the alveolar process and cementum are constantly subjected to remodeling and renovation during normal masticatory activities [6]. The fibroblasts in the periodontal ligament have similar features to osteoblasts and, standing out from the focal cell population, produce new tissue bone. Osteoclasts and cementoclasts remove alveolar bone and cementum, respectively. During the physiological process of mastication, dental and periodontal structures are subjected to both heavy and intermittent forces. In particular, the teeth are subjected to loads that vary from 1 to 2 kg when chewing soft food up to 50 kg for tough food. During heavy masticatory loads lasting maximum 1 second, the displacement of a single tooth in the periodontal space is prevented by the naturally incompressible fluid, and the loading force is transmitted to the alveolar process walls, which are consequently flexed. They generate currents stimulating bone regeneration and repair, thus allowing the adaptation of the bone architecture to the changed function.

### **4. Periodontal and bone responses to the application of orthodontic forces**

Bone and periodontal responses to orthodontic treatment mainly depend on the force duration and its intensity applied to the teeth. The orthodontic biological mechanisms can be evaluated considering two different theories: bioelectrical theory and pressure-tension theory. According to the first theory, the bone subjected to bending generates piezoelectric currents that determine changes in bone metabolism. These currents are constituted by electrons ( $e^-$ ) moving from side to side of the network of a crystalline material; thus, the orthodontic forces routinely produce an alveolar bone deflection, and these strains lead to changes in the periodontal ligament. For the theory of the pressure tension, the cell differentiation and the subsequent tooth movement are controlled by chemical signals. A continuous force induces a compression of the ligament in some areas, with reduction of oxygen tension and then of blood flow, whereas in other areas, a traction of the ligament with increased oxygen tension and equal or increased blood flow (**Figure 2**) [5].

Modifications of the irroration are accompanied by rapid chemical changes, which can stimulate the differentiation and activation of specialized cells for bone and periodontal remodeling. Cellular destruction and injury of capillaries lead to an inflammatory reaction followed by formation of new capillaries and connective cells. In particular, the compression of the periodontal fibers causes the "hyalinization" [7] characterized by the disappearance and/or pycnosis of cell nuclei and the convergence of the collagen fibers in a gelatinous-like substance. Reitan in his papers on the histological changes following orthodontic force application [8] reported that the hyalinization refers to cell-free areas within the PDL, in which the normal tissue architecture and staining characteristics of collagen in the processed histological material have been lost. He observed that the hyalinization occurred within the PDL following the application of even minimal force. After the elimination of the hyalinized tissue, a direct resorption occurs thanks to the activation of the osteoclasts from the PDL, and then, an indirect resorption with cellular elements from the blood flow occurred. The pressure exerted on the periodontal ligament is directly proportional to the reduction of the blood flow inside the PDL until a complete collapse of the blood vessels and to a consequent ischemia. If the pressure applied to the tooth is light and lasting for 1–2 seconds, the PDL is partially compressed to the displacement of the fluids outside of the periodontal space following the displacement of the tooth in its alveolus; after 3–5 seconds, the blood vessels are passively compressed by the pressure side and dilated from the tension side; fibers and cells of the PDL appear mechanically distorted. A slight pressure maintained for a few minutes causes changes in the blood flow and variations of oxygen tension, with a simultaneous release of prostaglandins and inflammatory cytokines. After at least 4 hours, metabolic changes occur by induction of several chemical modulators, increased cyclic Adenosine Monophosphate (cAMP), and cell differentiation within the periodontal ligament. After 48 hours, the orthodontic tooth movement starts after the alveolar

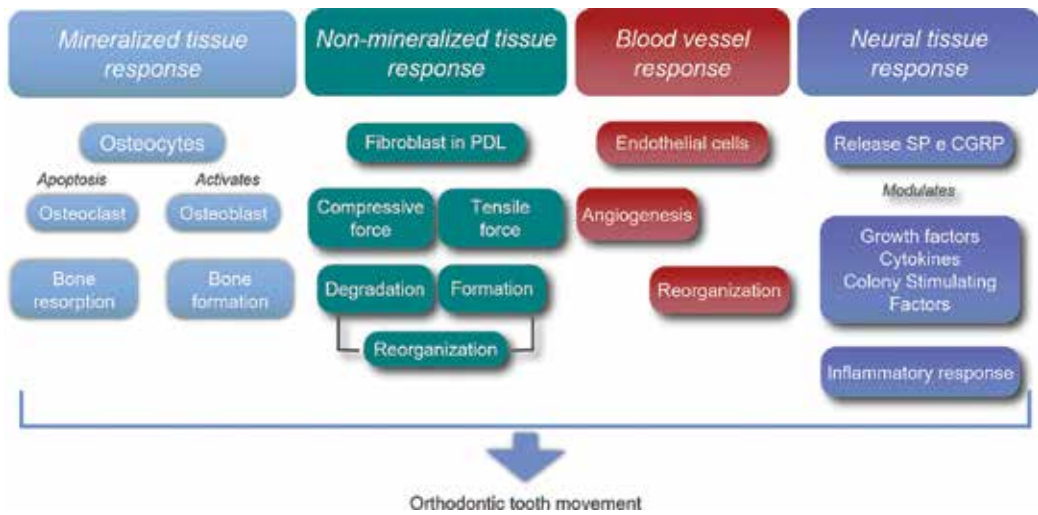


**Figure 2.** Compression and tension areas after force application.

bone remodeling that occurs through the combined activity of osteoclasts and osteoblasts. If pressure on dental structure is high, after 3–5 seconds, the blood vessels in the PDL collapse on the compression side, and after a few minutes, the interruption of blood circulation occurs in that area of PDL, with sterile necrosis and disappearance of the cellular component (areas of hyalinization). In this case, the remodeling takes place thanks to cells from contiguous areas, which begin to invade the necrosis causing an indirect resorption, since the action of osteoclasts starts from the outer part of the lamina dura. Consequently, the mechanisms of hyalinization and resorption indirectly involve an inevitable delay in the displacement of the tooth, in addition to the pain caused to the patient, due to the presence of ischemic and inflamed areas in the PDL. The ideal intensity for orthodontic forces is when it promotes tooth movement producing cell differentiation without the complete occlusion of the blood vessels in the periodontal ligament, and, therefore, the biological effect of an orthodontic force depends on its intensity and area of PDL involved, or by the pressure on the tooth.

Therefore, orthodontic tooth movement could be divided in three phases: the initial phase, the lag phase, and the postlag phase. The initial phase is characterized by immediate and rapid tooth movement and occurs from 24 to 48 hours after the first application of force. This rate is largely attributed to the displacement of the tooth in the PDL space. The lag phase lasts from 20 to 30 days and shows relatively little tooth displacement. This phase is marked by PDL hyalinization in the region of compression. No subsequent tooth movement occurs until the cells complete the removal of the main part of the necrotic tissues. The lag phase is followed by the postlag phase, during which the rate of movement increases [9].

The sequence of events following orthodontic tooth movement can be characterized using suitable biomarkers (**Figure 3**).



**Figure 3.** Effects of orthodontic force application on periodontal tissues.

## **5. Two different models “mice and men” to analyze the orthodontic tooth movement**

### **5.1. Mice as models of study of periodontal and bone tissue remodeling after orthodontic tooth movement**

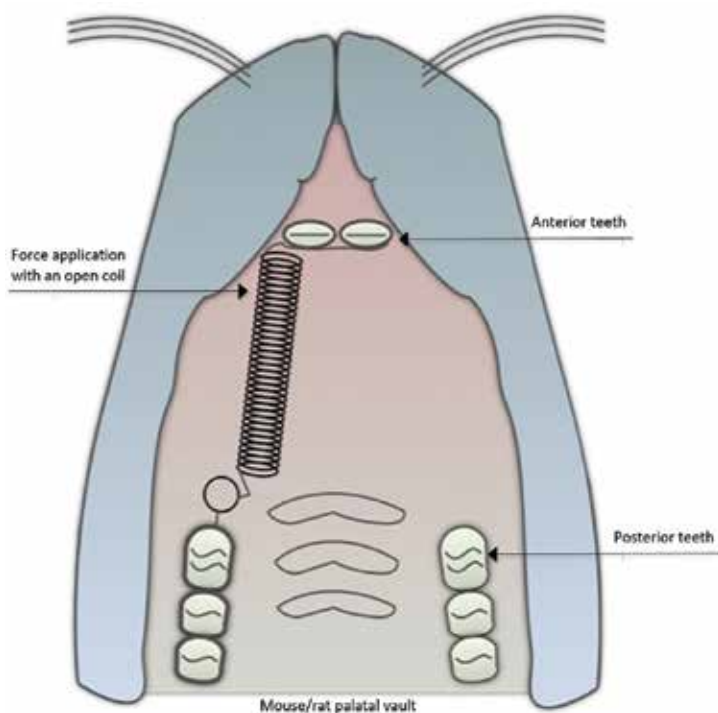
Up to now, a large number of studies in various species of animals have been carried out to evaluate the biological response on the periodontal ligament after a force application. Mice are the most widely used animals to study tooth movement (**Figure 4**), even if there are advantages or disadvantages [10].

To note among the disadvantages, the alveolar bone of mice is denser than in humans, and there are no osteons. Indeed, the animal osteoid tissue along the alveolar bone surface is less, a few mucopolysaccharides are contained in the extracellular matrix, and the calcium concentration is more controlled by intestinal absorption than by the bony tissue. Some disparities have also been reported in the arrangement of the peritoneal fibers, in the supporting structures, as well as in root formations that are faster. However, mice are considered a good model to study the orthodontic tooth movement consequences. They are relatively inexpensive facilitating the use of a large sample and the housing for a long period of time; the histological preparation of their material is easier than in other animals; most antibodies required for cellular and molecular biological techniques are only available for mice and rats; moreover, transgenic strains are almost exclusively developed in small rodents. The difference between mice and rats poses greater difficulty in placing an effective orthodontic appliance in the smaller mouth of a mouse. A systematic review on the literature for experimental tooth movement in rodents showed several shortcomings in part related to the physiology of the animals and on the other hand to the design of the orthodontic appliance [11]. In consideration of this critical evaluation, the animal model was useful to achieve the current knowledge on experimental orthodontic tooth movement.

### **5.2. Evaluation of bone and periodontal tissue responses to orthodontic movement in men with the gingival crevicular fluid**

The gingival crevicular fluid (GCF) is an exudate derived from epithelium lining of the gingival sulcus has been recognized for over a century. However, the exact nature of the fluid, its origins, and its composition have been the subject of controversy for decades [12, 13]. Investigations into the protein content of the GCF reported that in healthy gingival crevices, the GCF has a similar protein concentration to interstitial fluid, which was notably lower than in serum [14–16]. On the contrary, an inflamed gingiva has GCF with raised protein concentrations that are thus similar to those of serum [17]. Subsequently, upon local inflammation or injury, the GCF would become an inflammatory exudate. The increased GCF flow contributes to host defense by flushing bacterial colonies and their metabolites away from the sulcus, thus restricting their penetration into the tissue [18].

The range of the GCF constituents is very large, as it can contain both human and bacterial cells and many different molecules. Among the most representative cellular components of the GCF, there are the leukocytes, especially neutrophils, which have important roles in the



**Figure 4.** A schematic drawing of the animal model with the application of a force by an open coil between anterior and posterior teeth to induce a tooth movement.

antimicrobial defense of the periodontium [19]. However, while the bacterial and cellular components of the GCF are of primary concern for clinicians and researchers, its molecular contents represent a promising source of biomarkers in dentistry and in orthodontics for the monitoring of site-specific tissue remodeling leading to the tooth movement [20] and, as revealed more recently, for the evaluation of skeletal maturation on an individual basis [21].

The host molecular content of the GCF includes a large variety of molecules that have the potential to be classified as biomarkers of cell death, tissue damage, inflammation, bone resorption, bone deposition, and others, according to their specific biological functions. For several of these biomarkers, associations between their levels and specific clinical conditions have been shown along with the predictive value for the biomarkers; for instance, in terms of tissue destruction due to periodontitis. Several recent studies on orthodontic tooth movement have used biochemical assay analysis of GCF as a simple and noninvasive procedure for repetitive sampling from the same site [22].

#### 5.2.1. Methods of collection of gingival crevicular fluid

The GCF can be collected by different methods. The most used methods in the literature are (1) the gingival washing technique; (2) the capillary tubing or micropipettes; and (3) the use of absorbent filter paper strips.



#### 5.2.1.1. *The gingival washing technique*

This technique was described a long time ago [23]. The gingival crevice is perfused with a determined volume of an isotonic solution ejected from a microsyringe and then re-aspirated from the gingival crevice at the interdental papilla. The fluid collected represents a dilution of the GCF, and it will contain both cells and soluble constituents, such as plasma proteins. As a major disadvantage, this procedure can fail to recover all the instilled fluid or the GCF contents during the re-aspiration. Thus, an accurate quantification of the GCF volume or composition is not guaranteed as the precise dilution factor cannot be determined. This technique is particularly valuable for harvesting cells from the gingival crevice region.

#### 5.2.1.2. *Capillary tubing or micropipettes*

This collection method was described more than 40 years ago [24]. It consists on the use of capillary tubes of specific internal diameter inserted into the gingival crevice after the isolation and drying of the area. Due to the known internal diameter of the capillary tubes, it is possible to determine the exact GCF volume collected, through the measure of the GCF migration along the capillary tubing. However, it is difficult to collect an adequate volume of GCF in a short period, unless the sites are inflamed and contain large volumes of fluid. In fact, collection times from an individual site may exceed 30 minutes, thus making the capillary holding difficult and possibly traumatic for periodontal tissues. Moreover, this can cause the release of a serum-derived fluid that may alter volume and composition of the GCF [25]. A further disadvantage of this method is the challenging removal of the full GCF sample from the capillary tubes.

#### 5.2.1.3. *Absorbent filter paper strips*

The use of adsorbent paper strips represents the procedure most used for GCF collection today [25, 26]. In this procedure, the standardized absorbent paper strips are inserted into the gingival crevice and left *in situ* for 5–60 seconds. The advantages of the technique are that it is quick and easy to use and can be applied to individual sites, and it is the least traumatic when correctly used. The main variations are the reduced timing of sampling and the volume estimation of the collected sample. Because of this methodological variability, the data from different studies need to be interpreted with caution, considering how exactly the collection of the GCF was performed with the paper strips. The methods of collection may be broadly divided into the intracrevicular and extracrevicular techniques. The former depends on the strip inserted at least 3 mm into the gingival crevice or into a periodontal pocket [27], whereas in the latter, the strips are inserted until the “minimum resistance” is felt [28] in an attempt to minimize trauma. A problem with GCF collection and data interpretation may be the sample contamination by blood, saliva, or bacterial plaque. A careful isolation should be performed to minimize the potential GCF contamination. Before performing any biochemical analysis, the volume of the GCF sample must be determined. To achieve the recovery of strips, it is necessary to separate the GCF from the filter paper strips, and protein recovery is close to 100% using a centrifugal elution technique [29].

## 6. Biomarkers of periodontal and bone responses to orthodontic force application

Orthodontic tooth movement induces a series of orchestrated cellular and molecular events responsible for connective tissue remodeling and osteoclast activation [30].

Thus, the sequence of cascades following the mechanical stress from orthodontic appliances can be characterized using suitable biomarkers.

### 6.1. Pro-inflammatory cytokines: interleukin-1 (IL-1 $\beta$ ), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-(TNF- $\alpha$ ), and prostaglandins E (PGE1-PGE2)

IL-1 $\beta$  is one of the most abundant cytokines in the periodontium during the initial stage of orthodontic tooth movement [3]. In the early stages of tooth movement (at 12 and 24 hours), the following cells stained positively for IL-1 $\beta$ : fibroblasts, macrophages, cementoblasts, cementoclasts, osteoblasts, and osteoclasts [31, 32]. IL-1 $\beta$  seems to be primarily secreted by macrophages, whereas macrophage accumulation in compressed periodontal areas has been detected at later stages of the treatment. Thus, during the initial stage of tooth movement, IL-1 $\beta$  derives from other periodontal cell types, like the osteoclasts, as immediate response to mechanical stress.

The experimental tooth movement leads to significantly increased recruitment of cells belonging to the mononuclear phagocytic system. It was suggested that the neuroimmune interactions may be of primary importance in the initial inflammatory response, as well as the regenerative processes of the periodontal ligaments are incident to orthodontic tooth movement [33]. IL-1 $\beta$  is involved in the survival, fusion, and activation of osteoclasts. This role is significant since the rate of tooth movement correlates with the quantity of bone remodeling in the alveolar process [34]. Higher levels of expression of inflammatory cytokines and their related receptors have been shown after an inflammatory process induced by perforating the buccal cortical plate of orthodontically treated rats. The concentration of the IL-1 $\beta$  mRNA in the rats' periodontal ligament is increased within 3 hours after orthodontic force loading, mainly on the pressure side [35, 36].

The particular function as pro-inflammatory cytokine of IL-1 $\beta$  has been demonstrated by the administration of exogenous IL-1 receptor antagonist (IL-1RA) [37]. IL-1RA treated mice showed a 66% decrease in the levels of IL-1 $\beta$  when compared to the experimental tooth movement of vehicle treated mice, and this was associated with a reduction of the number of osteoclasts in the pressure side of periodontal tissues after histological characterization. Therefore, IL-1RA down-regulates orthodontic tooth movement because of the lower rate of tooth displacement in mice treated with IL-1RA therapy. IL-1 $\beta$  is also considered a potent inducer of IL-6 production: it overlaps with IL-6 and TNF- $\alpha$  in their actions [38]. IL-6 regulates immune responses in inflammation sites [39], and it has an autocrine/paracrine activity stimulating osteoclast formation and the bone-resorbing activity of preformed osteoclasts [40]. IL-6 production increases after 24 hours [41].

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is another pro-inflammatory cytokine shown to elicit acute or chronic inflammation and stimulate bone resorption [32]. TNF- $\alpha$  directly stimulates the differentiation of osteoclast progenitors to osteoclasts in concert with the macrophage-colony stimulating factor (M-CSF). Tuncer et al. [42] reported the increased levels of IL-8 at PDL tension sites and proposed it as triggering factor for bone remodeling.

Many studies of the gingival crevicular fluid have confirmed the increased levels of these pro-inflammatory cytokines during periodontal tissue remodeling after orthodontic tooth movement [41, 43, 44].

Other clinical and animal investigations have also identified a primary role of prostaglandins E (PGE1 and PGE2) in stimulating bone resorption [45, 46]. Prostaglandins are produced from the arachidonic acid, which in turn derives from phospholipids. The liberation of prostaglandins constitutes the first response to the pressure stimulus; it occurs when cells are mechanically deformed, and the consequent mobilization of membrane phospholipids leads to the formation of inositol phosphate (IP), an important chemical messenger. PGE2, in particular, is able to mediate inflammatory responses and induce bone resorption by osteoclastic cell activation [9]. The literature reports that prostaglandins directly stimulate osteoclast production and their capacity to form ruffled border and effect bone resorption. In addition, the PGE2 level in GCF reflects the biologic activity in the periodontium during orthodontic tooth movement, and it is significantly increased in both tension and compression sides [47].

## **6.2. RANK/RANKL/osteoprotegerin (OPG) system**

During orthodontic movement, a variety of proliferation markers are expressed: KI-67 and receptor activator of nuclear factor-Kappa  $\beta$  ligand (RANKL) [48, 49] indicate the recruitment of osteoclasts in compression areas, whereas Runx2 [50], Col1-GFP, and BSP-GFP expression cells express the increase of differentiated osteoblasts in tension areas [51]. To note, the TNF-related ligand, the receptor activator of nuclear factor-Kappa  $\beta$  ligand (RANKL), and its decoy receptor RANK, as well as the osteoprotegerin (OPG), were found to play important roles in the regulation of bone metabolism. RANKL is a downstream regulator of osteoclast formation and activation, through which many hormones and cytokines produce their bone resorption effect. In the bone tissue, RANKL is expressed on osteoblast cell lineage and exerts its effect by binding the RANK receptor on osteoclast lineage cells. This binding leads to rapid differentiation of hematopoietic osteoclast precursors to mature osteoclasts. OPG is a decoy receptor produced by osteoblastic cells in competition with RANK for RANKL binding. The biological effects of OPG on bone cells include inhibition of terminal stages of osteoclast differentiation, suppression of activation of matrix osteoclasts, and induction of apoptosis. Thus, bone remodeling is controlled by a balance between RANK-RANKL binding and OPG production. Kanzaki et al. [52] reported that OPG gene inhibited RANKL-mediated osteoclastogenesis and experimental tooth movement in rats. Thus, the inhibition of the activity of RANKL in its promoting osteoclast differentiation could be very helpful in preventing, for instance, tooth anchorage during orthodontic treatment and relapse during the posttreatment period.

### 6.3. Macrophages-colony-stimulating factors (M-CSFs)

Colony-stimulating factors (CSFs) comprise those related to granulocytes (G-CSFs), macrophages (M-CSFs), or to both cell types (GM-CSFs). They have a great implication in bone remodeling through osteoclast formation and thereby during tooth movement [9]. These molecules are specific glycoproteins interacting to regulate production, maturation, and function of granulocytes and monocyte macrophages. Therefore, M-CSF plays an important role during the early osteoclast differentiation, which increases the rate of osteoclastic recruitment and differentiation during initial phases of orthodontic tooth movement [50]. In particular, optimal dosages of M-CSF are correlated with measurable changes in tooth movement and gene expression, providing potential for clinical studies in accelerating tooth movement.

### 6.4. Vascular endothelial growth factor (VEGF) as a key factor of neovascularization

Vascular endothelial growth factor (VEGF) is the primary mediator of angiogenesis and increases vascular permeability during tissue neof ormation, always associated to the presence of blood vessels [53]. During orthodontic tooth movement, compressive forces induce angiogenesis of periodontal ligaments and the activation of the vascular endothelial growth factor [54]. A study performed the localization of VEGF *in vivo* in the periodontal tissues of 15 male Wistar rat during an experimental tooth movement. A compressive force at 150 mN was applied by a standardized compressive spring placed between the right and left upper first molars in each rat's mouth. The maxillary bone was analyzed with immunohistochemical staining. VEGF immunoreactivity was in vascular endothelial cells, osteoblasts, osteoclasts in resorption lacunae, in fibroblasts adjacent to hyalinized tissue, a local necrotic area in compressed zone, and in mononuclear cells in periodontal tissues from animals. VEGF mRNA was also found in fibroblasts and osteoblasts in tension area of mice periodontal ligament during experimental tooth orthodontic movement [55]. The protocol included 10 mice, divided between experimental and control animals, and provided the assessment of premaxillary bone frontal sections [56].

Therefore, VEGF has a relevant role in remodeling periodontal ligament as well as in bone resorption and formation.

### 6.5. Neuropeptides during neural tissue response to orthodontic tooth movement

During orthodontic tooth movement, a neurogenic inflammation occurs in the periodontium with an increased concentration of specific proteins. Somatosensory neurons disseminate signals from periodontal peripheral nerve fibers to the central nervous system. With application of physiologic orthodontic force, periodontal peripheral nerve fibers release calcitonin gene-related peptide (CGRP) and substance P, acting as neurotransmitters. Moreover, CGRP and substance P are vasodilators, inducers of increased vascular flow and permeability (diapedesis), and stimulators of plasma extravasation and leukocyte migration into tissues (transmigration).

