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PRINCIPLES OF PERIODONTOLOGY: ETIOLOGY, PATHOGENESIS AND RISK MODIFIERS IN PERIODONTAL DISEASE. REVIEW.

Maria-Alexandra Martu[1], Liliana Pasarin[1]*, Irina-Georgeta Sufaru[1], Ioana Martu[2], George-Alexandru Maftei[3], Stefana Nanu[4], Oana Bejan[5], Giorgio Nichitean [5], Odette Luca[2], Sorina-Mihaela Solomon[1].

[1]"Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Periodontology, Iaşi, Romania,
[2]"