CGRP activates the bone formation through osteoblast proliferation and osteoclast inhibition. Receptors for CGRP are revealed on osteoblasts, monocytes, lymphocytes, and mast cells. Receptor activation results in amplified intercellular communication, promoting cytokine (inflammatory mediator molecules) synthesis and release.

Healthy periodontal and alveolar bone innervation promotes maximum blood flow during orthodontic tooth movement, whereas denervation reduces blood flow and bone formation [57]. Substance P (SP) is another sensory neuropeptide released from the peripheral endings of sensory nerves. It can modify the secretion of pro-inflammatory cytokines from immunocompetent cells during periodontal tissue remodeling. Worthy of note, SP stimulated the production of PGE<sub>2</sub> [58].

#### **6.6. Enzymes reflecting biological activity in periodontium: caspase-1, $\beta$ -glucuronidase ( $\beta$ -G), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)**

In conjunction with the inflammatory process, there is an apoptotic process occurring to eliminate the hyalinized periodontal tissue formed during the early stages of orthodontic movement. Caspase-1 is the most important mediator of inflammation and apoptotic responses, activated by inflammatory signals as alterations in the intracellular ionic milieu. It has the role to process and activate pro-IL-1 $\beta$  and other pro-inflammatory cytokines. In a rat model under orthodontic treatment, caspase-1 mRNA expression is increased, and the level of caspase-1 changes with different temporal phases of orthodontic tooth movement [59]. An irreversible root resorption and deformation of periodontal tissues might emerge: an excessive local orthodontic force application or in some diseases like rheumatoid arthritis related to the hyperexpression of caspase-1. In these cases, a method to preserve the structure of periodontal ligaments may be the administration of the inhibitors of caspase-1 activity such as VX-765 [60] and Pralnacasan [61]. A biomarker of primary granule release from polymorphonuclear leukocytes is the lysosomal enzyme  $\beta$ -glucuronidase ( $\beta$ G). Increased levels of this enzyme have been found in the GCF of adolescents treated with rapid maxillary expander, thus during orthodontic and orthopedic movement. Moreover,  $\beta$ G, similar to other biochemical mediators as IL-1 $\beta$ , correlates to both direct and indirect application of mechanical stimuli, with an increased level that is higher following stronger forces [62].

Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are soluble enzymes usually confined to the cytoplasm of cells but released to the extracellular environment upon cell necrosis. The AST and LDH activities into the GCF have also been assessed during orthodontic treatment. The GCF AST activity is significantly increased in both the tension and compression sites at days 7 and 14. This amount is explained as a consequence of a controlled trauma that leads to an increased cell necrosis after the mechanical stress on the periodontal ligaments and alveolar bone. A low increase of GCF AST activity reflects the application of orthodontic force on teeth, particularly on compression side, while an occlusal trauma leads to a higher amount of enzymatic level [63]. The enzyme lactate dehydrogenase, likewise, is normally limited to cytoplasm, and it is only released extracellularly after cell death and tissue breakdown [64]. This enzymatic activity is greater in compression sites because of the typical process that occurs during periodontal remodeling resulting from orthodontic tooth movement: there is an early wave of resorption of 3–5 days followed by its reverse process of 5–7 days, and a late wave of formation that lasts for 7–14 days, on both the pressure and the tension sides of the alveolar wall.

### **6.7. Enzymes involved in bone cell activities: alkaline phosphatase (ALP) and acid phosphatase (ACP)**

The biological alteration due to the orthodontic tooth movement involves alterations above all in the surrounding bone tissue [65]. Bone metabolism is associated with alkaline phosphatase (ALP) and acid phosphatase (ACP) expressed by osteoblasts and osteoclasts, respectively. ALP is a ubiquitous tetrameric enzyme associated with the plasma membrane of cells, also found in liver, intestine, and placenta, and it is observed during healing of bone fractures and physiologic bone growth. The bone isoenzyme predominates in childhood and particularly during puberty [66]. These are 507 amino acid proteins encoded by the same gene but differ in their degree of glycosylation. The enzymes catalyze the hydrolysis of monoesters of phosphoric acid and a transphosphorylation reaction with large concentrations of phosphate acceptors [67]. Alkaline and acid phosphatases are released by injured, damaged, or dead cells into extracellular tissue fluid, and, in general, high enzyme activity is an expression of greater cellular activity. ALP activity is found at much higher levels in the periodontal ligament than in other connective tissues [68]. After an orthodontic force application, these enzymes are produced in the periodontium and diffuse in the site-specific GCF. Thus, the monitoring of phosphatase activities in the GCF could be suggestive of the tissue changes occurring during orthodontic tooth movement. In fact, experimental studies in rats and clinical studies in humans correlate alveolar bone remodeling with changes in GCF phosphatase activities [26, 69–72]. To identify and understand the enzymatic changes occurring during the early stages of orthodontic tooth movement and to coincide with initial and lag phases of tooth movement, the studies consider an orthodontic cycle of duration 21 days. It was observed that the ALP activity peaked on the 14th day in most patients, followed by a sharp fall by the 21st day. The activity decrease is related to removal of the hyalinized zone. When the enzyme activity is high, the tooth movement rate is greater. This implies that the ALP activity follows the rate of tooth movement during the initial phases. In the hard bony tissues, the ALP has been implicated in the process of mineralization. Active osteoblasts and osteocytes give an intense staining reaction for alkaline phosphatase. No enzyme activity is found in bone matrix, except when it is in close association with matrix-synthesizing cells. The osteogenic cells in the periodontal ligament react to the tensional forces with an increase in the maturation level. The fibroblast proliferation and collagen have been shown to increase in the tension sites. The ALP activity is lower in the compressed hyalinized zones of the periodontal ligament, whereas ACP activity is higher. After 7–14 days of orthodontic force application, the bone deposition occurs in both tension and pressure sites of the alveolar wall. The main bone remodeling activity at the early times in a remodeling cycle is resorptive, but in the later phase, resorption and deposition become synchronous. This might be due to increased acid phosphatase activity that has been observed in the early phases of orthodontic tooth movement. High levels of alkaline phosphatase have been described after 7 days, when bone deposition begins, and a significant peak occurs on day 14. It is obvious that, as a forerunner to bone formation, the number of fibroblasts and osteoblasts increases in areas of tension. This occurs as a result of increase in cell number by mitotic cell division. The histologic studies showed that in marginal tensional areas, cell proliferation occurs between 36 and 50 hours and lasts for 10–21 days. The tension causes shape changes, and osteoblasts move slightly apart. On the compression side, bone resorption would occur, and osteoclastic activity would be high with little or no osteoblastic activity.

To note, ALP activity is influenced by clinically detectable dental displacements and also by mechanical stress and gingival inflammation.

In conclusion, the analysis of the ALP associated with bone metabolism, under healthy gingival conditions, is a suggestive indicator of the histological and biochemical changes in bone turnover and therefore of the rate/amount of tooth movement. Moreover, the properties of the GCF ALP activity distinguishing between clinically moving and nonmoving teeth show that this enzyme should be further studied as a diagnostic tool in orthodontics [22].

## 7. Conclusions and clinical relevance

When exposed to different degrees of magnitude, frequency, and duration of mechanical loading, alveolar bone and periodontal ligaments show extensive macroscopic and microscopic changes. Force application on the tooth also alters periodontal tissue vascularity and blood flow, resulting in the local synthesis and release of various molecules, such as cytokines, growth factors, colony-stimulating factors, enzymes, and neurotransmitters [73].

A biomarker is a substance that can be objectively measured revealing any process occurring during a therapeutic treatment. The several potential biomarkers of the biological alterations after an orthodontic force application described in this chapter may be significantly useful to perform an appropriate choice of the mechanical force to achieve the right rate of tooth movement and to accelerate the orthodontic time, avoiding adverse effects such as root resorption or bone loss [74–76].

Finally, a clinical use of these biomarkers for the specialists may be mandatory to improve orthodontic therapies.

The different experimental and clinical methods for the collection and assessment of these potential biological markers were described in both animal and human models. GCF analysis, especially, offered several advantages for its simple, quick, and noninvasive collection.

Overall, a detailed knowledge of the ongoing process occurring in periodontal tissues during orthodontic procedures can lead to proper choice of mechanical loading with the aim of shortening the period of treatment and avoiding adverse consequences associated with orthodontic treatment.

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# Periodontal Considerations in Adult Orthodontic Patients

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69960>

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## Abstract

The relationship between periodontology and orthodontics consists of a highly complex, bidirectional and close interaction that is nowadays characterized by controversial scientific opinions and clinical approaches. The relevant increasing number of adult orthodontic patients which in most cases present already-compromised periodontal tissues has markedly highlighted the potential of orthodontic treatment in enhancing or deteriorating periodontal health and also the utmost relevance of peculiar periodontal planning prior and during orthodontic treatment. Since the progress in adult orthodontics trend is rapid, there is also an increasing need for evidence-based protocols that might guide clinicians through a comprehensive, interdisciplinary and successful treatment. This chapter has been compiled with the aim of providing orthodontists, periodontists and general practitioners with sound evidence-based protocols and valid clinical approaches that have proven to be successful for numerous patients over long follow-ups. It is structured following the steps for a correct therapy management, starting from comprehensive examination and diagnosis to before and during orthodontic treatment considerations, and finally analysing the present state of new adult orthodontic technologies.

**Keywords:** adult orthodontics, periodontal health, orthodontic therapy, interdisciplinary treatment, accelerated orthodontics, future orthodontics

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## 1. Introduction

Over the past three decades, the number of adult orthodontic patients has increased markedly. Recent surveys have reported that orthodontic treatment contributes to significant improvements in both professional and personal life, especially in adult patients who generally have undergone substantial loss of sustaining tissues, resulting in compromised function

and aesthetics. Based on the new-found self-confidence, the majority of these patients suggest orthodontic treatment to friends and relatives. Therefore, orthodontic treatment seems to be a promising emerging therapy to be integrated into a multidisciplinary dental treatment approach.

Apart from establishing a functional occlusion and improving dental and facial aesthetics, one of the major objectives of orthodontic therapy is enhancement and maintenance of periodontal health. In adult patients, the altered periodontal health might result in teeth loss, altered function and compromised aesthetics. Most of these patients present a variety of problems, which include teeth overeruption, migration, traumatic occlusion, irregular interdental spacing, consumed occlusal surfaces, irregular occlusal planes and loss of vertical dimension. In such complex and challenging clinical situations, an interdisciplinary treatment is mandatory. Unfortunately, in everyday orthodontic practice, insufficient emphasis is placed on comprehensive diagnosis prior to orthodontic therapy with particular attention to periodontal health and to its control and maintenance throughout the therapy. All attempts of limited treatment, with poor consideration of the whole picture will result in failure, relapse and very often aggravation of the pathology. The careful control of periodontal pathologies before, during and after orthodontic treatment, along with functional rehabilitation and patient's compliance can provide the most satisfactory results and long-term stability.

We present herein our step-by-step approach on pre-orthodontic and orthodontic treatment of periodontally compromised patients, through several detailed clinical cases and clear scientific protocols. Moreover, we consider several important issues concerning the bidirectional interaction of orthodontics and periodontology and discuss ways to optimize it. Potentials and limitations of such interaction are reflected. Furthermore, we summarize scientific evidence and clinical expertise on different techniques aiming enhancement and acceleration of adult orthodontic therapy, leading to conclusions of high relevance in terms of an effective and efficient therapy.

Guided by sound scientific principles and constructive clinical experience, it is vitally important to keep in mind that adult patients with orthodontic needs require individualized and tailored treatment plans to meet both clinical success and patient's expectations. The information presented in this chapter is gathered by considering these aspects and with the hope of providing investigators and clinicians with solid bases for the state of art and potential future directions of interdisciplinary treatments.

## **2. Periodontal considerations prior to orthodontic treatment**

### **2.1. Comprehensive diagnosis**

As all medical treatments, dental therapies might have two approaches, which are referred to as 'causal' and 'symptomatic'. It is of utmost importance that when possible, these approaches are considered complementary, not as an alternative to each other. Therefore, in all cases when causal factors can be identified, the treatment should focus on addressing them first. If these approaches are followed, the symptomatic spectrum might be easier to treat or even disappear.



In oral-related pathologies, many etiological factors are recognized and widely accepted, whereas sometimes their precise effects and multifactorial influences are difficult to quantify. Nevertheless, the identification of potential causal factors is the fundamental part of any diagnostic process and should be performed through an accurate clinical visit.

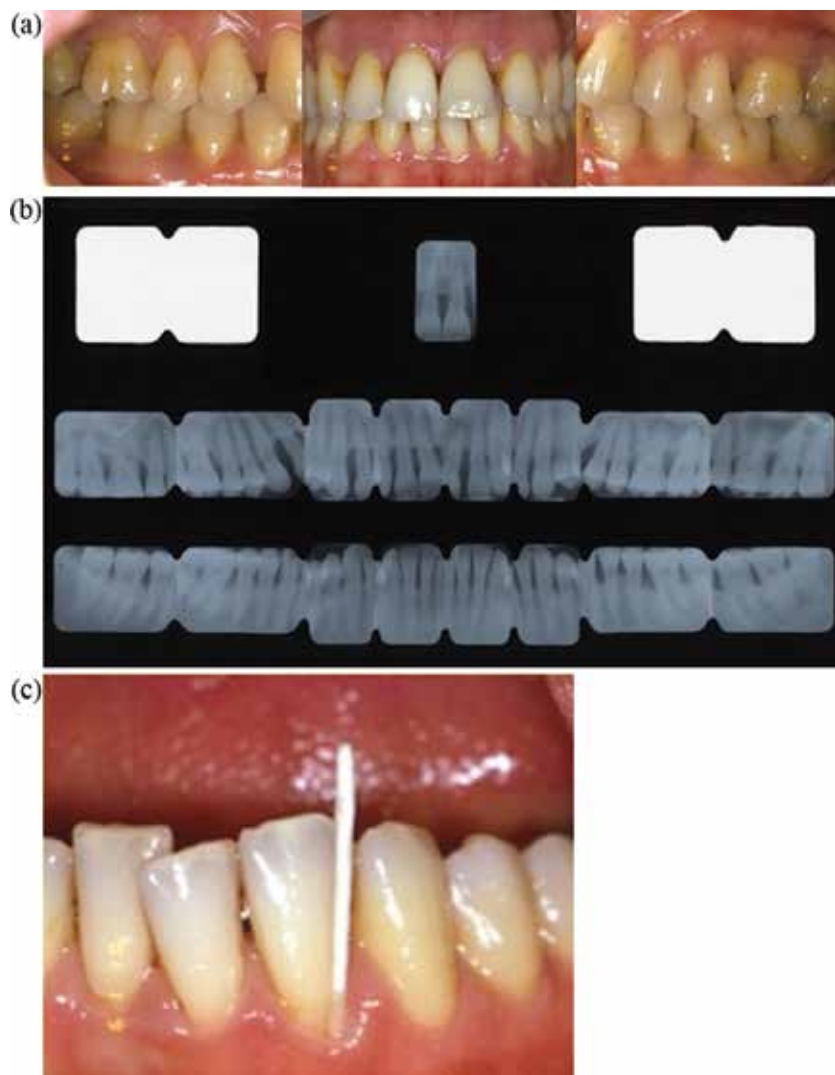
In case of particular malocclusions, such as severe deep bite or crowding, the direct causative relationships and influences on periodontal health are evident. Such influences are determined primarily by the pathological traumatic occlusion and unfavourable position of the tooth inside the bone envelope, and secondarily, by favouring the plaque accumulation which results in progression of plaque-induced periodontal breakdown.

This is not always the case for periodontal pathologies that trigger or predispose orthodontic problems. In the absence of a thorough clinical check and comprehensive causal diagnosis, numerous orthodontic symptoms might be wrongly treated without accounting for the underlying periodontal, often causative or predisposing factor. Such a situation is typically encountered in adult patient referring diastemas opening over time and teeth flaring (**Figure 1a**). If no detailed clinical and radiographic checks were performed, neglecting the periodontal health, the orthodontic treatment alone would have aggravated the periodontal state, resulting in an iatrogenic damage and the post-treatment stability would have been questionable.

The clinical check-up itself should always be preceded by a detailed anamnesis for identification of potential risk factors. Thereafter, it should include compilation of a periodontal chart including all relevant periodontal indexes, general radiographic estimation and, when deemed necessary, a detailed radiographic assessment through series of periapical radiographs (**Figure 1b**).

It is of utmost importance to consider the presence of highly compromised periodontal health in young patients as an indicator that might suggest need for further laboratory and genetic analysis in order to identify potential risk factors to periodontal pathology or important systemic pathologies (**Figure 1c**).

In terms of ethology, it is well established that periodontitis is a multifactorial condition and apart from microbial and environmental factors, its progression is determined also by genetic susceptibility [1]. Epidemiologic data suggest that approximately 10–15% of populations are susceptible to a quick progression from gingivitis to periodontitis which can hardly be explained by solely microbiological or external factors [2, 3]. Numerous genetic polymorphisms have been characterized and recognized to play a role here [4]. While the influence of genomic testing on non-surgical periodontal treatment outcomes has been recently questioned [5], it should be kept in mind that its consideration remains highly important for prevention measures, early diagnosis and better individualized treatment protocol. Moreover, when integrated into a multifactorial diagnostic scheme, it allows for a comprehensive estimation of risk factors. To-date epidemiological evidence is limited and further investigation is required in order to thoroughly understand the nature of this association and its clinical implications [3]. Nevertheless, since full genome sequencing is feasible, phenotypic and genotypic data can be used to improve ‘personalized’ treatment and public oral health [6]. Apart from genetic influences, other risk factors such as diabetes, smoking



**Figure 1.** Twenty-eight-year-old female patient (FG), whose major requirement is diastema closure and teeth alignment. (a) Intraoral aspect; (b) during periodontal probing, it is noticed the presence of infrabony defects in multiple sites on both maxilla and mandible. A complete series of periapical radiographs is performed, showing a diffuse bone loss. (c) Bacteriological analysis on collected gingival cervical fluid indicated high levels of *actinobacillus actinomycetemcomitans*, *porphyromonas gingivalis* and *prevotella intermedia*.

and stress should also be considered, controlled and integrated into the overall treatment plan. The destructive effects of smoking on periodontal tissues have long been recognized, and recent biochemical and genetic studies have clarified direct and indirect pathways of this association [7, 8]. Levels of matrix metalloproteinase-8 (MMP-8), which is involved in periodontal destructions, are significantly increased in smoking patients. Additionally,

immune response is modified and the pathogenesis of the disease is negatively affected [8]. A similar pattern is present in patients affected by type 2 diabetes mellitus suggesting that diabetic-smoker patients have increased periodontal breakdown and are prone to a more severe periodontitis [9].

## 2.2. Multidisciplinary treatment plan

Oral functional and aesthetic rehabilitation in adult patients is a complex process that requires exhaustive scientific knowledge, extensive clinical experience and, in most cases, a multidisciplinary treatment planning. Independently of what the role of orthodontic therapy might be in the multidisciplinary frame, each tooth movement should initiate and be fulfilled in the presence of a healthy periodontium. It has already been demonstrated that if orthodontic treatment is conducted in the presence of periodontitis, it causes major bone destruction and clinical attachment loss (**Figure 2a, b**).

General treatment guidelines for orthodontic patients with chronic periodontitis suggest initial non-surgical therapy for adequate plaque control and then reevaluation [10]. It is purported that critical pocket depth for maintaining periodontal health with no need for surgical intervention is 4–5 mm, but this should be carefully estimated considering patient compliance on adequate oral hygiene and specific needs for regenerative therapy [11].

The non-surgical periodontal treatment consisting of scaling a root planning results in gingival recession due to inflammation reduction (**Figure 3a**). It is advisable to inform the patient in advance regarding this effect as it might compromise aesthetics. Following the classical approach, in case of persistence of infrabony defects of 4–5 mm deep after the initial periodontal therapy, the open flap debridement and regenerative treatment are carried out (**Figure 3b–e**). Another more recent alternative approach at this stage is the performance of

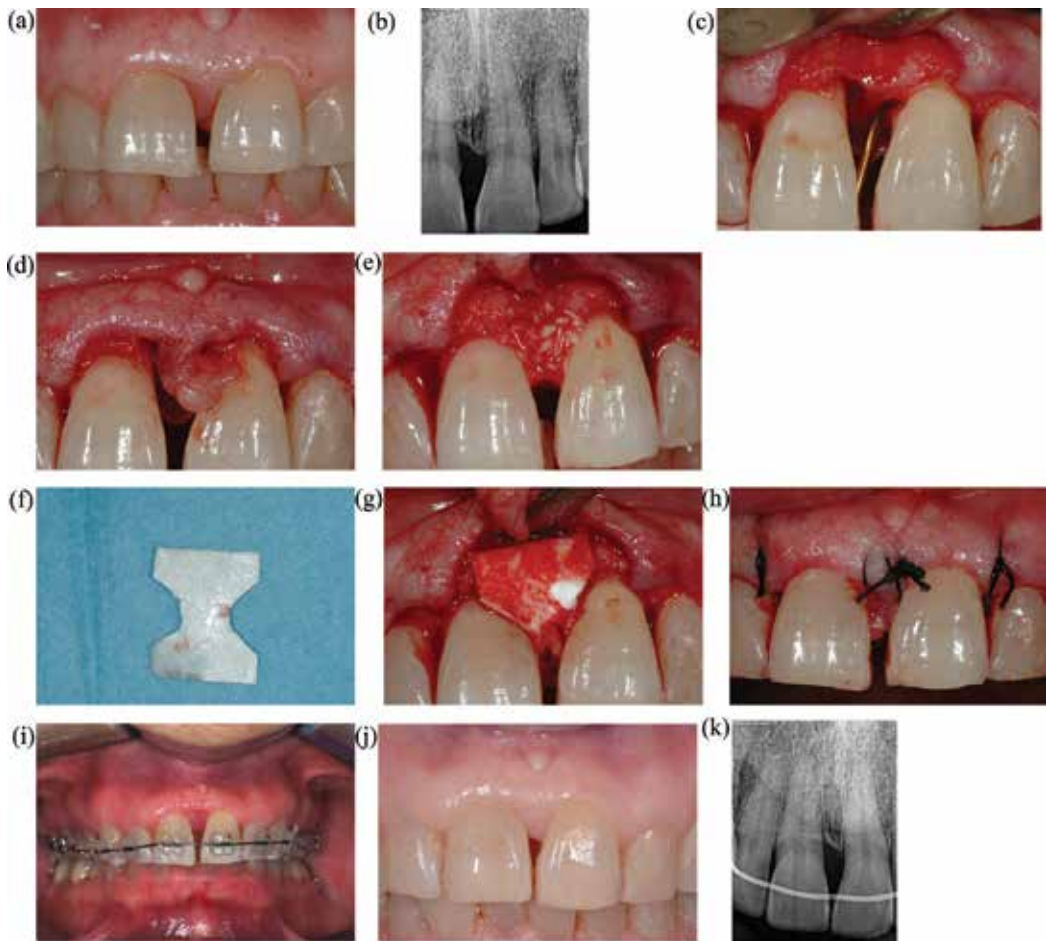


**Figure 2.** Twenty-two-year-old female patient having a fixed orthodontic appliance for 2 years. She was referred to us by an orthodontist who after one year of orthodontic therapy realised the gravity of the periodontal situation after performing a panoramic radiograph. The orthodontist refused to remove the orthodontic appliance, afraid that the patient would lose the teeth, being retained in their position only through the ligation to the archwire. (a) Panoramic radiograph showing the extensive bone loss in almost all maxillary teeth. (b) Periapical radiographs indicating the almost complete loss of periodontal support for multiple maxillary teeth.



**Figure 3.** Patient F.G. (a) Intraoral aspect 2 weeks after completion of periodontal causal therapy (scaling and root planing). Notice the gingival recession that follows the reduction of inflammation; (b, c) open flap debridement on vestibular and palatal side; (d) injection of enamel matrix derivatives; (e, f) flap adaptation and suturing on the maxillary and mandibular arch. Because of the post-periodontal therapy recession, the flap adaptation results more challenging; (g) orthodontic therapy during space closure phase; (h) panoramic radiograph before periodontal therapy; (i) panoramic radiograph during orthodontic therapy. Please notice the overall enhancement of bone level and reduction of infrabony defects. (j, k, l) intraoral aspects at the day of orthodontic appliance removal. The patient showed great compliance during the entire treatment.

deep debridement and regeneration in a unique phase intra-surgically proceeded only by superficial debridement in order to avoid gingival recession and to have the possibility to reposition the soft tissues more coronally. This would allow for unaltered aesthetics and better root coverage, especially considering the fact that flap adaptation is a crucial aspect of the healing quality (Figures 4a–g and 5a–g). Despite the preferred approach, the surgical stage should be followed by a healing time before orthodontic therapy commencement. This period would allow for connective tissue stabilization and remodelling, restoration of health and evaluation of patient's compliance [12]. If the regenerative therapy is integrated into an ongoing orthodontic treatment, the compromised teeth should be immobilized through rigid splinting in order to stabilize the clot and the regenerative material throughout the healing phase.



**Figure 4.** Thirty-eight-year-old female patient (T.A.), whose midline diastema was opened during the past 3 years. Major request was diastema closure. (a) Initial intraoral aspect; (b) initial radiograph; (c) deep manual debridement during surgical intervention. A supragingival debridement was previously performed; (d) sufficient gingival tissue for appropriate coverage of regenerative materials; (e) synthetic bone grafting; (f) membrane shaped extraorally following the area to be covered; (g) adaptation of the membrane in order to cover all grafting material; (h) suturing with coronal flap adaptation; (i) orthodontic treatment started 3 months after surgery; (j) clinical aspect at the end of orthodontic treatment; (k) radiograph at the end of orthodontic treatment with lingual fixed retainer.



**Figure 5.** (a) Gingivitis present during orthodontic therapy. (b) when the inflammation is limited only at the gingival level and the deep periodontal tissue is not compromised, after orthodontic appliance removal the gingival inflammation disappears. (c) Periodontal probing after orthodontic appliance removal indicates healthy periodontal state.

The regenerative therapy might follow several approaches, including open flap debridement, guided tissue regeneration (GTR) associated or not with different types of bone grafting, growth factors and more recently also stem cell-based therapy [13–15]. Utilization of mesenchymal stem cells in periodontal regenerative therapy has already resulted in promising outcomes that need to be further elucidated especially in terms of cell survival and efficient expression of programmed proliferative capacity to consent fully translation of mesenchymal stem cell-based therapy into clinical practice [15–22]. Irrespective of the chosen regenerative approach, the orthodontic treatment that follows should aim for elimination of occlusal traumatic contacts and establishment of a functional occlusion, which is one of the major factors for maintenance of periodontal health. Dental implant therapy for tooth replacement is incorporated after a thorough treatment planning followed by the positioning of teeth and roots into correct positions. If implants are planned and inserted before the end of orthodontic therapy, they might also be used as an anchorage unit for facilitating the orthodontic treatment. Maintenance of periodontal health is of utmost importance also for implants success and survival. Patients susceptible to periodontal pathologies are more prone to peri-implant inflammation and implant failure. Potential prosthetic restorations are usually performed at the end of orthodontic therapy, aiming reconstruction of lost tooth tissues or replacement of missing crowns.

Most of adult patients suffer also from enamel and dentine excessive consumption especially at the cervical area, which results in highly compromised aesthetics, hypersensitivity and important discomfort [23, 24]. Treatment of these cervical, non-carious defects is mainly focused on hypersensitivity reduction [25], whereas hard tissue reconstruction remains a controversial and challenging issue, especially considering the aetiology and progress of such pathology. Recent emerging technologies have proposed restoration of dental enamel using high repetition rate femtosecond lasers and novel iron-doped calcium phosphate biomaterials. During this procedure, the irradiated mineral transforms into a densified layer of acid-resistant iron-doped  $\beta$ -pyrophosphate, which bonds with enamel and dentine surface of non-carious lesions [26, 27]. This promising technology is yet to be fully developed for optimal clinical usage.

Attempts in reducing duration of orthodontic treatment especially in adult patients have recently resulted in therapies combining surgical and non-surgical approaches to orthodontics. Even if surgical adjunctive therapies have been studied for many decades, their overall benefit is still questionable, whereas non-surgical approaches do not provide solid scientific bases for incorporation into everyday clinical practice [28, 29]. Careful consideration of patient-centred aspects, treatment time and overall cost-benefits must be performed for a thorough estimation of these approaches.

Each adult patient is a challenging situation that in the majority of cases requires the periodontal and orthodontic therapy for denture preparation and later prosthetic therapy for full rehabilitation. In almost all cases of mature patients, orthodontic treatment alone would not result in the best possible approach for oral functional and aesthetic rehabilitation and sometimes it would also deteriorate the overall state of oral health.

It should be bared in mind that all orthodontic movements are guided by a periodontal dimension that should be of primary consideration during treatment planning and application of orthodontic biomechanics. On the other hand, correct functional occlusion and ideal tooth position are one of the major prerequisites for maintenance of periodontal health.



### 3. Interrelationship between periodontics and orthodontics in adult patients

#### 3.1. Periodontal considerations during adult orthodontic treatment

The presence of healthy periodontal tissues is of vital importance for undertaking any kind of orthodontic or prosthetic therapy. In adult patients, even if periodontal tissues might be reduced and have compromised sustaining capacity, they should be free of inflammation. All attempts in applying an orthodontic treatment on inflamed periodontal tissues will aggravate the periodontal state and result in an iatrogenic damage [30].

Components of orthodontic appliances predispose aggregation of bacterial plaque which, if not properly controlled through accurate hygiene regimen, might trigger gingivitis or its conversion into periodontitis [31, 32]. On the other hand, when a thorough oral hygiene regimen is applied, gingivitis is easily controlled, and when no other periodontal tissues get involved, it seems to disappear in long term after appliance removal (**Figure 5**) [12, 33]. Periodontal short-term effects after orthodontic therapy remain controversial [34], which suggest that in all cases orthodontic patients are at a higher risk for periodontal disease development. The increasing demand for orthodontic treatment and the occurrence of biofilm-related complications has positioned orthodontic treatment as a potential public health threat [35].

Independent of whether the periodontal health is compromised or not, in all adult patients, particular attention should be paid to avoid when possible bulk elements such as bands or other orthodontic components placed close to gingival margin that apart from plaque retention might cause marginal gingival injury and attachment loss. In this respect, orthodontic treatments based on removable appliances or aligners permit unimpeded oral hygiene and result in better periodontal health [36]. Nonetheless, while these aligners behave superiorly in terms of oral hygiene maintenance and periodontal health, other important considerations, such as occlusal interferences, gnathologic effects and postural ones, must be made before applying them.

According to the 'bone envelope' theory, orthodontic tooth movement should be performed within an anatomically and functionally periodontal limited space which is in any case to be respected in order to obtain the desired movement. If the periodontal space is violated, its connective tissue might react through a series of mechanisms such as bone dehiscence and fenestration, gingival inflammation and gingival recession. Apical migration of the gingiva (gingival recession) has an unpleasant effect in both aesthetics and cervical dentinal hypersensitivity. It might be caused by periodontitis, gingival trauma and specific anatomical conditions such as tooth crowding or muscular inserts. It has been demonstrated that in cases of tooth movement beyond the 'alveolar envelope' such as uncontrolled orthodontic movement, the recession might arise immediately but also years after, resulting in unpleasant post-treatment effects [37, 38]. It has been reported that factors related to the development or progression of recessions in adult patients are the presence of pre-treatment recessions, thin gingival biotype, reduced width of keratinized gingiva and visual gingival inflammation [39]. Other features, such as gingival margin thickness smaller than 0.5 mm and vestibular incisor inclination (over 95°), have also been associated with higher incidence and severity of recession [40]. In cases of pre-existing recessions, it is recommended that mucogingival interceptive surgery

be accomplished before orthodontic therapy to maintain the width of keratinized gingiva in the long term [41].

Every orthodontic movement induces remodelling and reorganization of periodontal tissues. It is demonstrated that light and continuous forces illicit non-destructive periodontal turnover, whereas heavy forces might result in necrotic periodontal tissue and further irreversible periodontal damage. Another consequence of these uncontrolled and heavy forces might be the root resorption, which is of extreme importance in teeth with reduced periodontium as further loss of periodontal support results in increased crown-root ratio and in compromised stability and aesthetics. Peculiar control of force levels has been outlined especially for orthodontic intrusion where the surface of force application is reduced. Intrusion also requires absolute control of inflammation and bacterial biofilms in order to avoid any aggravation or creation of infrabony defect and loss of attachment [42–45].

In most adult patients, the already-compromised periodontal tissue would need particular care in order to avoid further destruction and when possible aim for periodontal health enhancement.

Professional cleaning and examination of periodontal tissues should be performed routinely during the entire orthodontic treatment, following a personalized schedule that is determined by the individual risk factors and the specific treatment plan.

In all cases, orthodontic treatment should be suspended if patient fails to comply in maintaining the appropriate level of oral hygiene.

### **3.2. Orthodontics as a tool for periodontal health enhancement**

Orthodontic therapy on adult patients has become particularly popular in the last few decades, following the general trend of the modern life where overall aesthetic enhancement has become one of the major priorities. However, orthodontic treatment as a corrective tool that eliminates pathologic migration of teeth on periodontally compromised patients had been suggested long time ago in the literature, by authors such as Neustadt [46] Dummett [47] and Scoop [48]. Later studies conducted principally on animal models confirmed the beneficial effect of orthodontic therapy on periodontal health. The results of these animal studies demonstrated that movements of teeth with infrabony defects in an inflammation-free environment result in elimination of the bone defect through creation of a long epithelial junction [49, 50].

Extrapolation of animal studies' findings to human conditions has been questioned, mostly because of the specific pattern of attachment loss that occurs on humans [51]. However, it is well established that in the absence of periodontal inflammation, the biological process of bone resorption and apposition during orthodontic tooth movement might positively influence the healing of periodontal defects. Similarly, the positioning of teeth into correct occlusal relations, which allow for axial and physiological distribution of masticatory forces, results in reduced stress to periodontal tissues. Moreover, such an occlusal stability contributes in diminishing mesial migration of posterior teeth and related consequences such as incorrect



interdental contact points, flaring of incisor teeth and diastema opening. All these clinical symptoms are typical in adult patients, in which the malocclusion is aggravated by chronic periodontitis, resulting in the establishment of a vicious cycle where the two pathologies contribute to the exacerbation of each other (**Figure 6**). If the therapeutic approach does not account for both pathologies and does not follow a correct protocol starting with periodontal therapy and stabilization, the final result and its maintenance are highly questionable.

The orthodontic therapy can contribute to the improvement of periodontal health through reduction of intraosseous defects or furcation lesions [52–56]. It can also help in reducing gingival recession, levelling uneven gingival margins or rebuilding missing interdental papilla [12, 57, 58].



**Figure 6.** (a–e) The periodontal pathology has contributed in aggravating the malocclusion, characterized mainly by mesial tipping of posterior teeth, incisors flaring and diastema opening; (f–h) open flap debridement and regeneration; (i, j) soft tissue adaptation and suturing; (k) orthodontic therapy performed on a reduced periodontal tissue but with no active inflammation; (l) set of periapical radiographs after orthodontic alignment.

However, as reported by several recent systematic reviews, despite the major interest in trying to understand the beneficial effect of orthodontic therapy on periodontal health, the orthodontic scientific evidence still lacks solid protocols in this field and human studies are poor both qualitatively and quantitatively [44, 45, 54].

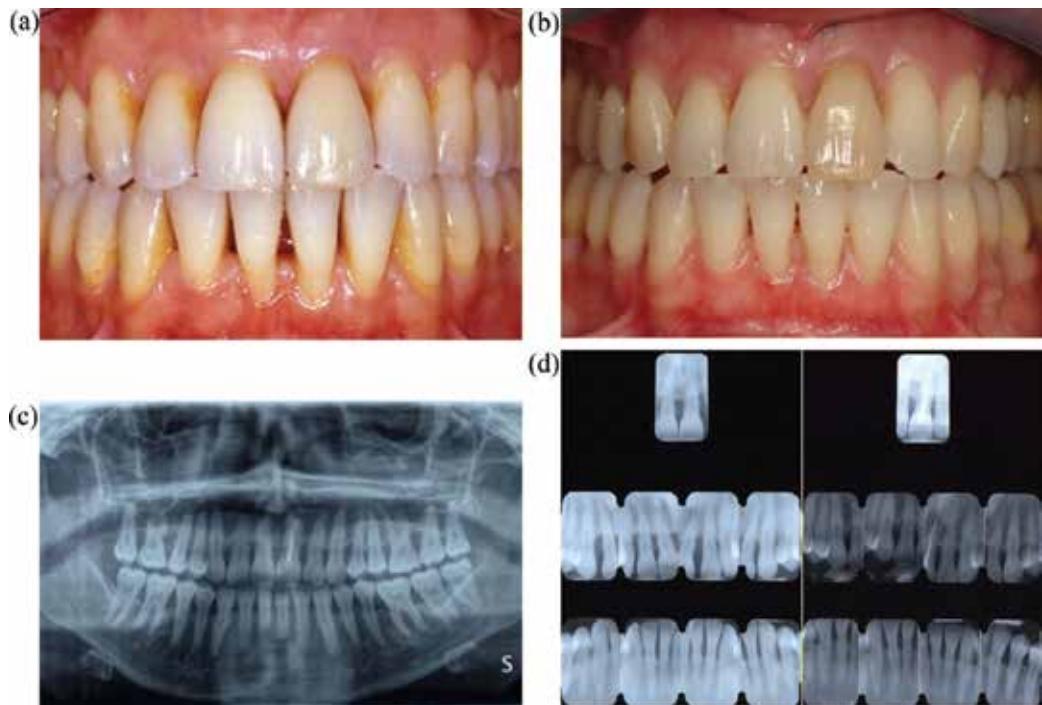
### 3.3. Post-orthodontic stability

Preservation of final orthodontic result is often considered as the third phase of overall orthodontic therapy and its major long-term goal. Post-orthodontic relapse has been mainly attributed to elasticity of gingival tissues that are compressed towards the direction of tooth movement. Considering this, the suggested retention period should exceed the time for remodelling of periodontal fibres, which usually ranges from 4 to 6 months [59, 60]. In order to enhance post-treatment stability attributed to soft periodontal fibres, many authors have suggested adjunctive interventions such as overcorrection, interproximal reduction or circumferential fibrotomy of supracrestal gingival fibres [61]. The variety of retainers is represented by the two big categories of fixed and removable ones. Scientific data report controversial opinions regarding the retentive method of choice for patients with reduced periodontal support. While the fixed retainer assures firm position and satisfying long-term stability, it does not allow for teeth to retain their physiological mobility, and in most cases, it impedes maintenance of good oral hygiene which is of fundamental importance for these patients [62]. In all cases, bulk fixed retentions that block easy access through interdental spaces should be avoided and patients should be given exhaustive instructions for appropriate oral hygiene maintenance (**Figure 7**).

Finally, despite the importance of considering all periodontal and mechanical features of retentive devices, the authors of the present manuscript consider of primary importance for long term retention a final orthodontic result that assures occlusal stability, meaning correct teeth positioning, physiological movement with no interferences or dislocating pre-contacts [63]. It is extremely relevant to recognize the importance of a good balance between static and dynamic occlusion and all related craniofacial structures represented by temporomandibular joint (TMJ) and the neuromuscular system for both oral rehabilitation and long-term stability. A final orthodontic result that respects these parameters is the best tool for physiological retention, with no need for external mechanical elements (**Figure 8**).



**Figure 7.** (a, b) Bulk lingual and vestibular splinting of teeth, which do not allow for appropriate oral hygiene maintenance. (c) Radiographic image showing the complete loss of periodontal support.



**Figure 8.** (a) Clinical check at 7 years of follow-up. (b) Clinical check at 13 years of follow-up. (c) Panoramic radiograph at 13 years of follow-up. (d) Periodontal radiographic status before the therapy and 13 years after the therapy with no fixed orthodontic retainer.

## Acknowledgements

The authors acknowledge the financial support of EU-Marie-Curie-IAPP LUSTRE (324538) project.

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# Immune Strategy

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# Periodontal Disease and Autoimmunity: What We Have Learned from Microbiome Studies in Rheumatology

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69012>

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## Abstract

The oral cavity is home to vast populations of commensal microbial organisms which constitute the 'healthy oral microbiome.' Periodontitis is a destructive, infectious, inflammatory condition affecting the gums. Initially, a biofilm structure develops, causing localized inflammation. This biofilm is then colonized by certain anaerobic bacteria, including the 'red complex' organisms. There is an increasing interest in the communication between these organisms and host immune surveillance, a dialog which may play an important role in the development of autoimmune diseases. Studies have shown an association between periodontitis and other inflammatory conditions including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and systemic lupus. The advent of accessible 16S ribosomal sequencing has led to exciting developments in the characterization of the human microbiome and the ability to study this interaction in more detail. The trans-mucosal communication between periodontitis and host immunity may provide avenues of discovery regarding the etiology and progression of rheumatic diseases.

**Keywords:** *Porphyromonas gingivalis*, gums, biofilm, periodontal membrane, microbiome, rheumatology, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, systemic lupus erythematosus, osteoarthritis

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## 1. Introduction

It is known that the human body is covered with microbes. Until recently, many of these have been thought of as indolent, 'commensal' organisms, living in harmony with the host and thought of little relevance. If we consider that a single human being consists of approximately 1 trillion human cells but 10 trillion bacterial cells and, at a genetic level, 20,000 human

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genes but between 2 and 20 million bacterial genes, the indolence of these bacteria has to be questioned. There is therefore increasing interest in the roles played by these microorganisms on host immunology and physiology.

The oral mucosa is home to vast populations of these 'commensal' microbial organisms. It is therefore an arena which is ripe for communication between foreign organisms and host immune surveillance, a dialog which may play an important role in the development of autoimmune diseases.

The thrust of this chapter is to provide an update on the current literature regarding the role of microbiota in rheumatological conditions including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, osteoarthritis and systemic lupus.

## 2. The human microbiome

The term 'microbiome' refers to the sum of bacterial communities that colonize a particular area [1]. In humans, this includes almost any surface, for example, the skin, gut, airways, genitourinary tract and oral cavity. It is estimated that the human body is comprised of 1 trillion human cells [1], with genetic material accounting for approximately 20,000 human genes [2]. The bacteria which colonize the human body are estimated to contribute a further 20 trillion cells and between 2 and 20 million bacterial genes [1]. This does not include estimates of fungi and viruses which inhabit the human corpus. This has led to conjecture that a human is not simply 'an organism' but rather 'a superorganism' comprised of multiple separate organisms. Whether this is the case or not, it is certainly conceivable that the microorganisms that colonize the various niche environments of the human body contribute to physiology, biochemistry and immunity.

Study of the bacterial microbiome was previously limited by culture-dependent techniques which are limited in their ability to identify beyond the most prevalent organisms or that are amenable to culture in the media available. However, there have been exciting developments in the characterization of the human microbiome with the relatively recent advent of accessible 16S ribosomal sequencing.

Prokaryotic cells contain 70S ribosomes which consist of larger 50S and smaller 30S subunits [3]. The latter is comprised of 22 ribonucleoproteins and 16S ribosomal RNA. Due to the slow rate of evolution and highly conserved primer binding sites, 16S ribosomal RNA has been developed as a tool for phylogenetic studies [4]. With developments in the processes of RNA extraction, amplification with 16S rRNA primer and high-throughput sequencing, this has become an increasingly available method of bacterial identification, providing proportionally quantitative data on all bacteria in a sample. Identification libraries are growing and initiatives including the human microbiome project (HMP) [5] in the USA and metagenomics of the human intestinal tract (MetaHIT) [2] in Europe aim to catalogue the microbiomic constituents of the human body in health and disease. Of course, in order to identify changes in the microbiome related to disease, the spectrum of normality must first be established.

The human microbiome represents a previously unexplored arena which may provide discoveries relating the etiology, progression and management of human disease. However, it must be recognized that the horizons of human-microorganism interaction are vast and include bacterial proteomics, metabolomics, not to mention the human virome. Much endeavor is required to delineate the role played by these organisms in human diseases, including rheumatoid arthritis and periodontitis.

### **3. Periodontitis and the oral microbiome**

#### **3.1. Periodontitis**

The periodontium is made up of the gingiva and the underlying attachment tissues. Gingivitis is a reversible form of gingival inflammation characterized by redness, swelling and bleeding. Periodontitis occurs once the inflammation extends to the deeper tissues including the periodontal ligament and alveolar bone. The loss of periodontal attachment and bone produces deepening of the gingival sulcus (periodontal pocket) and progressive loosening of teeth eventually leading to their loss [6].

The prevalence of periodontitis varies internationally; however, approximately 10–15% of the global adult population are affected by the condition [7]. The etiology is multifactorial and is composed of genetic predisposition, bacterial dysbiosis associated with a specific local and systemic host response and environmental factors [8] Although there is a genetic element to periodontitis, it is usually polygenic and so difficult to predict. Known risk factors include smoking, age, diabetes mellitus, educational level, gender and immunological diseases (e.g., HIV) [7, 9].

Plaque-induced periodontitis is the most common presentation. Initially, a biofilm structure develops which causes localized inflammation in the form of gingivitis. This biofilm is then colonized by anaerobic bacteria which causes further inflammation and neutrophilic activation. Matrix metalloproteinases are spilled, leading to tissue destruction, exacerbating the attachment loss and deepening the periodontal pocket. These results in further anaerobic colonization, soft tissue destruction, alveolar bone loss and ultimately tooth loss [8].

Management of periodontitis is aimed at excellent oral hygiene with twice-daily brushing of teeth and use of interdental brush and flossing. Plaque removal with scaling and debridement can be used to prevent excessive buildup of plaque. Low-dose doxycycline can inhibit matrix metalloproteinases such as collagenase and therefore reduce tissue damage. In severe cases and nonresolving gingival inflammation, there are surgical options which could allow for some regeneration of the lost soft and hard tissues [8].

#### **3.2. The oral microbiome**

The oral microbiome is composed of the microorganisms found in the oral cavity, or as defined by Joshua Lederberg 'the ecological community of commensal, symbiotic, and pathogenic

microorganisms that literally share our body space [10]. Although about 280 species have been cultured, it is estimated that they make up less than half of the bacterial species present in the oral cavity and the true number is likely to be between 500 and 700 [11, 12].

The advent of next generation sequencing has allowed the development of the human microbiome project. Findings suggest that although each individual body site is colonized by a characteristic microbiome, it is the individual who is colonized which is the primary factor affecting the bacterial makeup [11]. In the mouth, there are three distinct bacterial communities: the buccal mucosa, gingivae and hard palate forming one; the saliva, tongue, tonsils and throat forming another; and lastly the supra- and subgingival plaque [13].

Ninety-six percent of the bacterial community of the mouth is the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochaetes* and *Fusobacteria* [11, 14]. The composition of bacteria in ‘healthy’ and ‘diseased’ sites in the mouth is shown in **Table 1**.

Of these bacteria, there is the most evidence that *Porphyromonas gingivalis* has a key role to play in the pathogenesis of periodontitis and that with ‘accessory pathogens’ it alters the host immune response [8]. For example, *P. gingivalis* has specifically been implicated in blocking complement activation and inhibiting complement function [9].

‘Healthy’ sites; more gram-positive organisms	Dental caries	Gingivitis; gram-positive aerobes, facultative anaerobes, gram negatives	Periodontitis; gram-negative and anaerobic organisms
<i>Actinomyces</i>	<i>Streptococcus mutans</i>	<i>Actinomyces</i>	<i>Porphyromonas gingivalis</i> *
<i>Streptococci</i>	<i>Streptococcus sobrinus</i>	<i>Streptococci</i>	<i>Porphyromonas endodontalis</i>
<i>Veillonella</i>	<i>Actinomyces</i>	<i>Treponema</i>	<i>Tannerella forsythia</i> *
<i>Granulicatella</i>	<i>Lactobacillus acidophilus</i>	<i>Synergistetes</i>	<i>Treponema denticola</i> *
<i>Corynebacterium</i>	<i>Bifidobacterium</i>		<i>Aggregatibacter actinomycetemcomitans</i> *
<i>Fusobacterium</i>	<i>Propionibacterium</i>		<i>Anaeroglobus geminatus</i>
<i>Gamella</i>	<i>Veillonella</i>		<i>Eubacterium saphenum</i>
<i>Rothia</i>			<i>Filifactor alocis</i>
<i>Porphyromonas</i>			<i>Prevotella denticola</i>
<i>Prevotella</i>			<i>Prevotella nigrescens</i>
<i>Staphylococcus</i>			<i>Fusobacterium nucleatum</i>
<i>Lactobacterium</i>			
<i>Haemophilis</i>			
<i>Peptostreptococcus</i>			

\*Red-complex organisms implicated in periodontitis [11–14].

**Table 1.** Selection of bacteria found in oral microbiome in health and disease.



### 3.3. Measures of periodontitis

In order to carry out high-quality studies investigating the association of periodontitis with other conditions, it is imperative to have valid and reliable measures of periodontitis.

There are a number of measures, shown in **Table 2**, which have been developed, looking at both gingivitis severity and periodontal disease [15, 16].

These measures have significant limitations. Multiple confounders such as age, smoking and immunosuppressant therapy can make them very difficult to interpret [7, 17]. Some measures under- or overestimate periodontal disease as they are affected by gum recession alone. However, the most significant limitation is that there is no single gold standard definition of a threshold for a diagnosis of ‘periodontitis’. Most studies use varying thresholds which has a significant impact on prevalence rates and means studies need to be compared with caution [18].

	Measure	Definition	Method	Characteristic
<b>Gingivitis</b>	Bleeding on probing (BOP)	Bleeding of gingiva on gentle probing	Bleeding within 10 seconds, following gentle probing of gingival crevice	Assessment of gingivitis
	Bleeding index	BOP, calculated as an index	Number of sites BOP, divided by the total number of available sites multiplied by 100	Assessment of gingivitis
	Loe and Sillness Gingival index	Degree of gingival inflammation, characterized by erythema, hypertrophy and bleeding	Degree of inflammation given score out of four. The scores of four areas of the tooth are averaged	Assessment of gingivitis
<b>Periodontitis</b>	Missing teeth	Number of missing teeth	Count missing teeth	Crude, may be due to reasons other than periodontal disease
	Alveolar bone loss	Loss of the bone which supports the teeth	Periapical radiograph	Associated with tooth loss secondary to periodontitis
	Probing depth (PD)	Depth of the periodontal pocket	Measure the distance from the gingival margin to the base of the pocket	Measure of current periodontitis
	Clinical attachment level (CAL)	Measurement of the position of the soft tissue in relation to the cementoamel junction (CEJ)	Calculated using the probing depth and the level of the gingival margin	Measure of cumulative periodontal disease

**Table 2.** Gingivitis and periodontal disease clinical measures [15, 16].

#### 4. Periodontitis and systemic inflammation

The interest in the systemic inflammatory profile of patients affected by periodontitis has risen in the last 30 years to investigate the plausibility of the hypothesis that periodontal infection might have an impact on the systemic health. Particularly, the effect of periodontitis on the cardiovascular system but more recently a large spectrum of conditions such as neurodegenerative diseases, diabetes mellitus and rheumatoid arthritis, has been investigated. All these diseases are characterized by an increased systemic inflammatory profile, and periodontitis could contribute to their onset and progression by elevating pro-inflammatory markers. Consistent evidence from observational studies has suggested that severe periodontitis is associated with increased serum levels of C-reactive protein (CRP), moderate leukocytosis, as well as increased serum levels of interleukin-1 (IL-1) and IL-6 [19–23]. Furthermore, elevated serum levels of CRP have been associated with presence of keystone periodontal pathogens [24].

A recent systematic review confirmed a weighted mean difference of 1.56 mg/l ( $p < 0.00001$ ) of CRP levels between cases with periodontitis and controls [25]. Further, the review reported on data from six intervention studies, concluding that periodontal treatment produced a 0.50 mg/l (95% CI 0.08–0.93) ( $p < 0.02$ ) reduction of CRP serum levels. A recent meta-analysis on the effects of periodontal therapy and systemic inflammation reported a 0.23-mg/l reduction in CRP levels after treatment ( $-0.231$ ;  $p = 0.000$ ) [26]. Interestingly, a further analysis confirmed that the anti-inflammatory effect of periodontal treatment was more evident in clinical trials involving patients with periodontitis and other comorbidities like diabetes [27]. Individuals with periodontitis also show increased serum concentration of IL-6 and TNF- $\alpha$  compared to controls. Intervention trials reported inconclusive results on the effect of periodontal treatment on serum levels of these markers [28].

Different plausible mechanisms by which periodontitis could cause an increase in systemic inflammatory levels have been suggested. Firstly, multiple studies have reported the production of inflammatory cytokines in the periodontal pocket [29] and it has been postulated that these mediators could reach the bloodstream leaking from the inflamed periodontal lesions. Some of these mediators (i.e., IL-6) could have an effect on distant tissues and organs such as the liver, triggering an acute-phase response. However, for the time being, there is a limited evidence supporting this mechanism [30].

Secondly, bacteria colonizing the periodontal pockets and/or their by-products have been detected in the peripheral circulation [31]. A disruption of the subgingival epithelium could lead to short-lived bacteremia. This could trigger an immune response as well as allow bacteria to directly impact on the vasculature or distant organs (like the liver or kidney). In support of this hypothesis, periodontal bacteria DNA [32] and viable pathogens [33] have been detected in human atherosclerotic plaques. Thirdly, several antibodies induced by periodontal pathogens might trigger a molecular mimicry with cross-reactive antibodies recognizing host antigens.

Lastly, periodontitis and other comorbidities such as cardiovascular diseases and diabetes, share many common risk factors, including obesity and smoking [34, 35] which both have an effect on the systemic inflammatory profile [36]. This might account for a spurious association between periodontitis and systemic inflammation. However, the majority of the observational

and experimental evidence available seems to support the concept that periodontitis could independently contribute to systemic levels of different inflammatory mediators and that periodontal treatment could result in their reduction. The systemic low-grade inflammation generated by periodontal infection might represent the link between periodontitis and systemic conditions [37, 38].

## **5. Periodontitis and rheumatoid arthritis**

### **5.1. Rheumatoid arthritis**

Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint inflammation and destruction with extra-articular features including rheumatoid nodules, pulmonary disease, vasculitis and neuro-inflammation [39]. It can lead to chronic disability, early mortality, systemic complications and high socioeconomic burden on society as a whole [40]. The prevalence of RA is 0.5–1.0% [13] with apparent variation according to latitude and urban/rural habitation [41, 42].

It is currently classified according to the 2010 American College of Rheumatology/European League against rheumatism criteria by joint involvement, positive serology for rheumatoid factor or anti-citrullinated protein antibodies (ACPA), raised acute-phase reactants and the duration of symptoms [43]. Treatment regimens traditionally include corticosteroid to induce remission used in combination with maintenance of disease-modifying antirheumatic drugs, though have been much improved by the intervention of biologic agents.

### **5.2. Pathogenesis of rheumatoid arthritis**

The exact etiology of RA is unknown; however, it is thought to be secondary to an interaction between genetic attributes and environmental exposures.

Indeed, genetic studies in twins have estimated approximately 60% heritability of rheumatoid arthritis [44]. Genetic polymorphisms including HLA-DRB1 [45] are implicated. However, other susceptibility alleles including genes involved in the differentiation of T cells, selection of antigens and peptide affinity have been identified by genome-wide association studies.

In terms of environmental insults, smoking has long been associated with RA [46] as have social factors including lower socioeconomic class and education but other areas of interest include silica, exogenous infections, periodontitis and the microbiota of mouth, gut and lung [47].

Autoantibodies to the Fc portion of immunoglobulin are known as rheumatoid factor and antibodies that are known to form against citrullinated proteins are called anti-citrullinated protein antibody (ACPA) or anti-cyclic citrullinated peptide (anti-CCP) antibodies. If either of these are present, they confer 'seropositivity,' which is seen in 70–80% of patients [48]. In addition to forming an element of the diagnostic criteria [43], seropositivity is also associated with more aggressive disease and with the earlier development of erosions. Rheumatoid factor is limited in diagnostic application by low specificity; however, ACPA is 95–98% specific [49].

ACPA has been shown to be present in RA patient sera up to a decade prior to the development of the disease [50] although the amount of ACPA and inflammatory cytokine levels rise sharply a few months before the synovitis presents [51]. It is therefore hypothesized that as well as citrullination of endogenous proteins, a second inflammatory 'hit' is required to stimulate the development of RA. Citrullinated proteins are also associated with other environmental factors such as smoking and pathological conditions including periodontitis.

Peptidyl arginine deiminase (PAD) causes the posttranslational modification of arginine to citrulline. It is hypothesized that this citrullination leads to amino acid chains being recognized as autoantigens, which leads to the development of autoantibodies and the subsequent autoimmune damage that is the signature for rheumatoid arthritis. PAD is produced by human cells, for example, in the lung; however, it is also produced by the microbe *P. gingivalis* [52].

Mucosal surfaces provide a rich opportunity for cross-talk between the immune system and the microorganisms which inhabit them. It is supposed that these microbes are not simply left unattended and indolent, but are held in a constant state of tension by physiological barriers (mucus and immunoglobulin A), epithelial tight junctions, innate immune surveillance (by macrophages and dendritic cells) and adaptive immune response.

The mucosal-joint axis hypothesis is supported by the fact that 20% of chronic inflammatory bowel disease patients develop an enteropathic arthropathy and that reactive arthritis can develop in response to chlamydial and dysenteric organism. A possible mechanism is therefore that disruption in pathogenic-commensal bacterial balance at the mucosal surface leads to innate activation and pro-inflammatory cytokine release, leading to a lasting adaptive immune response. If we then consider that some bacterial epitopes are shared with cartilage [53], it is possible that the adaptive immunity, in response to transmucosal exposure to a microorganism, could lead to sustained autoimmunity.

### 5.3. *P. gingivalis* and rheumatoid arthritis

PAD production by *P. gingivalis*, an anaerobic prokaryote, has been demonstrated in vitro. Due to this organism's role in the development of periodontal disease and the association of rheumatoid arthritis with periodontitis, it has been hypothesized that *P. gingivalis* provides a causal link between periodontal disease, citrullination and RA [54].

The temporal nature of this association is a point of conjecture, though it has been shown that non-RA (as defined by the American College of Rheumatology (ACR) criteria) patients with risk factors for the development of RA such as first-degree relatives and ACPA positivity had a higher concentration of anti-*P. gingivalis* antibodies [55]. This finding suggests that *P. gingivalis* (one of the 'red complex' organisms) appears prior to the development of rheumatoid arthritis in an 'at risk' population. Indeed, an etiological role of *P. gingivalis* is implicated by the recent finding that anti-*P. gingivalis* antibodies are significantly higher in RA cases compared to controls and, similar to ACPA, are present in sera years before the onset of symptoms of inflammatory arthropathy [56].

In addition to an etiological role, there is longitudinal evidence to suggest that *P. gingivalis* PAD may attenuate response to biologic Disease-modifying anti rheumatic drugs [57].

Together, these elements suggest a role for this organism in various aspects of rheumatoid disease and pronounce *P. gingivalis* as a viable avenue for further research.

#### 5.4. Periodontitis in rheumatoid arthritis

Epidemiological studies have shown a strong association between periodontitis and RA [58, 59], though these have been hampered by their cross-sectional nature, variability in definition of periodontitis and dental endpoints, the extent of oral examination and the limited RA information collected. However, despite the above, there remains a significant amount of robust evidence to support the association between periodontitis and RA including a recent meta-analysis of 17 studies and over 150,000 participants, found a significant association between RA and periodontitis with a relative risk of 1.13 (95% CI 1.04–1.23,  $p = 0.006$ ) compared to healthy controls [60].

Shared risk factors, including cigarette smoke, provide possible confounders; however, an increased risk of periodontitis has been demonstrated in a nonsmoking RA group [61], and a comparison of periodontitis in osteoarthritis versus RA demonstrated that RA patients were twice as likely to have moderate to severe periodontitis independent of smoking, age or sex [62]. In addition, there is evidence to suggest that periodontitis responds to RA treatment [63].

#### 5.5. Oral microbiome in rheumatoid arthritis

The association of periodontitis and *P. gingivalis* with rheumatoid arthritis has led to a great deal of interest in the oral microbiome in rheumatoid disease. Indeed, oral bacterial DNA and antibodies to *P. gingivalis*, *T. forsythia* and *P. intermedia* have been demonstrated in synovial fluid and the site of inflammation in rheumatoid arthritis [64]. It is also interesting that the medications with an antibacterial action against these species (including levofloxacin and clarithromycin) seem to have a beneficial effect in RA [65].

Animal studies have demonstrated a possible role periodontal bacteria in inflammatory arthritis. In a murine model of collagen-induced arthritis, oral infection with bacteria associated with periodontitis in humans developed exacerbated signs of inflammatory arthropathy, raised levels of matrix metalloproteinase 3 and histological evidence of active arthritis [66].

A study by Scher and colleagues used 16S ribosomal RNA sequencing to investigate oral microbiota from the subgingival plaque in patients with new-onset, Disease-modifying anti rheumatic drug naïve rheumatoid arthritis [67]. They found significantly higher levels of *Prevotella* and *Leptotrichia* species in the new-onset RA group compared to controls, which was robust to adjustment for periodontal disease status. In addition, *Streptococcus* and *Corynebacterium* genera were reduced in the new-onset RA group compared to healthy controls. They also investigated a chronic RA (Disease-modifying anti rheumatic drug treated) group and found higher levels of red complex bacteria in the new-onset RA group compared to the established disease group. These findings illustrate the changes in subgingival microbiota over time, and further work is required to delineate any independent roles of these changes in the microbial landscape.

Investigations of the oral microbiome are potentially hampered by the site of sampling as the oral microbiome varies depending on the region sampled. The gingival sulcus, tooth surface, hard and soft palate, saliva and tonsils are home to significantly different compositions of bacterial populations at a baseline. With the addition of the increased expertise required to acquire samples, there are far fewer studies investigating the oral microbiome than the gut microbiome in RA.

### 5.6. Gut microbiome in rheumatoid arthritis

The gut microbiome is the most densely populated microbial environment in the human body and is the most extensively studied. The benefit of extensive investigation is that the spectrum of the bacterial constituents of a 'normal' gut microbiome is more clearly defined [68] than in the oral cavity.

Disruption of the gut microbiome has been demonstrated in patients with rheumatoid arthritis compared to controls [69], and preceding work in animal models has highlighted possible etiological mechanisms of action.

For example, manipulating the gut microbiome by instilling segmented filamentous bacteria into the small intestine of mice induces CD4 T cells which produce the pro-inflammatory cytokine IL-17 in the intestinal lamina propria [70]. The experimental creation of small bowel bacterial overgrowth leads to reactivation of resolved arthritis in a rat model [71]. The resultant proposed hypothesis is that bacterial overgrowth leads to systemic, transmucosal absorption of Lipopolysaccharide which deposits in the liver and joints, leading to an inflammatory response.

Subtraction of microbiota as well as addition can lead to a pro-inflammatory phenotype, as in an adjuvant-induced arthritis, rat model, gnotobiotic (germ-free) rats demonstrated significantly greater joint inflammation than those with normal gut microbiota [72].

Analysis of the fecal microbiome of patients with rheumatoid arthritis found that haemophilus species were significantly reduced and lactobacillus species were significantly increased [69]. This study also showed that the intestinal dysbiosis was attenuated by Disease-modifying anti rheumatic drugs. *Prevotella copri* is raised in new-onset RA [73] and correlated with shared epitope genes, drawing attention to the organism as a potential environmental trigger for the development of RA.

The rapidly engorging literature regarding the gut microbiome and rheumatoid arthritis acts as encouragement to investigate other mucosal surfaces, and, with the marked associations with periodontitis and oral bacteria, the oral microbiome is a viable candidate for investigation.

### 5.7. Potential interventions

In addition studies to delineate the role of the microbiome in the etiology and progression of RA, it is worth considering the possible therapeutic interventions which could be developed.

Antibiotic administration is an apparent start, and doxycycline (which was historically used to treat RA) when used in conjunction with methotrexate has been shown to outperform

methotrexate alone [74]. In patients with mild-to-moderate RA, minocycline was shown to significantly improve joint swelling, tenderness and erythrocyte sedimentation rate in a placebo-controlled trial [75]. However, the use of broad spectrum antibiotics introduces the risk of depleting all elements of the microbiome and opening the door to hostile organism invasion.

Other postulated interventions include probiotics, fecal microbiota transplant [64], harnessing bacterial secretions and single-target approaches to modify bacterial composition and by-products. Although they are exciting opportunities for research, these approaches are still in relative infancy.

## 5.8. Conclusion

There are strong data to support the association between rheumatoid arthritis, periodontitis and *P. gingivalis*. The role of mucosal immunity in the development of autoimmunity has been demonstrated extensively in the gut, and similar pathogenic processes could occur in the oral mucosa, though further investigation is required to clarify the role played by the oral microbiome in RA.

## 6. Periodontitis and ankylosing spondylitis

### 6.1. Ankylosing spondylitis

Ankylosing spondylitis (AS) is a chronic inflammatory condition primarily affecting the spine and sacroiliac joints. It is characterized by inflammatory back pain caused by enthesitis, bone erosion and new bone formation. Clinical features also include peripheral arthritis, anterior uveitis and rarely lung fibrosis, heart block and aortic regurgitation [76].

The overall prevalence of AS is between 0.1 and 1.4% with most cases coming from Europe [77]. Onset occurs young with 80% of patients presenting before the age of 30 and less than 5% present after the age of 45. Men are 2–3 times more commonly affected than women [77].

The diagnosis of AS is based on clinical features and presence of sacroiliitis on X-ray or MRI [78]. Treatment options are still limited, and emphasis is placed on physiotherapy and nonsteroidal anti-inflammatory medications, with anti-TNF therapy reserved for those who experience a persistently high level of disease activity. However, these have many potential side effects [78] and further understanding of the etiology of AS is needed in order to develop other treatment options.

### 6.2. Pathogenesis of ankylosing spondylitis

More than 90% of the risk of developing ankylosing spondylitis is determined genetically [79]. An important contribution of this is from human leucocyte antigen B27 (HLA-B27), a class 1 surface antigen encoded by MHC which has a role in presenting antigens to CD8 T cells. HLA B-27 is present in 90–95% of patients with AS, and the risk of developing AS in

HLA-B27-positive individuals is 2–5%. This is increased to 15–20% in HLA-B27-positive first-degree relative of AS patients [77].

The remainder of the risk comes from environmental factors. There is already substantial evidence to support a role of bacteria in the pathogenesis of AS. Reactive arthritis, another subtype of spondyloarthropathy, is triggered by genitourinary infections or enteritis caused by gram-negative enterobacteria. It is believed that the persistence of microbial antigens in the synovium of these patients contributes to the propagation of inflammation [77]. The possible role of these bacteria in the pathogenesis of AS is highlighted by the fact that 10–20% of HLA B27-positive patients with reactive arthritis develop the full clinical picture of ankylosing spondylitis [80]. Furthermore, 54% of HLA B27-positive patients with Crohn's disease develop ankylosing spondylitis, possibly due to the inflammatory processes in the gut allowing interaction of the gut bacteria and immune system [81]. TNF-alpha and T cell response is thought to be an important driver of inflammation in AS.

### 6.3. Periodontitis and ankylosing spondylitis

The similarities in the pathogenesis of ankylosing spondylitis and periodontitis have led to the hypothesis that periodontitis may allow oral bacteria access to the immune system and perpetuate inflammatory processes in those patients with genetic susceptibility. HLA A9 and B15, both associated with susceptibility to AS, may also be a susceptibility factor in aggressive periodontitis [82]. In particular, T lymphocyte-driven inflammation certainly plays a role in periodontitis [83] as well as in AS [84]. Interleukin (IL)-2, IL-6 and TNF- $\alpha$  are all raised in AS [85] as well as being implicated in periodontitis [86]. In fact, anti-TNF therapy in AS patients leads to a significant improvement in periodontal disease markers [87].

Ratz et al. performed a meta-analysis in 2015 of 6 studies comparing periodontitis measures in cases of AS and controls, and ranging in size between 90 and 40,926 participants [18]. All studies showed a positive correlation between AS and periodontitis severity, but only two showed statistical significance. On meta-analysis, the risk of developing AS in those with periodontitis was almost double with an overall odds ratio of 1.85 (CI 1.72–1.98). Despite no significant difference in probing depth and Clinical attachment level (CAL), there was a significant association in Bleeding on probing (BOP) with those with AS ( $p = 0.0005$ ) [18].

Other studies since have looked further at the association between spondyloarthropathies, of which AS is a subtype, and periodontitis. In a group of 30 patients with spondyloarthropathy of which 8 had a diagnosis of AS, those patients with more than 5 years of evolution of disease had significantly worse periodontal disease [88]. In contrast, another group found that in 79 spondyloarthropathy patients, of which 19 had AS, levels of insertion loss were lower compared to the control group [89]. The association with *P. gingivalis* is still not certain, with contrasting findings that antibody titers were higher and lower in AS compared to controls [89, 90]

Recently, the oral microbiome has been studied in patients with axial spondyloarthritis. Patients were matched for age, gender and ethnicity to healthy controls. Interestingly, although patients had significantly greater prevalence of periodontitis (PPD  $\geq 4$  mm at 24



sites), a higher plaque index and higher mean bleeding on probing [91], there was no difference in either community structure or in diversity of organisms in the plaque bacterial communities analyzed. However, it is important to note that the small sample size in this study made it unlikely that any small effect size would be measured and so further larger studies are warranted to investigate this.

#### **6.4. Conclusion**

Overall, evidence does suggest an association between periodontitis and AS. When small studies were combined in meta-analysis, a significant odds ratio was calculated. Shared pathogenic mechanisms may explain some of this association, but the details of the underlying processes remain largely unknown.

## **7. Periodontitis and psoriatic arthritis**

### **7.1. Psoriatic arthritis**

Psoriatic arthritis (PsA) is an inflammatory arthritis associated with the presence of psoriasis, usually prior to joint involvement. Ninety-five percent are a peripheral arthritis, usually involving more than five joints in an asymmetrical pattern. The spine and sacroiliac joints are also affected in about 5% of patients [92].

Psoriatic arthritis is uncommon in the general population affecting <1%. However, it occurs in up to 30% of patients with psoriasis. It occurs equally in men and women and most commonly in those between 30 and 50 years of age [93].

Diagnosis is made clinically by the presence of inflammatory joint pain, personal or family history of psoriasis and typical X-ray findings. The treatment can be subdivided firstly into the treatment of psoriasis and secondly into the treatment of arthritis with Non-steroidal anti-inflammatory drugs, disease-modifying anti rheumatic drugs and biologics [94]. Again, the treatment options for PsA are limited and further understanding of disease etiology may lead to alternative treatments.

### **7.2. Pathogenesis of psoriatic arthritis**

Similarly to the other seronegative spondyloarthropathies, psoriatic arthritis is thought to be caused by an environmental trigger in a genetically primed individual. Indeed, about 15% of first-degree relatives of a patient with psoriatic arthritis will also be affected and studies have elucidated genetic variants [93]. The most important of these are on the human leucocyte antigen 1 (HLA-1) which is involved in presenting antigens to CD8 T cells. Variants such as HLA-Cw6, HLA-B27 and HLA-B39 are associated with specific phenotypes of PsA. These variants highlight that psoriatic arthritis is not just a subset of psoriasis and provide important clues as to the pathogenesis of PsA in which the CD8 T cell plays a key role [93].

It is hypothesized that the molecules encoded by these HLA variants may recognize self-antigens in the synovium and enthesis. However, the true pathogenesis is likely to be more complex as T cell expanded clones in the synovial tissue of patients with psoriatic arthritis lack common motifs to explain a single trigger. Fitzgerald and Winchester propose that in fact CD8 T cells are stimulated through NK receptors which respond to molecules produced in inflammation and stress [93]. In support of this, both physical trauma and immunization with rubella vaccine have been shown to proceed development of psoriatic arthritis. Stimulation of the inflammatory response leads to infiltration of T cells and cytokines including TNF- $\alpha$ , IL-1, IL-6, IL-12, IL-15, IL-17, IL-18 and IF- $\gamma$  in the synovium and ultimately to the clinical finding of synovitis [93].

### 7.3. Periodontitis and psoriatic arthritis

The characteristic lymphocytic infiltration of the joint with activated T cells and thus with the secretion of the pro-inflammatory cytokines IL-1 and TNF- $\alpha$  potentiates abnormal bone remodeling and the activation of matrix metalloproteinases in PsA. Interestingly, chronic periodontitis has a similar cytokine profile which has led to research investigating the relationship between them.

In 2013, Ustun et al. compared the periodontal status of 51 patients with PsA to that of healthy controls. Clinical attachment level, the gold standard measure of periodontitis severity and past disease activity, was significantly greater in those with PsA ( $p = 0.037$ ). Although not statistically significant, probing depth was greater in those with PsA [95]. In another study, probing depth was statistically greater in patients with PsA [96]. A much larger Danish nationwide cohort study including 6428 patients with PsA found that incidence rates of periodontitis were significantly greater in patients with PsA compared to reference population. Of note, periodontitis rates were also greater in patients with PsA compared to psoriasis alone [97].

One cause for this may be the trapping of oral bacterial DNAs in synovial fluid. Mean number of oral bacterial species is significantly higher in both sera and synovial fluid of PsA patients, and periodontitis-associated species *P. gingivalis* and *Prevotella nigrescens* have been exclusively detected in PsA sera and synovial fluid [98].

A systematic review in 2016 presented 10 studies which all showed an association between psoriasis and periodontitis [99]. Eight of these, with between 33 and 115,365 cases, found measures of periodontitis including probing depth and CAL were increased in those with psoriasis compared to controls [95, 100–106]. A large population cohort study concluded that self-reported alveolar bone loss and loss of teeth increased risk of subsequent psoriasis [104]. Furthermore, these studies concluded that the presence of psoriasis was associated with greater severity of periodontitis.

### 7.4. Conclusion

Although there are still a limited number of studies, those which have been carried out provide strong evidence for an association between PsA and periodontitis. Importantly, they also highlight the distinct association of PsA, rather than psoriasis, with periodontitis.

## **8. Periodontitis and systemic lupus erythematosus**

### **8.1. Systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) is a multi-systemic, chronic inflammatory condition which primarily affects connective tissues and is associated with specific serological signatures. Clinical features are extremely varied but common features include facial rash, oral ulcers, photosensitivity and nonerosive and nondeforming arthritis. Almost all body systems can be affected including renal, neurological, cardiac, respiratory, ophthalmic, gastrointestinal and hematological.

The estimated prevalence of SLE in the USA is 51/100,000, with females being affected 9 times more commonly than males. It is more common in people from Afro-Caribbean and Latin American descent. Most diagnosis is between the ages of 16–55 years with about 30% diagnosed younger than 16 or older than 55 years [107].

Diagnosis is based on clinical assessment and laboratory measures. Antibody tests including ANA, Anti-ds DNA and Anti-Sm may aid diagnosis but need to be used in the context of the clinical presentation [107].

Treatment for SLE depends on clinical presentation and disease activity, but the backbone is immunosuppression. However, given that the major cause of mortality in SLE is infection and malignancy which have both been attributed to long-term immunosuppression, there is a need to further understand disease etiology and find ways to prevent disease initiation and progression.

### **8.2. Pathogenesis of systemic lupus erythematosus**

SLE occurs in genetically susceptible individuals, in whom an inflammatory response is triggered by a secondary stimulus. There is a strong genetic predisposition, and siblings of patients with SLE are 30 times more likely to have SLE than the general population [107]. Certain genetic variants which alter the inflammatory process and lead to impaired clearance of immunoglobulins have been implicated. Estrogen-driven stimulation of humeral activity may at least in part explain the female predominance [108]. Known environmental factors include UV light, demethylating drugs and infections such as EBV virus. These lead to the initiation of apoptosis and impaired clearance of cells. The nucleic acids are transferred to endosomal sensors, leading to the activation of endosomal toll-like receptors (TLRs) and dendritic cells which produce IFN alpha. These inflammatory mediators activate the inflammatory cascade, immune complex production and vascular damage. Immune complexes cause tissue destruction by deposition in tissues [109].

### **8.3. Periodontitis and systemic lupus erythematosus**

Several case reports have suggested that patients with SLE have a greater severity of periodontitis [110–114]. The similar mechanisms of tissue destruction for periodontitis and SLE could explain a potential association. Polymorphisms in the Fcγ receptor leading to impaired

clearing of immune complexes have already been implicated in susceptibility to both periodontitis and SLE [115]. The inflammatory cytokine profile is similar in periodontitis and SLE. For example, IL-18 levels are increased in patients with SLE and correlate with SLE disease activity index (SLEDAI) [116], while there is a significant correlation between IL-18 levels and periodontal parameters [116]. Interestingly, higher levels of IF- $\gamma$ , IL 10, IL-17, IL-1 $\beta$  and IL-14 found in healthy patients with periodontitis are also present in patients with SLE even in the absence of periodontitis [117]

TLRs which modulate the inflammatory response to microorganisms in periodontal disease have also been implicated in SLE. Activation of TLRs which respond to specific pathogen-associated molecular patterns (PAMPs) such as Lipopolysaccharide produced by bacteria, triggers cell signaling pathways and release of pro-inflammatory cytokines. The overexpression of TLR-4 leads to autoimmune lupus and is essential for the production of anti-DsDNA antibodies found in SLE [108]. Therefore, the influence of the microorganisms in periodontal disease may affect the expression of TLRs in SLE and stimulate the autoimmune process.

Periodontitis is common in patients with SLE, with frequency varying between 60 and 93.8% [113, 118]. One Japanese study found that the frequency of periodontitis in SLE patients was 70% as compared to 30% in the general population [115].

Cross-sectional studies have found that periodontitis is more common in SLE than in controls [119–125]. A comparison of periodontal status in 105 SLE patients and geographically matched samples of the Adult Dental Health Survey in the UK found that, with adjustments for age and sex, patients with SLE were significantly more likely to have periodontitis with an OR 7.25 (95% CI 3.84–13.68) [121]. In another study, SLE patients had a significant 1.69-fold increased odds (CI 1.37–3.25) of having periodontitis defined by CAL > 3 mm [125]. These findings have been replicated in juvenile SLE patients where periodontal parameters were significantly higher than in controls [122]. Interestingly, SLE disease activity as measured by SLEDAI index correlates with periodontal condition and is a significant predictor of periodontitis [125, 126].

Three further studies have found nonsignificant findings that periodontitis is more prevalent in SLE patients [119, 123, 121]. The lack of statistical significance may be due to small sample size, use of immunosuppressants which can affect periodontal disease activity, and age. In fact, one study did highlight the fact that although there was no statistical difference in periodontal status, SLE cases were significantly younger than controls and so one could conclude that periodontitis is premature in SLE patients [120]. A study which did not find that periodontitis is more prevalent in SLE patients may have been due to the use of anti-inflammatory agents and the fact that controls were skewed to being older than cases [127].

No differences in bacterial species have been found in SLE patients compared to controls when specific periodontitis-associated bacteria have been examined [116]. However, there have been no studies to date which have sequenced the oral microbiome in SLE patients.

Nonsurgical treatment of periodontitis in patients with SLE significantly improves both periodontitis measures and SLE disease activity index (SLEDAI) at 3 months [128]. This suggests

treatment of periodontitis leads to a reduction in SLE disease activity and periodontitis may be an important factor in maintaining the inflammatory process in SLE.

#### **8.4. Conclusion**

There are some very compelling biological arguments including the shared pathogenesis and specifically a possible role of TLRs which could explain the link between periodontitis and SLE. To date, the evidence from case control studies suggesting an association is promising. However, further work with larger studies and identification of the oral microbiome in SLE patients is required to explain the role of periodontitis both in the initiation and in the maintenance of the inflammatory process in SLE.

### **9. Periodontitis and osteoarthritis**

Osteoarthritis (OA) is a chronic degenerative condition of the joints characterized by cartilage loss, bone remodeling and periarticular muscle weakness. In contrast to RA, PsA, AS and SLE, there is not thought to be a primary inflammatory element in the disease process in OA though, as a patient group, they are considered to be good controls for the inflammatory arthropathies due to a similar demography [129, 54].

Osteoarthritis is extremely common, affecting more than 10% of people over the age of 45 [130]. Diagnosis is made on clinical symptoms and signs, and typical X-ray findings can be helpful although not required.

OA usually occurs due to chronic stress on the joint but can be accelerated by trauma, infection, crystal deposition and inflammatory arthritides. Interestingly physical factors alone are not responsible for the development of OA. In fact, family history is a significant predictor of disease development and genome-wide association studies have identified multiple significant loci associated with OA [131].

There are elements of oral microbial flora that are of interest. A recent study has demonstrated the presence of periodontal bacteria in the joints of patients following joint replacement that may play a role in loosening and replacement failure [132]. However, compared to RA, the risk of periodontitis is significantly lower in OA [62] and no association has been found between OA and mild, moderate or severe periodontitis [133].

### **10. Conclusion**

The advent of 16S sequencing techniques has allowed further study of the interaction between the oral microbiome and inflammatory arthritides. It is clear that periodontitis is associated with systemic inflammation. The underlying mechanisms for this are less clear but may be due to the interaction of microbes with the immune system in the periodontal pocket or as a result of bacteremia. There is strong evidence to suggest an association between

periodontitis and rheumatoid arthritis with PAD producing *P. gingivalis*, having a key role in this interaction. There is also accumulating evidence for other inflammatory arthritides including ankylosing spondylitis, psoriatic arthritis and systemic lupus erythematosus. Common pathogenic mechanisms may explain this, but details of these interaction still need to be elucidated. Studies have been hampered by different measures of periodontitis, small sample size, multiple confounders and their cross-sectional nature. Further longitudinal studies which address these issues are needed. In the future, there may be a role for antibiotics, probiotics or interventions, targeting specific bacteria in these autoimmune conditions.

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# Immunopathogenesis of Chronic Periodontitis

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69045>

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## Abstract

Periodontitis is a chronic inflammatory condition characterized by destruction of non-mineralized and mineralized connective tissues. The pathogenesis of periodontitis involves a complex interplay between periodontopathogens and the host immunity, greatly influenced by genetic and environmental factors. Failure in the inflammation resolving mechanism leads to establishment of a chronic inflammatory process, resulting in the progressive destruction of bone and soft tissue. The aim of this chapter is to summarize the role of innate and specific immune response involved in pathogenesis of periodontitis. Cells and inflammatory mediators, those participating in inflammatory process of the ligamentous supporting structure and in resorption of alveolar bone, will be presented.

**Keywords:** periodontal diseases, immune response, cytokines, chemokines, immune cells

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## 1. Introduction

Periodontitis is one of the most common and a complex infectious disease of the oral cavity. Studies suggest that up to 60% of the population is affected by the common form of the disease, termed chronic periodontitis. Periodontitis is a multifactorial disease, with participation of bacterial, environmental, and host factors. The disease is characterized by an inflammatory response to commensal and pathogenic oral bacteria. Due to bacterial infection, gingival tissues become inflamed, characterizing gingivitis, and if left untreated, periodontal supporting tissues can be slowly destroyed by the action of the inflammatory process, which characterizes periodontitis. In the course of periodontitis, teeth lose their ligamentous supporting structure

to the alveolar bone, the alveolar bone is resorbed, and the teeth become mobile. In its severe form, it may lead to tooth loss.

There are two major forms of periodontitis, chronic periodontitis (CP) and aggressive periodontitis (AP). CP is a slowly progressing disease, which can be categorized as mild/early, moderate or severe based on clinical criteria, such as probing pocket depth (PPD) and clinical attachment loss (CAL). AP occurs in 1–3% of the population and is characterized by rapid rate of disease progression in an otherwise clinically healthy patient, absence of large accumulations of plaque, and familial inheritance.

Traditional and fundamental procedures for diagnosis of periodontitis include visual examination, tactile sensation, PPD, CAL, plaque index, bleeding on probing, and radiographic assessment of alveolar bone loss. Periodontal bleeding reveals the ulcerated area of subgingival tissue, whereas probing depth reveals the extent of the area of tissue covered by subgingival biofilm. Although different estimates for quantification of the area of periodontal injury have been reported in the literature, the inflammatory challenge of periodontal disease usually consists of an affected area of 15–72 cm<sup>2</sup> [1].

Bacteria have been considered to be the initiating factors to trigger periodontitis. Especially, Gram-negative anaerobic bacteria including *Porphyromonas gingivalis* (*P. gingivalis*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) have been strongly implicated in disease. Localized aggressive periodontitis is associated with *A. actinomycetemcomitans*, while generalized forms of chronic disease involve *P. gingivalis*, and also *Tannerella forsythia*, *Prevotella intermedia*, *Treponema denticola*, and *Fusobacterium nucleatum* (*F. nucleatum*) [2]. *P. gingivalis* has been implicated to be one of the most important periodontal pathogens, and its important features in mediated CP include the ability to adhere to and invade host cells, to disseminate through host cells and tissues, and to subvert host immunological defense mechanisms. The cell wall components of *P. gingivalis*, especially lipopolysaccharide (LPS), can cause direct destruction of periodontal tissues and trigger a wide range of immune responses, including the production of proinflammatory cytokines, antiinflammatory cytokines and chemokines, which might be important in periodontitis development [3]. A distinct systemic response was observed among different strains of *P. gingivalis* *in vivo*: strains that induced expression of high levels of IL-4 were shown to induce alveolar bone loss, whereas strain that increased IL-10 did not significantly promote bone resorption in mice [4].

Current evidence supports the importance of several factors increasing onset and progression of periodontal diseases, including smoking, diabetes, hormonal changes, and osteoporosis. Other potential interactions with periodontal disease are those involving obesity, adverse pregnancy outcomes, cardiovascular diseases, psychosocial factors and socioeconomic status [5].

In this way, the aim of this chapter is to summarize the role of innate and specific immune response involved in pathogenesis of chronic periodontitis. The neutrophils, monocytes/macrophages, and T and B lymphocytes and inflammatory mediators, including cytokines, those participating in inflammatory process of the ligamentous supporting structure and in resorption of alveolar bone, will be discussed.

## 2. Immunopathogenesis of chronic periodontitis

The host response during periodontitis involves the innate and adaptive immune system, leading to chronic inflammation and progressive destruction of tooth-supporting tissues. The pathogenesis of periodontal disease involves a complex interplay between periodontopathogens and the host immunity, greatly influenced by genetic and environmental factors (as smoking). Dental plaque is necessary, but not sufficient for disease, where an exacerbated, poorly specific and effective inflammatory response is mounted. Failure in the inflammation resolving mechanism leads to establishment of a chronic inflammatory process, resulting in the destruction of bone and soft tissue.

The host inflammatory response is mediated mainly by neutrophils, monocytes/macrophages, and T and B lymphocytes. Neutrophils are the first cells to arrive at the inflammatory infiltrate. The innate response involves the recognition of microbial components by Toll-like receptors (TLRs) expressed by host cells in the infected microenvironment. When the resolution of inflammation is not achieved, antigen-presenting cells are activated by bacterial products and interact with naïve T helper cells (Th0), driving their differentiation into several subsets, such as Th1, Th2, Th17, and Treg. T lymphocytes are central to adaptive immunity and provide help for B cells to generate specific antibodies. T cell receptor recognition of peptide antigen in the context of major histocompatibility complex can result in CD4 T cells activation. Activated Th0 may differentiate into either Th1 lymphocytes expressing Interleukin (IL)-2, interferon-gamma (IFN- $\gamma$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ); or Th2 lymphocytes, expressing IL-4, IL-5, and IL-13; or Th17 expressing IL-17A, IL-17F, IL-21, and IL-22. Activated and effector T cells may become memory T cells. T regulatory (Treg) cells are responsible for mechanisms of tolerance: the T cells CD4<sup>+</sup> CD25<sup>+</sup> have been shown to be higher in periodontitis than gingivitis, suggesting the importance of immunoregulation in periodontitis.

### 2.1. The innate immune response

The innate immune response constitutes the first line of defense and is able to recognize non-self microorganisms triggering immune response to eliminate them.

Unlike adaptive immunity, innate immunity does not recognize every possible antigen. Instead, it recognizes a few highly conserved structures present in many different microorganisms such as LPS, peptidoglycans, bacterial DNA, double-strand RNA, N-formylmethionine found in bacterial proteins, the sugar mannose and proteases (named pathogen-associated molecular patterns, PAMPs). The defense cells have pattern-recognition receptors (PPRs) for these PAMPs and trigger immediate response against the microorganism. PPRs are found on inflammatory cells and on periodontal tissue resident cells, such as epithelial cells (ECs), gingival fibroblasts (GFs), periodontal ligament fibroblast (PDLF), dendritic cells (DCs), and osteoblasts (OBs). These receptors include TLRs, nucleotide-binding oligomerization domain (NOD) proteins, cluster of differentiation 14 (CD14), complement receptor-3, lectins, and scavenger receptors. PAMPs can also be recognized by a series of soluble pattern-recognition receptors in the blood that function as opsonins and initiate the complement pathways.

After bacterial challenge, the resident cells recognize PAMPS, via TLRs, and initiate an orchestrated signaling events resulting in the production of proinflammatory cytokines and chemokines, and recruitment of inflammatory cells. The TLR4 has been shown to specifically recognize LPS from gram-negative bacteria. Cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$  and IL-6, within periodontal lesions, orchestrate the cascade of destructive events that occur in the periodontal tissues. These events include the production of inflammatory enzymes and mediators (such as matrix metalloproteinases and prostaglandins), and osteoclast recruitment, and differentiation through receptor activator of nuclear factor- $\kappa$ B ligand (RANKL)-dependent and -independent pathways resulting in irreversible hard and soft tissue damage and in chronic periodontitis.

These cells involved in innate response and molecular factors involved in the local inflammatory reaction will be discussed later.

### *2.1.1. Cells involved in innate immune response*

#### *2.1.1.1. Neutrophils and monocytes/macrophages*

Neutrophils, also called polymorphonuclear leukocytes (PMN), are one of the first responders of inflammatory cells to migrate toward the site of periodontal inflammation. The nucleus of neutrophils is segmented into three to five lobules, and the cytoplasm contains granules of two types: specific granules which are filled with enzymes such as lysozyme, collagenase, and elastase; and azurophilic granules which are lysosomes containing microbicidal substances as enzymes, defensins and cathelicidins. After periodontal pathogens successfully overcome epithelial barriers and invade soft tissues, signals from bacteria and gingival epithelial cells, human mesenchymal stem cells, connective fibroblast, and resident macrophages induce the production of cytokines and chemokines by the gingival epithelium. The chemoattractant molecules (such as IL-8 and IL-1 $\beta$ , and serum-derived plaque activated C5a) increase the expression of adhesion molecules, the permeability of gingival capillaries, and the migration of neutrophils through the junctional epithelium and into the gingival sulcus [6].

The interaction between the oral microbiota and neutrophils is a key determinant of oral health status. Neutrophils form a "wall" between the junctional epithelium and the pathogen-rich dental plaque providing a robust secretory structure (reactive oxygen species [ROS] and bactericidal proteins) and a phagocytic apparatus. However, this protection is not without cost because neutrophils from periodontitis patients are hyperreactive and contribute to tissue destruction: ROS and the enzymes released by cytoplasmic granules degrade the structural elements of tissue cells and extracellular matrix and cause tissue damage [7, 8]. A recently discovered innate defense strategy of neutrophils is the release of DNA to the extracellular environment, where the web-like DNA threads trap and kill microorganisms by means of DNA-bound antimicrobial proteins and peptides. These neutrophil extracellular traps (NETs) are also known to arise in periodontal tissues and purulent pockets. NETs represent a host defense mechanism but may also cause host tissue injury [9]. On the other hand, alterations in neutrophils function result in an acute, severe, and generalized clinical phenotype of periodontitis. These alterations can involve different functions, such as adhesion capabilities, chemotaxis response, and phagocytic function [10]. Added to this, neutrophils

with normal functions in individuals with alterations in the IL-8 production and/or mutations in Duffy antigen receptor chemokines (DARC) expression on erythrocytes predispose those individuals to periodontitis [11]. It occurs because adsorption of IL-8 onto erythrocytes by DARC leads to an increased recruitment of leukocytes from the blood to the tissue compared to individuals without DARC on the erythrocytes.

Monocytes/macrophages belong to mononuclear phagocytes system. They are also important innate immune cells at infection sites in patients with CP. These cells produce large amounts of proinflammatory cytokines, antiinflammatory cytokines, and chemokines, including TNF- $\alpha$ , IL-1 $\beta$ , IL-8, and IL-10. These chemokines produced by these cells attract neutrophils; however, neutrophils also produce chemokines, which attract macrophages to inflamed periodontal site. Thus, a crosstalk exists between these innate cells. Macrophages are important in triggering the specific immune response serving as antigen-present cells that display antigens to and activate T lymphocytes. Added, monocytes/macrophages can differentiate into osteoclasts [12].

#### *2.1.1.2. Fibroblasts*

Periodontal ligament fibroblast (PDLF) and gingival fibroblasts (GF) are the main cells of the soft connective tissue. In conjunction with infiltrating inflammatory cells, GFs take part in the inflammatory process in the periodontium and contribute to the disease persistence. After microorganisms breach the epithelial barrier, these cells produce cytokines and degradation molecules. Expression of matrix metalloproteinases (MMPs) became accentuated. Fibroblasts from periodontitis patients expressed higher mRNA of IL-1 $\beta$ , IL-6, and TIMP-3 and lower mRNA of IL-4 [13]. PDLF also produces IL-8, TNF- $\alpha$ , macrophage inflammatory protein (MIP)-1 alpha, and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), which are regulators on inflammation and alveolar bone loss. When in cell-cell contact with osteoclast precursors, PDLF upregulated osteoclastogenesis-related genes and significantly increased the number of osteoclast-like cells [14].

#### *2.1.1.3. Dendritic cells*

Dendritic cells (DCs) are the most important antigen-present cells for activating naïve T cells. These cells function in both innate and adaptive immune responses and are a link between these two components of host defense. They are part of myeloid lineage and arise from the same monocyte precursor. DCs have long membrane projections and phagocytic capabilities. Similar to macrophages, they express receptors that recognize bacteria and respond by secreting cytokines. They produce IL-12 and IL-18 that consequently promote interferon-gamma (INF- $\gamma$ ) secretion by natural killer (NK) cells and latter by T cells. In response to activation by microorganism, conventional DCs become mobile, migrate to lymph nodes, and display antigens to T lymphocytes.

#### *2.1.2. Molecular factors involved in the local inflammatory reaction*

In the innate immune response, the bacterial activation of resident cells culminates in a production of proinflammatory cytokines involved in periodontal immunopathogenesis. In order

to eliminate the bacteria, the inflammatory process is triggered; however, the inflammation culminates in periodontal tissue lesions and alveolar bone resorption [15].

#### 2.1.2.1. Toll-like receptors (TLRs)

TLRs play an important role in the recognition of pathogens. TLRs are a family of transmembrane proteins expressed by immunologically competent cells. They recognize and bind to PAMPs derived from bacterial plaque. TLRs are evolutionarily conserved proteins with a highly conserved intracellular toll-interleukin receptor (TIR) domain involved in protein-protein interaction and signaling activation. The extracellular domain, with leucine-rich repeats (LRRs) is related to ligand recognition. LRR motifs vary among TLRs. After PAMPs recognizing, TLRs initiate the activation of several transcription factors including nuclear factor-kappa B (NF- $\kappa$ B) and activator protein 1 (AP-1) through the mitogen-activated protein kinase (MAK) cascade. NF- $\kappa$ B is a key transcription factor complex that appears to play a critical role in the regulation of an acute inflammation. NF- $\kappa$ B enters the nucleus of the cell and induces expression of proinflammatory mediators including adhesion molecules (ICAM-1, VCAM-1, and E-selectin), enzymes (COX-2, 5-LO, CPLA, and iNOS), cytokines (IL-1, TNF, IL-6, GM, and G-CSF), and chemokines (IL-8, RANTES, MCP-1, eotaxin, and MIP-1 $\kappa$ ). An increase in the recruitment of leukocytes is generated [16, 17].

TLR-2, 3, 4 and 9 are much expressed in gingival tissues of periodontitis patients, and TLR-2 and TLR-4 expressions are increased in severe disease states, suggesting that these receptors have an increased capacity to signal and influence downstream cytokine expression. TLR-4 is present on antigen presenting cells, fibroblasts, and keratinocytes of gingival epithelium. TLR4 has been shown to specifically recognize LPS from gram-negative bacteria and thus recognize PAMPs of periodontopathogens such as *P. gingivalis*, *A. actinomycetemcomitans*, and *V. parvula*. Toll-like receptor-4 acts in cooperation with its coreceptors CD14 protein and MD-2 complex, as well as TLR4 adaptor proteins such as myeloid differentiation primary response gene 88 (MyD88) playing an important role in maintenance of periodontal healthy. However, over production of proinflammatory cytokines due to chronic stimulation of TLRs may lead to tissue damage. LPS-triggered TLR4 activation leads to an increased secretion of proinflammatory cytokines, mediators and matrix metalloproteinases (MMPs) from a variety of cells including monocytes, macrophages, neutrophils, lymphocytes, and gingival fibroblasts. Repeated stimulations of these receptors may also lead to development of tolerance to bacterial products. TLR-2 engagement has been shown to trigger production of proinflammatory and antiinflammatory cytokines [18, 19].

CD14 is a myeloid differentiation marker found primarily on monocytes and macrophages, although low levels are also found on neutrophils and endothelial cells. CD14 is a coreceptor for LPS. The CD14 gene is located on chromosome 5q31.3 and encodes two forms of protein: one is anchored to the membrane glycosylphosphatidylinositol (mCD14) and is a 55-kDa glycoprotein, and another is a soluble form (sCD14) found in body fluids. CD14 lacks the transmembrane domain and is unable to transmit LPS signaling. The major function of CD14 is to serve as an initial receptor for LPS and facilitate the binding of LPS to TLR4/MD-2 complex. In



addition, CD14 also recognizes other PAMPS such as lipoteichoic acid. Proinflammatory and antiinflammatory effect of CD14 has been shown indicating dual roles in response to Gram-negative bacteria [20, 21].

#### 2.1.2.2. *Matrix metalloproteinases (MMPs)*

The matrix metalloproteinases (MMPs) are a structural and functional family of zinc-dependent extracellular proteinases, which are responsible for the remodeling and degradation of tissue extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycans. To date, at least 26 members of MMPs have been identified and they are grouped according to their structural properties and substrate specificity in collagenases (MMP-1, -8, -13, -14), gelatinases (MMP-2 and -9), stromelysins, matrilysins, and membrane type matrix metalloproteinases. The balance between MMPs and their endogenous inhibitors (tissue inhibitor of matrix metalloproteinases, TIMPs) controls the MMP activity. MMPs play an important role in tissue degradation observed in periodontitis, and elevated levels of MMP-1, -2, -3, -8, and -9 have been detected in gingival crevicular fluid, peri-implant sulcular fluid, and gingival tissue of periodontitis patients. MMP-1 is produced by periodontal resident cells and is considered as central in the physiological remodeling of extracellular matrix in wound healing. MMP-8, mainly produced by neutrophils, is associated to periodontal collagen destruction and represents the major collagenase in gingival and crevicular fluids; its levels can differentiate periodontitis from gingivitis and healthy sites. MMP-9 has been associated with collagen breakdown and periodontal inflammation. MMP-13 is little or not expressed on normal tissues, but its upregulation has been involved in periodontitis progression and bone loss. MMPs can be released or activated during periodontal disease by proinflammatory cytokines, as TNF- $\alpha$  and IL-1 $\beta$ , reactive oxygen species, and proteases; IL-18 may be related to be involved in the regulation of MMPs [22–24].

#### 2.1.2.3. *Cytokines*

The resident cells and migrating cells release cytokines during inflammatory response involving in chronic periodontitis. Endothelial cells and GF produce IL-8, a chemokine that is neutrophils chemoattractant and that increase the monocytes adhesion. Neutrophils (the migrant cells) and PDLF produce IL-1, IL-6, and TNF- $\alpha$ . GF also produces IL-6 and TNF- $\alpha$ . PDLF and also bacterial antigens could promote the expression of the RANKL by the osteoblasts. DC IL-12p40, IL-18, IL-6, and TGF- $\beta$  (transforming growth factor beta) act as antigen-present cells to T lymphocytes. The role of this cytokines will be described later.

After this initial primary response, activation of T cells by APCs initiates the adaptive response. Subsequently, the effector mechanisms of innate immunity are improved by adaptive immunity involving an efficient loop for microbial clearance. Chronic periodontal inflammation perpetuates and amplifies itself through numerous autocrine and paracrine loops of cytokines, acting on cells within the periodontal tissue.

## 2.2. The adaptive immune response

When the resolution of inflammation is not achieved, adaptive immune response is initiated. Therefore, APCs are activated by bacterial products and interact with naïve T helper cells (CD4 T cells, Th0: CD45RA+), driving their differentiation into several subsets (CD45RO+), such as Th1, Th2, Th17, and Treg. Adaptive immune cells and their cytokines have important participation in pathogenesis of the chronic periodontitis, including the resolution of infection, tissue damages, and osteolytic process.

### 2.2.1. T cell subsets enrolled in the pathogenesis of CP

For a long time, periodontitis lesions were conceptually defined based on a Th1/Th2 paradigm. Currently, Th1 and Th2 responses, added to the Th17 subset, have been related to the disease progression and bone resorption. T cell lymphocytes (CTLs) are primarily involved in the cell-mediated immunity. CTLs were named because their precursors, which arise in the bone marrow, migrate and mature in the thymus. The two major T cell subsets are CD4 (T helper) and CD8 CTLs, which express a highly diverse and clonally distributed antigen receptor called the  $\alpha\beta$  receptor. Both effector cells usually express surface protein indicative of recent activation, such CD25, a component of the receptor for the T cell growth factor IL-2. Th cells express surface molecules as CD40 ligand (CD154) [25].

#### 2.2.1.1. T-helper 1 (Th1 cells)

Th1 cells are generated under the influence of interleukin-12 and/or interferon- $\gamma$  (INF- $\gamma$ ) signaling that leads to the activation of the transcription factor T-bet. In diseased gingival tissues, Th1 cells have been predominant. Activated Th1 cells secrete INF- $\gamma$ , present in high levels in human and experimental periodontal lesions. INF- $\gamma$  is associated to onset and progression of lesions. Besides, INF- $\gamma$  stimulates osteoclast formation and bone loss via antigen-driven T cell activation or through the chemoattraction of RANKL cells. Also, INF- $\gamma$  can contribute to the migration of CD4/80+ cells, monocyte/macrophage-like phenotypes, a potential osteoclast precursor subpopulation [26, 27].

#### 2.2.1.2. T-helper 2 (Th2 cells)

Th2 cells are generated under the influence of interleukin-4 and the activation of the transcription factor GATA-3. IL-4 secreted by Th2 exhibits antiinflammatory and suppressive properties related to the induction of IL-10 and suppression of Th1 response. In pathogenesis of periodontitis, it can be associated with the ability to inhibit the production of tissue degrading factors such as MMPs and the major osteoclastogenic factor RANKL. Therefore, IL-4 could attenuate the soft and mineralized tissue destruction. However, IL-4 levels were found lower in crevicular fluid from periodontitis patients.

Added to this fact, Th2 cells could be related to B cell function and humoral immunity in the periodontal lesion. B cells seem to contribute to periodontal disease development since B cell deletion prevented alveolar bone loss in mice after infection with *P. gingivalis* and because

the majority of B cells in periodontal lesions are RANKL+ [28]. IgG is the more frequent antibody class present in the gingival crevicular fluid and gingiva of patients with periodontitis; therefore, IgA and IgM are also found. IgM and IgG classes were related to autoimmunity in periodontitis where high titers of anticollagen type I, antifibronectin and antilaminin were found [26, 27].

#### 2.2.1.3. *T-helper 17 (Th17 cells)*

Th17 cells are generated under the influence of IL-6/IL-21/IL-23/TGF- $\beta$  and the activation of the transcription factor ROR $\gamma$ T. After TGF- $\beta$  and IL-6 driver Th17, IL-23 amplifies the phenotype. Th17 cells have been linked to the development of pathological inflammatory disorders. Cytokines secreted by Th17, such as IL-17 and IL-22 are crucial for host protection against many extracellular pathogens. Furthermore, IL-17 contributes to innate and specific immunity by recruiting inflammatory cells and immobilizing macrophages in inflamed tissue. The consequence is an abundance of other inflammatory cytokines as IL-1 $\beta$  and TNF- $\alpha$ , and RANKL. Th17 cells have been linked to several autoimmune disorders and are also linked to the development of pathological inflammatory disorders, and their presence was demonstrated in the gingival tissue from patients with periodontitis [29, 30].

#### 2.2.1.4. *T regulatory cells (Treg cells)*

Treg cells are generated under the influence of IL-10/TGF- $\beta$  and the activation of the transcription factor forkhead box P3 (FOXP3). They also expressed as a  $\alpha\beta$  antigen receptor. Treg cells specifically regulate the activation, proliferation, and effectuating functions of activated T cells. There are two types of Treg: (i) endogenous or natural (Treg), which are derived from the thymus and control autoreactivity; (ii) adaptive or induced (aTreg or iTreg), which regulate responses upon antigenic exposure in the periphery. In noninflammatory tissues, they are in resting state. The cytokines TGF- $\beta$ , IL-10, and cytotoxic T lymphocyte-associated molecule 4 (CTLA-4) are supposed to mediate the suppressive activity in peripheral tissues and attenuate periodontal diseases progression and protect the bone resorption. Treg cells are shown to attenuate RANKL expression by other activated T cells. Treg cells were identified in periodontal tissues; however, deficiency of Treg cells was observed in periodontitis [31].

Another subset of Treg cells includes the Tcreg. In animal models and under noninflammatory conditions, murine osteoclasts can recruit naïve CD8 T-cells and activate these T-cells to induce CD25 and FoxP3 (Tcreg). Tcreg can potently and directly suppress bone resorption by osteoclasts. The activation of CD8 T cells by osteoclasts also induced the cytokines IL-2, IL-6, IL-10, and interferon (IFN- $\gamma$ ). Individually, these cytokines can activate or suppress osteoclast resorption [32].

#### 2.2.1.5. *T CD8 cells*

After naïve cells are activated, they became larger and proliferate, and are called lymphoblasts. Some of these cells differentiate into CD8 CTLs. CD8 cells have cytoplasmic granules filled with proteins that, when released, kill the cells that the CD8s recognize. They are also

called cytotoxic T cells. In an animal models, which allow investigating the stages of periodontitis, CD8 T cell knockout mice showed no significant change in bone loss after infection with *P. gingivalis* [33].

The activated T helper subsets, their produced cytokines and their general functions are summarized in **Table 1**.

### 2.2.2. The adaptive immunity in the pathogenesis of chronic periodontitis

Adaptive immune responses to most immunogens can begin only after the immunogen has been captured, processed, and presented by an APC to naïve T helper cells. The reason of this is that T cells only recognize immunogens that are bound to major histocompatibility complex (MHC) proteins on the surfaces of other cells. There are two different classes of MHC proteins. Class I MHC proteins are expressed virtually in all somatic cell types and are used to present substances to CD8 T cells. Class II MHC proteins, on the other hand, are expressed only by macrophages, dendritic cells and a few other APCs, and are necessary for antigen presentation to T helper cells. The interaction of APC-T cells is dependent on other membrane receptors. The TCR complex confers specificity because it contains the antigen-specific receptor. The interaction is enhanced through coreceptors including CD27, CD28, CD40 ligand, or inhibited by coinhibitor receptors such as CTLA4, programmed cell death protein (PD1), and CD28 induced costimulator (ICOS) [25].

	Stimulating cytokines	Differentiated T cells subsets and respective transcriptional factors ( <i>italic</i> )	Produced cytokines *	Regulatory cytokines	Host defense	General roles in diseases
Naive TCD4 T cells	IL-12 IL-18 INF- $\gamma$	<b>T<sub>H</sub>1</b> <i>T-bet, STAT1, STAT4</i>	INF- $\gamma$ LTA RANKL	IL-4 IL-10, TGF- $\beta$	Intracellular pathogens	Tissue damage associated with chronic infections
	IL-2 IL-4	<b>T<sub>H</sub>2</b> <i>GATA-3, STAT6</i>	IL-4 IL-15 IL-13	INF- $\gamma$ TGF- $\beta$	Extracellular pathogens Humoral immunity	Allergic diseases Autoimmune diseases
	IL-6 TGF- $\beta$	<b>T<sub>H</sub>2</b> <i>2AHR</i>	IL-22	TGF- $\beta$	Extracellular pathogens	
	IL-6 TGF- $\beta$ IL-21	<b>T<sub>H</sub>17</b> <i>ROR<math>\gamma</math>T, STAT3</i>		IL-2 IL-4 IL-27 INF- $\gamma$	Homeostasis at mucosal sites/ inflammation	Organ-specific autoimmunity

The cytokines produced by these T cells subsets determine their effector functions and roles in diseases. The cytokines also participate in development and expansion of the respective subsets.

**Table 1.** T cell subsets differentiation and general functions in immune response.

The acquired immune response is known to be important for periodontal disease development. Specific microbial components activate APCs, such as DC, and both migrate to local lymph nodes. In APC, immunogen become enclosed within membrane-lined vesicles in the cytoplasm and within these vesicles, undergo a series of alterations called antigen processing and a limited number of the resulting peptides are noncovalently associated to MHC class II proteins, and transported to APC surface, where it is detected by CD4 T cells. After specific antigen recognition, activation phase is initiated by the sequences of events induced in lymphocytes. The Th0 differentiation is dependent of the local cytokine milieu. Then, the CTL proliferate, leading to expansion of the clones of antigen-specific CTL and the amplification of the response. The pattern of cytokines expressed determines subsequent polarization of a distinct antigen-specific CTL response. Next, CTL differentiates to cells that function to eliminate foreign antigens: some T cells (CD4) differentiate into cells T helper subsets, that activate phagocytes to kill intracellular antigens, or in others T cells (CD8) that directly lyses cells that are producing foreign antigens, and also B cells, which transform to plasma cells that secreted specific antibodies. The effector phase is the stage that leads to antigen elimination. In this phase, inflammatory response is amplified after recruitment of specific and nonspecific effector cells (lymphocytes, macrophages, neutrophils) and their soluble products (lymphokines, monokines, complement, kinines, arachidonic acid derivates, mast cells—basophile products).

Characteristic markers of Th1, Th2, Th17 and Treg cell subsets have all been described in diseased periodontal tissues. Therefore, the exact crosstalk that occurs among Th cytokines in periodontal disease and its impact on disease outcome is still to be determined. However, it is clear that Th cells are essential for periodontal destruction because the absence of B cells does not impede LPS-induced bone resorption.

There are tissue resident memory cells within the oral mucosa. When compared with primary immune response, the recall of memory is faster and shows fewer requirements for antigen presentation by MHC and costimulation. In gingival tissues, IL-15 is found in abundance and seen to be responsible for the survival and proliferation of memory cells.

### **2.3. Cytokines in the pathogenesis of chronic periodontitis**

Cytokines are low molecular weight water-soluble glycoprotein secreted by hematopoietic and nonhematopoietic cells in response to infection, and they are important key molecules and signal mediators in the pathogenesis of periodontitis. The cytokines involved in immunopathology of periodontal disease act in a highly complex coordinated network and play role in the maintenance of specific leucocytes on periodontal tissue, in the osteoclastogenesis activation and stimulation of bone resorption. Several of the cytokines have been demonstrated to serve either as proinflammatory (IL-1, IL-12, IL-17, IL-18, TNF- $\alpha$ ) or antiinflammatory (IL-4, IL-10) mediators of the inflammatory process [34].

#### *2.3.1. Interleukin-1 (IL-1)*

IL-1 is a potent proinflammatory mediator and bone-resorbing cytokine formerly known as the osteoclast-activating factor. The *IL1* gene cluster is located on chromosome 2q13–q21

(<https://www.ncbi.nlm.nih.gov/gene/3553>) [35]. In the IL-1 superfamily members, two agonists and one antagonist members are highlighted: IL-1 $\alpha$  and IL-1 $\beta$  (proinflammatory cytokines), and IL-1/IL-receptor antagonist (antiinflammatory cytokine). Between them, IL-1 $\beta$  is the most studied due its role in immunoinflammatory diseases. IL-1 $\beta$ , even as IL-1 $\alpha$ , is able to bind and activate interleukin-1 receptors resulting in the recruitment of several intracellular adapter molecules, including MyD88, NF $\kappa$ B, AP-1, and mitogen activated protein kinase (MAPks). This interaction leads to transcription of genes of intercellular adhesion molecule-1 (ICAM-1) that incites the innate immune response, and genes of lymphocyte function associated antigen-1 (LFA-1) that culminates in a greater migration of leukocytes in tissue direction. Once secreted, IL-1 may activate lymphocytes, incite macrophage chemotaxis and prostaglandin production, and stimulate osteoclastic resorption of bone [36, 37].

In periodontitis, IL-1 is detected in the periodontal tissue and in the gingival crevicular fluid and is important in the metabolism of collagen, in bone destruction, and other inflammatory processes. IL-1 $\beta$  is secreted mainly by macrophages and resident cells. IL-1 could mediate the gingival and periodontal tissue destruction and bone resorption by different ways: (i) IL-1 $\beta$  and IL-1 $\alpha$  stimulate the release of lysosomal enzymes such as metalloproteinase which degrades the extracellular matrix; (ii) IL-1 $\beta$  stimulates the production of prostaglandin E2 (PGE2) by fibroblasts and osteoblasts (OB). PGE2 stimulates bone resorption mediated by RANKL expression in OB; and (iii) IL-1 $\beta$  enhances RANKL expression on OB [38–40].

### 2.3.2. Tumor necrosis factor: alpha (TNF- $\alpha$ )

TNF- $\alpha$  is a very potent proinflammatory cytokine with a pleiotropic effect on both immune and skeletal systems. It is encoded by *TNF* gene located on chromosome 6 (<https://www.ncbi.nlm.nih.gov/gene/7124>) [41] and is primarily produced by activated T cells, monocytes/macrophages, and fibroblasts during inflammation. It is expressed as a transmembrane protein with 26 kDa or as a soluble TNF form with 17 kDa. When binding in the TNF receptors, TNF-RI of 55-kDa or TNF-RII of 75-kDa, the intracellular signaling pathways are activated via c-Jun, NF- $\kappa$ B, and calcium signaling leading to biological cellular response of inflammation. This response includes: (i) increase of adhesion molecules for leukocytes, as ICAM-1 and E-selectin; (ii) recruitment of leukocytes; (iii) increase of vascular permeability; (iv) increase the production of metalloproteinase and proinflammatory cytokines; and (v) osteoclasts differentiation dependent and independent of RANKL. The inappropriate or excessive TNF- $\alpha$  production can be harmful to tissue [42, 43].

TNF- $\alpha$  was detected in higher levels in saliva and crevicular fluid of patients with periodontal disease and its role and contribution to inflammation, loss of connective tissue attachment, and alveolar bone resorption has been well documented [44].

### 2.3.3. Interleukin-6 (IL-6)

IL-6 is a pleiotropic cytokine not only exerting immunological effect but also functioning in hematopoiesis, bone metabolism, and tissue regeneration. The human *IL6* gene is located in

the short arm of chromosome 7 (7p21; <https://www.ncbi.nlm.nih.gov/gene/3569>) [45]. IL-6 is a cytokine produced by T cells, B cells, monocytes/macrophages, endothelial cells, GF, OB and, periodontal ligament cells. It has multifunctional properties and is secreted in response to bacterial LPS or IL-1 $\beta$  and TNF- $\alpha$  stimulus. The IL-6 binding receptor (IL-6R) is located in membrane of cells and dimerizes with two gp130 subunits when IL-6 binds. Besides this, there is also a soluble form of receptor, and both receptors when activated are able to promote biological effects into the cells. IL-6 in promoting inflammation, it can also be involved in the regulation of tissue destruction. IL-6 induces the production of inhibitors of MMPs, suppresses IL-1 $\beta$  and TNF- $\alpha$  expression, and induces IL-1 receptor antagonist. Another positive point that favors the reduction of tissue damage is that IL-6 can stimulate fibroblasts to produce collagen and glycosaminoglycan. IL-6 plays role in B cells differentiation, T cell proliferation, and acute phase proteins expression. In addition, IL-6 in synergism with TNF- $\alpha$  is able to induce the differentiation of osteoclast progenitors directly or stimulate the stromal cells to produce RANKL.

This cytokine exerts an important effect in the pathogenesis of periodontitis, mainly in bone metabolism. During the development of CP, multiple biological actions could be mediated by the IL-6, including hematopoiesis, angiogenesis induction, immunocyte activation, and osteoclast differentiation. The presence of IL-6 in serum, gingival crevicular fluid, and saliva suggested an altered production of IL-6 in patients with CP [46, 47].

#### 2.3.4. *Interleukin-17 (IL-17)*

The IL-17 family contains six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25) and IL-17F, and five receptors, IL-17RA-RD and SEF. Interleukin-17A is most homologous to IL-17F and the genes encoding them are proximally located on chromosome 6p12 (<https://www.ncbi.nlm.nih.gov/gene/3605>). The IL-17F activity is similar to IL-17A, but significantly weaker, and is related to induce the expression of various cytokines, chemokines, matrix metalloproteinases, antimicrobial peptides, and adhesion molecules by human fibroblasts, and airway epithelial cells and vein endothelial cells. IL-17A, IL-17B, IL-17C, IL-17D, and IL-17F are considered proinflammatory cytokines, and IL-17E is believed to have antiinflammatory properties. IL-17A, IL-17F and IL-22 are involved in neutrophilia, tissue remodeling, tissue repair, and production of antimicrobial products [48].

Many studies have demonstrated the presence of IL-17 in periodontal tissues, crevicular gingival fluid, saliva, and plasma of patients with periodontal disease. IL-17 contributes to inflammatory bone pathology and bone resorption by different ways: (i) stimulating the production and expression of TNF- $\alpha$  and IL-1 $\beta$  by human macrophages and IL-1 $\beta$  by OB; (ii) stimulating secretion of IL-6, CXCL8/IL-8, and PGE2 by fibroblasts, epithelial, and endothelial cells; (iii) increasing the expression of RANKL on OB; and (iv) stimulates the differentiation and activation of OC. In periodontal environment, IL-17 potentiates the innate immunity, mobilizing macrophages and neutrophils, and increasing TLR responsiveness in gingival epithelial cells. IL-17, when combined with IFN- $\gamma$ , may modulate GF in periodontal disease by triggering the release of other proinflammatory, metalloproteinase and neutrophil-mobilizing cytokines [49].

### 2.3.5. Interleukin-18 (IL-18)

IL-18 is a member of IL-1 cytokine family and is codified by *IL18* gene located on chromosome 11q22 (<https://www.ncbi.nlm.nih.gov/gene/3606>) [50]. This proinflammatory cytokine is released at sites of chronic inflammation by APCs, such as macrophages and DC, and non-immune cells, such as epithelial and osteoblastic stromal cells. Its receptor IL-18RC is complex with two chains, the IL-18R $\alpha$  and the IL-18R $\beta$  chains; and the  $\beta$  protein contains the motif for sign-transducing. IL-18 has the property of stimulating both Th1/Th2 responses, depending on the immunological context. When the IL-12 is present, IL-18 drives to Th1 response, and in its absence, the Th2 cells' response is stimulated. IL-12 is able to increase the expression of IL-18R $\beta$ . One of the major actions of IL-18 in Th1 response is to enhance the release of IFN- $\gamma$  by TCD4<sup>+</sup> cells and natural killer (NK). INF- $\gamma$  acts as a positive regulator of Th1 differentiation through increased transcription of T-bet. In addition, proinflammatory properties of IL-18 are due to promotion of the increase of cell adhesion molecules, nitric oxide synthesis, and chemokines production. IL-18 induces the production of Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13, stimulating allergic inflammation, and inducing PGE<sub>2</sub> production [36, 51].

There are low evidences according IL-18 participation in periodontitis. Some studies show that IL-18 is an inhibitor of OC formation by indirect effects mediated by T cells and granulocyte macrophage colony-stimulating factor (GM-CSF). GM-CSF binds to preosteoclast and inhibits its proliferation and differentiation. The IL-18RC was found on T cells and OC. The concentration of IL-18 was found increased in the gingival crevicular fluid and serum of gingivitis, aggressive, and chronic periodontitis [52].

### 2.3.6. Interleukin-10 (IL-10)

IL-10 is considered an antiinflammatory cytokine playing a key role in the regulation of immune mechanisms. The *IL10* gene (<https://www.ncbi.nlm.nih.gov/gene/3586>) is mapped on chromosome 1q31-q32, in a cluster with closely related interleukin genes, including IL-19, IL-20, and IL-24, and has several regulatory promoter sequences within the 1.3 kb region upstream of the transcription start site [53].

IL-10 is produced by monocytes, macrophages, and T cells and plays a role in the regulation of proinflammatory cytokines such as IL-1 and TNF- $\alpha$ . During bone loss, IL-10 was correlated to inhibition of OC formation direct and indirectly. Directly, IL-10 inhibits osteoclast progenitors by an effect associated with decreased RANK-induced activation of nuclear factor-kappaB and expression of NFATc1, c-Fos, and c-Jun. Indirectly, by decreasing RANKL and increasing osteoprotegerin in dental follicle cells. IL-10 also had protective role toward periodontal tissue destruction, inhibiting MMPs. However, it has stimulatory effect on B lymphocyte and may also stimulate the production of autoantibodies. Autoantibodies may play a role in periodontitis. The high production of IL-10 by Treg was found in gingival tissue [54].

## 2.4. Immunopathogenesis of bone resorption

Skeletal homeostasis depend on a dynamic balance between the activities of the bone forming osteoblasts (OB) and bone-resorbing osteoclast (OC). The RANK/RANKL/OPG regulating



system controls the bone resorption and deposition activity that occur during this bone remodeling. The receptor-activator of nuclear factor-kappaB (NF-κB) ligand (RANKL) binds to RANK on osteoclast precursors (monocyte/macrophage) causing them to differentiate into active cells (OC) that secrete enzymes that degrade bone. OPG (osteoprotegerin) is a soluble decoy receptor of RANKL that prevents the RANK-RANKL interaction. OPG inhibits the osteoclastogenesis and induces osteopetrosis when overexpressed [55]. In periodontitis, high levels of RANKL and low levels of OPG have been detected in gingival crevicular fluid. In response to LPS from periodontopathogens, for instances *P. gingivalis* and *A. actinomycetem-comitans*, RANKL expression has been increased in periodontal ligament fibroblasts; however, its major source is the activated immune cells, mainly Th1, Th17, and B cells [15, 56].

Several cytokines can synergize with RANKL in promoting osteoclastogenesis and increasing RANKL/OPG ratio. Cytokines, such as IL-1, TNF-α, IL-6, and IL-17, have the ability to stimulate bone resorption, whereas others, such as IL-4, IL-10, and TGF-β, act as inhibitors. TNF-α and also IL-1 have been shown to play an important role in periodontal bone loss in a dependent and independent RANKL pathway: TNF-α could promote the proliferation and differentiation of osteoclast precursors via NFATc1 activation. TNF-α also could lead to inhibition of OB differentiation. The TNF-related apoptosis inducing ligand (TRAIL) is expressed on OB after infection and induce the apoptosis of these cells. IL-17, when in high concentration, promotes osteoclastogenesis by enhanced RANKL expression on osteoblasts and CD4 T cells [57, 58].

The immune cells and cytokines produced by them involved in the protective and aggressive roles during CP are summarized in **Table 2**.

Immune response	Cells	Characteristic produced cytokines*	Protective actions	Deleterious actions
Innate immunity	Neutrophils	TNF-α IL-1 IL-6	No evidence	Proinflammatory RANKL inducers
	PDLFs	TNF-α IL-1 IL-6	No evidence	Proinflammatory RANKL inducers RANKL+
	Monocyte/ macrophage		No evidence	Proinflammatory RANKL inducers OC differentiation
Adaptive immunity	T <sub>H</sub> 1	INF-γ	Antiosteoclastogenic	Proinflammatory RANKL+
	T <sub>H</sub> 2	IL-4	Antiosteoclastogenic	B cells lesions and autoimmunity B RANKL+
	T <sub>H</sub> 17	IL-17	No evidence	Proinflammatory RANKL inducers RANKL+
	Tregs	IL-10 TGF-β	Antiosteoclastogenic	No evidence

PDLFs: periodontal ligament fibroblasts; OC: osteoclasts.

**Table 2.** Immune cells and their characteristic cytokines involved in tissue damage and bone resorption in chronic periodontitis.

### 3. Immunomodulation and therapeutic approaches

Therapeutic approaches in periodontitis attempt to eliminate the periodontal plaque, manage the inflammation and minimize the tissue damage. The conventional treatment of CP is the mechanical removal of infectious agents in gingival tissues, which resulted in bacteria resistance and disease recurrence. Considering the complexity of periodontitis, immunopathogenesis and clinical manifestations of disease, many different approaches could be considered in order to interfere during immunity response. Amongst them, blocking the cytokines activity may be a promissory intervention. The effect of TNF- $\alpha$  and IL-1 antagonists on periodontitis showed a significant reduction of inflammation and bone resorption when administered either systemically or locally in the gingiva. The administration of antiTNF- $\alpha$  or TNF- $\alpha$ /Fc fusion protein had demonstrated significant reduction of the clinical and radiographic signals in rheumatoid arthritis and periodontal diseases [59]. IL-11 has antiinflammatory effects by induction of inhibition of TNF- $\alpha$  and other proinflammatory cytokines, thus the use of recombinant human IL-11 in the animal models showed significant reduction in the rate of clinical attachment and radiographic bone loss [60]. Similarly, the use of RANKL inhibitors in periodontitis, although limited in animal models [61] or *in vitro* assays [62], demonstrates a protective effect on bone resorption. The inhibition of PGE2 formation by nonsteroidal antiinflammatory drugs decreases osteoclasts formation and alveolar bone loss in humans and in animal experiments [63]. The United State Food Drug Administration approved the host modulator drug denominated subantimicrobial dose doxycycline (SDD), which inhibits matrix metalloproteinases and results in reduced progression of disease [64].

### 4. Genetic variants and the periodontitis risk

The development of periodontitis relies on multiple factors, and it is estimated that 50% of the expression of periodontitis could be attributed to genetic factors. The multifactorial etiology of the periodontitis and the development of sequencing technology enabled us to discuss whether the variations of host's immunity affect the occurrence and development of the disease. Several immune response genes have been related to the protection or predisposition to CP, such as those responsible for bacterial antigens presentation to lymphocytes, as well as soluble mediators or membrane receptors that initiate or amplify the inflammatory process and bone loss.

#### 4.1. Human leukocyte antigen (HLA)

HLA comprises of high polymorphic cell-surface molecules that have a key role in antigen presentation and activation of T cells. Because of the capability to bind periodontopathogens peptides, HLA represents an important factor of risk or resistance to CP development. A metaanalysis focusing on Caucasian case-control studies demonstrated no associations between HLA and CP, although for aggressive periodontitis, HLA-A\*09 and B\*15 appeared to represent susceptibility factors, and HLA-A\*02 and B\*05 were potential protective factors

[65]. However, other studies provide evidence that class I and II HLA polymorphisms are associated with chronic periodontitis [66].

## 4.2. Pattern recognition receptors (PRRs)

PRRs play an important role in the recognition of periodontopathogens and genetic variations within the genes encoding them. They may have an important influence on immune response and in the pathogenesis of periodontal diseases [67].

TLR polymorphism may alter host susceptibility to periodontitis. The main studied polymorphism was related to *TLR4* gene because TLR-4 is responsible for LPS recognition. *TLR4* was associated with susceptibility of the periodontitis, although conflicting results are related [68]. *TLR2* and *TLR9* (except some TLR9 haplotypes) were not associated to CP development [69].

The *CD14* -159C>T and -260 C>T promoter polymorphisms are located upstream from the major transcriptional site, affecting the transcriptional activity and CD14 density. Individuals homozygous for the mutation have increased serum levels of soluble sCD14 and an increased density of CD14 in monocytes. Thus, patients without mutation lead to a reduced expression of the CD14 receptor and may be more susceptible to CP. No association of CD14 polymorphism and CP was found in a metaanalysis study [70].

Mannose-binding lectin (MBL) polymorphisms were also associated to the severity of CP. Therefore, it is possible to affirm that periodontitis susceptibility was partly controlled by PRR polymorphisms involved in the innate immunity [71].

## 4.3. Cytokines

### 4.3.1. *IL1*

The *IL1* genotypes appear to be the most studied genetic polymorphisms in CP. Associations between *IL1* family polymorphisms and CP were initially assessed by Kornman et al. [72]. The authors observed that the simultaneous occurrence of *IL1A* -889 and *IL1B* +3953 polymorphisms was associated with severe periodontitis in nonsmokers. To date, the following *IL1* genetic polymorphisms have been studied in association with CP: *IL1A* -889 (in linkage disequilibrium, LD, with +4845), *IL1B* -511 (in LD with -31), *IL1B* +3954 (also mentioned as +3953), and *IL1RN* VNTR (in LD with +2018). Different from others *IL1* SNPs, *IL1RN* VNTR variant-alleles seem to decrease gene transcription or the protein production levels. Taken altogether, the *IL1* gene cluster polymorphisms cannot be considered as risk factors for CP in the worldwide population. However, in Caucasians, an association between CP and *IL1A* -889 and *IL1B* +3953 mutate-allele may be genetic risk factors. The *IL1* promoter SNPs and periodontitis might reflect subpopulation effects and have to be interpreted with care [72–74].

### 4.3.2. *TNF*

Several case-control studies in both Caucasians and nonCaucasians have investigated genetic polymorphisms in the *TNF* gene as putative risk factors for periodontitis. SNPs in the gene

encoding *TNF* are mainly studied in the promoter region at positions 1031, 863, 857, 376, 308, and 238 and also in the first intron at position +489. *TNF* -308 G/A and A/A genotypes were associated with increased CP risk in Asians, Caucasians, and nonsmoking Asians [75].

#### 4.3.3. *IL6*

The *IL6* -174G>C was found to influence IL-6 expression and production, and the individuals with carrier mutation present low *IL6* gene transcriptional activity and low plasma levels of IL-6. Thus, the polymorphism may hamper individual's defense against periodontal pathogens. Many studies had been found that *IL6* -174 polymorphism may be associated with CP susceptibility and *A. actinomycetemcomitans* infection. However, a metaanalysis that included this polymorphism did not show any association for this polymorphism with CP [76, 77].

#### 4.3.4. *IL17*

Two *IL17* polymorphisms had been found associated to diseases: *IL17A* G197A (rs2275913) and *IL17F* T7488C (His161Arg, rs763780). The *IL17A* 197A allele correlates to more efficient IL-17 secretion and higher affinity for the nuclear factor activated T cells (NAFT), which is a critical regulator of the *IL17* promoter gene. IL-17F activity is similar to IL-17A, but weaker, and the variant form of IL-17 protein suppresses the expression and the activity of wild type. *IL17A* AA genotype and A allele were associated with worse clinical and inflammatory periodontal parameters. *IL17F* polymorphisms were not associated to CP [78].

#### 4.3.5. *IL10*

Several promoter polymorphisms have been described in the *IL10* gene: -1087 (-1082), -819 (-824), -627, -592 (-597), and -590. There is strong linkage disequilibrium between *IL10* 1082G>A, 819C>T, and 592C>A, and they form two common haplotypes on the basis of *IL10* -592 polymorphism. The allele A of the -592 has been associated with decreased synthesis of IL-10 *in vitro* and *in vivo*, and when present could modify the synthesis of IL-10 in response to inflammation. IL-10 has a protective role toward periodontal tissue destruction; therefore, the *IL10* -592 polymorphism may be less protected against bacterial challenge and contribute to a relative increase in the risk for CP [79].

## 5. Conclusion

Chronic periodontitis is an inflammatory disease of the teeth-supporting tissues. The imbalance between periodontopathogens and host factors is responsible for the transition of gingivitis into periodontitis and is estimated that 50% of the expression of periodontitis could be attributed to genetic factors. The genetic polymorphisms may in some situations cause a change in the protein or its expression possibly resulting in alterations in innate and adaptive immunity and may thus be deterministic in disease outcome. Thus, development and regulation of the immune response influence disease progression or resolution, and each person may have an individual response to the bacterial challenge. Most individuals are

resistant to the disease and will not develop CP. The disease progression depends on the increased production of proinflammatory cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF $\alpha$ ), metalloproteinases, and prostaglandins or decreased production of antiinflammatory cytokines (IL-10, TGF $\beta$ ) and inhibitors of metalloproteinases. The knowledge of the immunopathogenesis mechanisms in the chronic periodontitis could contribute to new therapeutic approaches.

## Acknowledgements

The authors wish to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná and the financial support by Laboratory of Immunogenetics-LIG-UEM (00639/99-DEG-UEM).

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# Advances in Periodontal Therapy

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# Polycaprolactone-Based Biomaterials for Guided Tissue Regeneration Membrane

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69153>

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## Abstract

Guided tissue regeneration (GTR) is a clinical procedure promoting regeneration of periodontal tissues. In general, this technique provides spaces for periodontal cells to repopulate and regenerate in the periodontal defect by physically preventing an invasion of gingival tissues in the affected area. Although various reports certify clinical success of GTR, high variation of favourable outcome among studies leads to the investigation to improve clinical GTR efficiency for periodontal tissue regeneration. Recent development of GTR membrane aims to augment bioactivity for facilitating and enhancing tissue healing and regeneration. Various approaches are examined, for example, the release of growth factor, the incorporation of bioactive ceramics and the delivery of antimicrobial agents. Polycaprolactone (PCL) is widely used in biomedical application due to its acceptable biocompatibility and degradability. Physical characteristics are easy to manipulate. Various forms and shapes are simple to fabricate. PCL can be employed as GTR membrane and scaffold filling in the periodontal-defect area. Bioactive PCL could be fabricated by various techniques to enhance periodontal tissue regeneration. The present chapter reviews the bioactive approaches for GTR membrane, and the potential utilization of PCL for GTR application is described.

**Keywords:** guided tissue regeneration, periodontal tissues, polycaprolactone, biomaterials

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## 1. Introduction

The guided tissue regeneration (GTR) aims to regenerate the periodontal tissue apparatus in its original architecture. GTR procedure is well established and has proven to be a

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successful clinical procedure to regenerate periodontal tissues [1]. Treatment is conducted by applying a membrane barrier over the affected area in order to exclude the gingival tissues, connective tissues and epithelial tissues from the defect area, allowing specific cells to regenerate in the affected site [1]. Various materials have been employed and investigated in both clinical and experimental setting [2]. However, the results from systemic review and meta-analysis demonstrated that guided tissue regeneration technique exhibited highly variable between and within the studies [3, 4]. In human, the weighted-average bone-filling ration in the infrabony defect treated by GTR alone ranges from 42 to 77%, implying the variety of response [5]. One possibility of this discrepancy is the different types of membrane employed in the studies [4]. Thus, it suggests that the clinical available membranes are still needed for further improvement to efficiently promote periodontal tissue regeneration.

In order to advance the healing capability of the periodontal tissues, the membrane modification is widely investigated. In this regard, the development of drug/bioactive agent-containing membrane has been developed. Various specific agents, such as bioactive ceramics, antimicrobials, growth factors and small molecules, have been added into the membrane aiming to facilitate and/or enhance periodontal tissue regeneration [2, 6]. Many studies have proven the incremental effect imposed by the combination of these agents with traditional guided tissue regeneration membranes [6].

Polycaprolactone (PCL) has been introduced as a candidate biomaterial for tissue regeneration. It has many properties that satisfied the criterion for GTR membrane. For example, it exhibits biocompatibility properties and is not toxic [7]. It has been widely investigated as a scaffold material for tissue-engineering application [8, 9]. In addition, it has been approved for clinical application, for example, suture materials, confirming the biocompatibility and safety in clinical use. Besides, the physical characteristics (e.g. strength and degradability) could be easily manipulated. Further, a precise control of membrane architecture could be simply fabricated. PCL also has less chance to induce immunological reaction. Together, it may imply the potential use of PCL as a material-based for GTR membrane.

## **2. Periodontal tissue healing and regeneration**

Like healing processes of the other tissues, periodontal tissue-healing processes are divided into four phases: inflammation, proliferation, matrix formation, and remodelling [10]. First, the stability of blood clot at the defect site is crucial for periodontal tissue regeneration as it supports cell migration and proliferation in the affected area [10, 11]. However, periodontal tissue healing requires a unique healing process due to the complex nature of periodontal apparatus, which composes of cementum, periodontal ligament and alveolar bone. In addition, the distinctive periodontal ligament character requires the formation of collagen fibril embedding on the root surface of the teeth and alveolar bone. This contributes as another



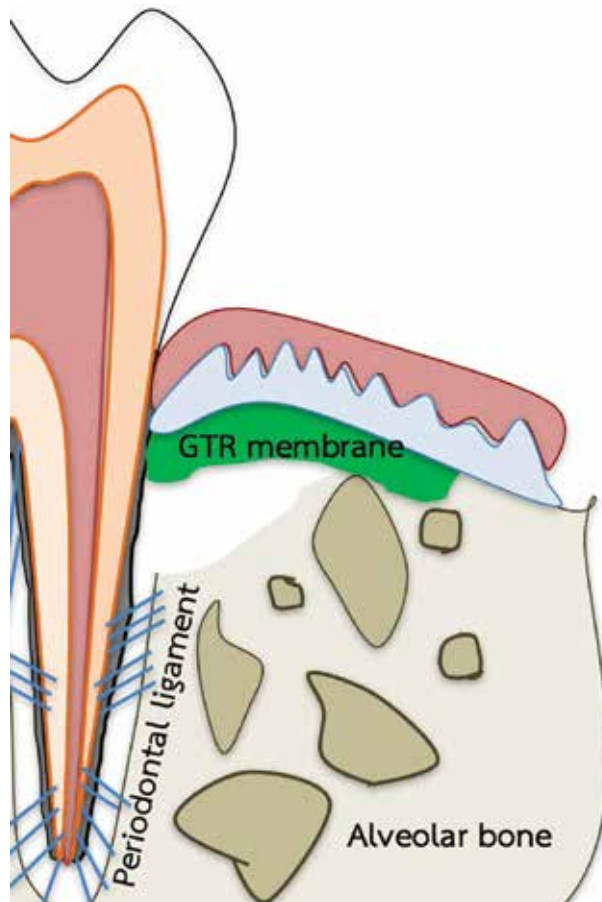
unique factor for periodontal tissue healing [10]. It is postulated that connective tissues recognize the exposed root dentin as a foreign body. Therefore, the formation of parallel collagen fibres is noted along the root surface [10]. The exposed root dentin provides a substrate for cementoblast-like cells to attach and differentiate. Further, cementoblasts form extracellular matrix where collagen fibres are anchored [10].

There are several cell types involved in periodontal regeneration/healing, for example, periodontal fibroblasts, osteoblasts, gingival fibroblasts and epithelial cells. A majority of cells in periodontal tissues is fibroblasts [12]. Fibroblastic cell population contains stem/progenitor cells that can differentiate into fibroblast, osteoblast and cementoblasts, depending on the stimulator [13–15]. These cells further participate in the regeneration and healing of periodontal tissues. However, it has been reported that gingival fibroblasts exhibited significantly higher *in vitro* wound-healing rate than periodontal ligament cells [16], implying that the speed of periodontal tissue healing is relatively slower than gingival tissues. Further, the bone healing is relatively slower than epithelium [15, 17]. Thus, normal periodontal tissue healing results in the formation of long junctional epithelium rather than the organized periodontal tissue formation [17]. This information indicates the complex and distinct regeneration/healing process of periodontal tissues. The control of specific time for each cell type to migrate into periodontal defect area is critical in the success of periodontal tissue regeneration/healing.

### 3. Guided tissue regeneration

GTR is one of the procedures that could ultimately regenerate the damaged periodontal tissues and restore them to a functional state [18]. GTR procedure is accomplished by placing the GTR membrane over the defect. This membrane acts as a physical barrier separating the gingival tissues and epithelium from the periodontal defect, allowing the required cell population (periodontal ligament cells and osteoblasts) to formulate a new attachment apparatus and functional periodontal tissues (**Figure 1**) [1, 17–19]. At the same time, the migration of epithelial cells and gingival fibroblasts is prevented. Thus, the formation of long junctional epithelium healing is attenuated [12].

GTR is well known for its successful clinical uses in an intrabony and furcation defect treatment [1, 18]. A systematic review of literature reports that GTR treatment results in better clinical outcomes than open-flap debridement procedure, for example, the improvement of clinical attachment levels, the reduction of probing depth and gingival recession [4, 20, 21]. Although other benefits of GTR for other types of periodontal defects are not as recognizable, GTR remains a beneficial treatment [1]. In this regard, GTR therapy results in a higher amount of clinical attachment gain than a therapy of accessing flap alone [22]. The disadvantage of GTR is that the result of the treatment varies due to the difference of host response, patient's oral hygiene, surgical technique and the lack of biological property to facilitate periodontal healing [1, 23–27].



**Figure 1.** GTR membrane is positioned over the periodontal defect to separate gingival tissues and epithelium from the affected area, allowing the regeneration of periodontal tissues.

#### 4. Guided tissue regeneration membrane materials

In general, like other biomaterials, GTR membrane should exhibit a biocompatibility. The degradation ability should match with the rate of new tissue formation, and the degradation product should not elicit host-inflammatory response. GTR should have the suitable mechanical and physical properties to maintain its shape *in vivo* and to easily manipulate. In this regard, GTR should have the adequate strength to maintain its form as a separating barrier and the clinical manageability [2, 6]. Materials for GTR membrane can be roughly categorized into two groups according to their degradation property, namely non-resorbable and resorbable materials. Both types of GTR materials exert similar clinical results [28, 29]. The non-biodegradable material currently available is polytetrafluoroethylene (PTFE) and methylcellulose acetate [1]. Major disadvantage of non-resorbable-guided tissue regeneration membranes is owing to the need for re-entering into the surgical site in order to remove the placed membranes, thus creating extra pain and discomfort to patients [2, 6, 29, 30].

Due to the disadvantage of the non-resorbable membrane, the resorbable membranes are created in order to eliminate the second surgery to remove the membrane [28]. The bioresorbable membranes can be further grouped into two categories: the natural and the synthetic membranes. The advantage of the natural membrane is that these can be degraded by normal physiological or pathological process *in vivo* [6]. In addition, the natural-derived membranes inherit biological properties that can induce or maintain biological activity of local cells and tissues. Collagen is widely used as a resorbable GTR membrane as it is an abundant structural protein in various types of connective tissues [31]. Although collagen exhibits low immunogenicity, the antigenic response and autoimmunization are noted [29, 31]. Another drawback of collagen is the fast degradation period leading to the failure of GTR treatment due to the downgrowth of epithelial cells, forming long junctional epithelium [6].

A resorbable membrane made from synthetic materials has the advantage in several aspects. Firstly, the desirable physical and chemical properties can be altered with simple methods [32]. Secondly, fabrication methods are controllable, easy and reproducible. In general, synthetic materials are biocompatible but the degradation products of some synthetic materials induce tissue reaction [33]. Many types of polyester-based material have been clinically utilized as synthetic resorbable membranes [2]. Synthetic materials for GTR membrane include polyglycolic acid (PGA), polylactic acid (PLA), polydioxanone (PDS), and polycaprolactone. PGA is an alpha-polyester. PGA is able to hold their mechanical strength for 2–4 weeks after implantation [32]. PLA has higher solubility in organic solvents than PGA because of its molecular structure. PLA has an amorphous poly(D,L-lactide) which is useful for further application in drug delivery. PGA and PLA can be copolymerized to form high-molecular weight copolymers [32]. Periodontal ligament cells attach better on PLA and co-PLA-PGA than on PTFE, and cell proliferation is observed on PLA and co-PLA-PGA but not on PTFE, implying their biocompatibility [34]. PDS is a homopolymer of p-dioxanone. PDS can maintain its strength for 4–8 weeks and completely resorb in 4–6 months [32]. PDS membrane treatment for human infrabony defects demonstrated the reduction of probing depth and the increase of vertical clinical attachment levels as well as bony filling in the defect sites [35]. These results are similar to those defects treated with polylactide acetyltributyl citrate [35].

## 5. Bioactive-guided tissue regeneration membrane

With the evolution of tissue-engineering approach, the recent development of GTR membranes is not only used as a physical barrier but also used as a delivery device of specific agents such as antimicrobials, growth factors and stem cells [6]. This development of bioactive GTR membranes aims for facilitating the regeneration and healing of periodontal tissues [6]. This type of membrane is considered as bioactive-guided tissue regeneration membrane. The first approach is to incorporate antimicrobial agents with GTR membrane to attenuate the risk of bacterial infection, leading to the reduction of inflammation process [36]. The bacterial contamination and infection could effect on the healing and regeneration outcome and it has been shown that bacterial infection may be associated with gingival recession and impediment

Subjects	Materials	Agents	Results	Reference
Human	ePTFE	Tetracycline (3%)	Additional gain of clinical periodontal attachment	Zarkesh et al. [45]
Dog	PGA and PLA copolymer	Doxycycline (25%)	More pronounced new bone formation and less crestal bone resorption	Chang and Yamada [46]
Human	Collagen	Minocycline	Not significantly beneficial	Minabe et al. [47]
Dog	Polytetrafluoroethylene (ePTFF)	Platelet-derived growth factor-BB (PDGF-BB)	Effectively promoted periodontal regeneration	Cho et al. [48]
Dog	Polytetrafluoroethylene (ePTFF) (GORE-TFX)	Platelet-derived growth factor-BB (PDGF-BB)	Effectively promoted periodontal regeneration with reproducibility	Park et al. [49]
Rat	Poly L-lactide (PLLA)	Platelet-derived growth factor-BB (PDGF-BB)	Enhanced regenerative efficacy	Park et al. [50]
Human	Collagen	1. Recombinant human platelet-derived growth factor-BB (rhPDGF-BB) 2. Platelet-rich plasma (PRP) 3. Commercially available enamel matrix derivative (cEMD) 4. Peptide P-15 (P-15)	1. cEMD effectively used to treat intra-osseous defects 2. The combined use of rhPDGF-BB and P-15 has shown beneficial effects in intra-osseous defects 3. PRP and graft combinations are not beneficial	Trombelli and Farina [51]
Dog	Polytetrafluoroethylene (ePTFF) (GORE-TFX)	Recombinant human transforming growth factor-beta1 (rhTGF-β1)	Restricted potential to enhance alveolar bone regeneration in conjunction with guided tissue regeneration	Wikesjö et al. [52]
Dog	Collagen	Basic fibroblast growth factor (bFGF)	Enhance periodontal regenerative results, both mineralized and non-mineralized tissues	Rossa et al. [53]
Dog	Sandwich membrane: collagen and gelatin	Basic fibroblast growth factor (bFGF)	Active vascularization and osteogenesis Successful regeneration of the periodontal tissues in a short period of time	Nakahara et al. [54]
Human	Cellulose	human fibroblast growth factor-2 (FGF-2)	Efficacious in the regeneration of human periodontal tissue	Kitamura et al. [55]
Rat	Alginate/nanofibre	Recombinant bone morphogenetic protein-2 (rhBMP-2)	Effective in repair of critical-sized segmental defect	Kolambkar et al. [56]

**Table 1.** Studies on the bioactive-guided tissue regeneration membrane.

of attachment gain [37, 38]. Local minocycline application in combination with GTR treatment results in the significant higher clinical attachment gain [39]. In addition, GTR loaded with metronidazole reduces inflammatory response *in vivo* [40]. Further, the antimicrobial-incorporated GTR membrane has been shown to improve the attachment of periodontal ligament cells by effective oral pathogen eradication [41]. The second approach is to incorporate bioactive calcium phosphate in GTR membrane [6]. The addition of hydroxyapatite improves the biocompatibility and osteoconductivity of GTR membrane [42, 43]. These composite membranes also enhance osteoblast cell proliferation *in vitro* [42, 44]. In addition, the different response could be obtained by varying the concentration of calcium phosphate in the composite membrane [42].

The last approach is to incorporate with growth factor. Growth factors regulate various biological processes, for example, cell differentiation, cell proliferation, angiogenesis and chemotaxis, resulting in the promotion of tissue healing and regeneration. Various growth factors have been identified as factors enhancing periodontal tissue healing. The exemplification of these growth factors is platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-I), fibroblast growth factor-2 (FGF-2), transforming growth factor  $\beta$ -1 (TGF $\beta$ -1), bone morphogenetic protein-2 (BMP-2), bone morphogenetic protein-4 (BMP-4), bone morphogenetic protein-7 (BMP-7), bone morphogenetic protein-12 (BMP-12) and enamel matrix derivative (EMD). The example of the development of bioactive GTR membrane is demonstrated in Table 1.

## 6. Polycaprolactone in guided tissue regeneration

PCL is a semi-crystalline, aliphatic polyester [57]. The structure of PCL comprises a repeating unit of one ester group and five methylene groups (Figure 2). PCL has an excellent biocompatibility and slow degradation rate [7, 58]. In regard to many studies, there is no evidence revealing that PCL could potentially induce any cytotoxic effects nor accumulate in human

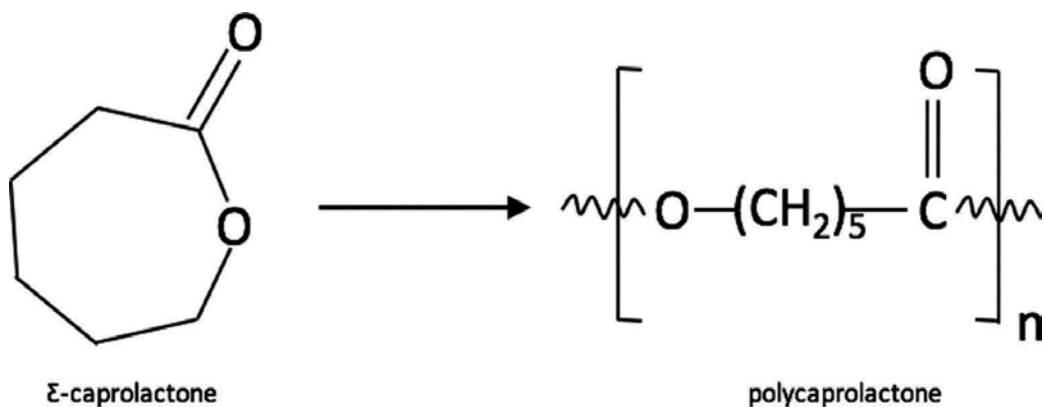
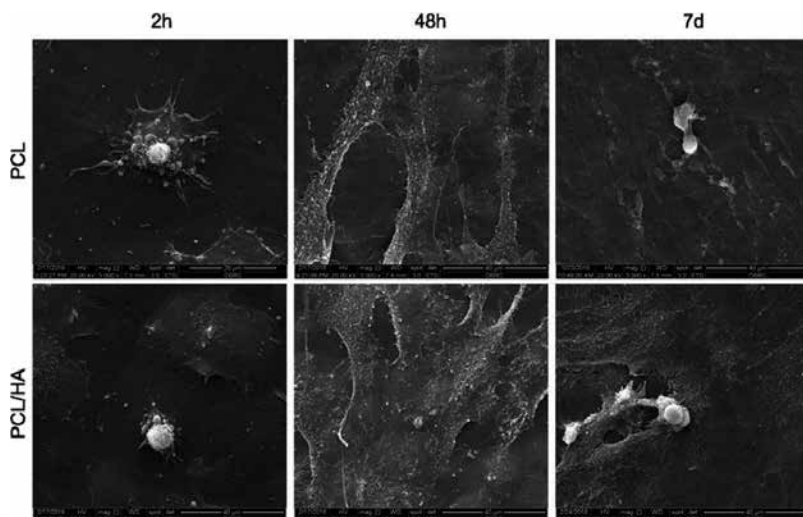


Figure 2. Structure of polycaprolactone.

body [59]. Its ester linkages can be hydrolysed and excreted under normal physiological conditions. The degradation rate of PCL is slower than other aliphatic polyester [60]. In this regard, the degradation of PCL and its copolymers can be altered with different form and molecular weight of the materials. The high-molecular-weight ( $\geq 50,000$  g/mol) PCL requires 3 years to degrade in host [60].

Therefore, PCL is a practicable option for many applications in tissue-engineering approaches. PCL been approved by the Food and Drug Administration (FDA) for several medical applications, for example, suture materials and subdermal contraceptive implants [57, 61, 62]. It has been applied as a beneficial biomaterial for drug delivery devices [63, 64]. The drug-releasing property is able to be controlled [64]. Thus, the biological activity could be lengthened [8]. For example, PCL was employed as wound-dressing materials, which released chemical anti-septic agent [65]. In dentistry, PCL has been introduced as root canal-filling materials. It was noted that PCL-filled root canal gave a predictable seal in an aqueous environment [66]. PCL is also employed as materials for bone tissue-engineering scaffolds that could be used for bone augmentation [58, 67–69]. Furthermore, PCL composites are recognized for its significant uses in tissue-engineering scaffolds in order to regenerate bone, ligament, cartilage, skin, nerve and vascular tissues [57]. PCL-based biomaterials have demonstrated the osteoconductive properties as they support various cell proliferations and differentiations, including bone marrow-derived mesenchymal stem cells (BMSCs), dental pulp stem cells (DPSCs) and adipose-derived mesenchymal stem cells (ADSCs) in PCL scaffold which was confirmed [68, 69]. Further, PCL implantation in murine calvarial defect model does not significantly increase the total IgG levels as compared with sham surgery group, demonstrating the immune compatibility of PCL-based materials [70].

As aforementioned, there has been a development in manufactured membrane used for GTR in order to meet its basic requirements. PCL is considered as satisfactory candidate for GTR due to its useful properties such as biocompatibility, proper mechanical strength, biodegradability and ease of fabrication [71–73]. Many studies investigated on the effectiveness of PCL membrane in GTR reveals an improvement of bone formation in the presence of noticeable bone cell attachment and proliferation [74, 75]. PCL and hydroxyapatite-incorporated PCL membrane were biocompatible and able to support human periodontal ligament cell attachment, spreading and proliferation (**Figure 3**). It was also shown that nano-apatite-incorporated PCL membrane facilitates osteoblast-like cell proliferation and differentiation [76]. Moreover, hydroxyapatite and gelatin nanocomposite-incorporated PCL supported osteoblast proliferation, induced alkaline phosphatase activity and enhanced mineralization [77]. For further study, the researcher has invented a new polymer/calcium phosphate composite for guided tissue regeneration use. Osteoblast alkaline phosphatase activity and expression of osteoblast marker gene, which indicates the promotion in bone maturation, have been recorded as the result [78]. The basic fibroblast growth factor-releasing heparin-conjugated PCL membrane has been successfully developed and exhibits biocompatibility. This basic fibroblast growth factor-releasing PCL membrane promotes human osteoblast-like cell attachment, proliferation and differentiation as compared



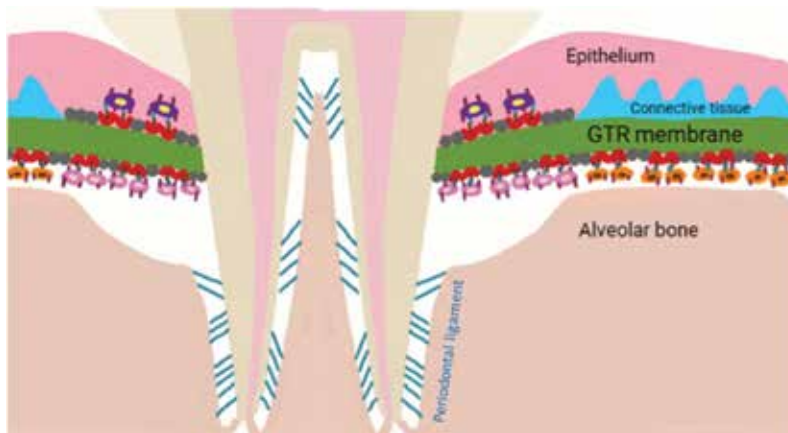
**Figure 3.** Scanning electron micrographs demonstrated the morphology of human periodontal ligament cell attachment, spreading and proliferation on polycaprolactone (PCL) and hydroxyapatite-incorporated PCL (PCL/HA) membrane. At 2 h, cells exhibited lamellipodia extension and completed cell spreading covering the surface was noted at 48 h after seeding. Cell monolayer was observed on the membrane at day 7.

with the naïve PCL membrane [79]. Metronidazole-incorporated PCL-based membranes decrease inflammatory response, determined in subcutaneous implantation model as compared to the unmodified PCL membrane [40, 80]. According to these studies, PCL become an appropriate material for the use of GTR membrane and advantageous prototype for further clinical membrane invention [76].

Beside GTR membrane, PCL has been developed as bone-defect-filling materials aiming to promote bone regeneration in periodontal defects. The scaffolds aim to support periodontal ligament and alveolar bone cell migration and repopulation in the affected site, facilitating the regeneration process. Three-dimensional PCL scaffolds can be fabricated by a modified solvent casting and particulate-leaching techniques, resulting in the highly porous and interconnected structure in PCL scaffolds [81]. Hydroxyapatite incorporation in PCL scaffolds exhibited biocompatibility and degradability [67]. These scaffolds have osteoconductive property which enhanced primary human osteoblast response *in vitro* and promoted bone formation in rat calvarial defect *in vivo* [67]. The incorporation of hydrophilic polyethylene glycol into hydrophobic PCL enhanced the overall hydrophilicity and cell culture performance of PCL/PEG copolymer as an optimal guided tissue regeneration material [82]. PCL/PEG scaffolds supported growth and osteogenic differentiation of human periodontal ligament cells *in vitro* [70]. Huynh et al. demonstrated that PCL/PEG scaffolds incorporated with epigenetic-modified human periodontal ligament cells could promote bone formation in calvarial defect [70]. Together, these findings strongly support the potential application of PCL as the potential guided scaffold in periodontal tissue regeneration therapy.

## 7. Notch signalling as a potential bioactive molecule in guided tissue regeneration of periodontal tissues

Notch ligands, Jagged1, promote cell differentiation towards osteoblast lineages of human periodontal ligament stem cells and bone marrow-derived mesenchymal stem cells [83–85]. Other studies also demonstrated that Jagged1-immobilized surface could reduce epithelial cell proliferation and enhanced epithelial cell differentiation [86, 87]. In addition, in rafted organ culture model, Jagged1-coated porous biomaterial significantly reduced the formation of epithelial tongue [87]. In other words, Jagged1 could prevent epithelial cells migration down into the dermis. For this reason, Jagged1 is considered as a beneficial molecule to be coated on a guided tissue regeneration membrane to enhance periodontal tissue formation. The schematic diagram of the propose idea is demonstrated in **Figure 4**. The Jagged1-coated PCL membrane firstly acts as a physical barrier to prevent epithelial down-growth into periodontal-defect site. In biological events, Jagged1 inhibits proliferation and induced the differentiation of epithelial cells. Further, Jagged1 promoted osteogenic differentiation of periodontal ligament cells and alveolar osteoblast cells. Together, these effects prevent the epithelium downgrowth in the lesion and promote the formation of alveolar bone, leading to the achievement of successful guided tissue regeneration.



**Figure 4.** Schematic diagram of Jagged1-coated PCL membrane for GTR therapy.

## 8. Conclusion

The present chapter reviews the biological basis of GTR membrane in periodontal tissue healing and regeneration. In the past, GTR acts as a physical barrier to allow required cells to facilitate periodontal tissue formation. Recently, bioactive GTR membrane has been investigated and developed aiming to fabricate membrane that not only act as a physical barrier but also induce biological events to enhance periodontal tissue regeneration. PCL has been introduced



as candidate materials for bioactive GTR membrane due to its biocompatibility and simple fabrication procedure. The modification with other agents/biomolecules could be easily constructed. With the use of proposed Notch ligands, PCL-decorated Jagged1 could be beneficial to promote periodontal tissue formation. However, further investigations are indeed required.

## Acknowledgements

The authors are supported by the Faculty of Dentistry Research Fund, Chulalongkorn University.

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*Edited by Pachiappan Arjunan*

*Periodontitis - A Useful Reference* is a comprehensive book compiled by a team of experts with the objective of providing an overview of the basic pathology of “periodontitis” and its implication on oral health and general systemic health. Periodontitis has become a global health burden in recent days. It is noteworthy that oral health is being considered as the mirror of general health and the study of oral-systemic health connections has advanced among scientists, clinicians, and the public as well. We wish the array of chapters that highlights the importance and impact of periodontal health could be a useful guide for the community of public, students, and clinicians.

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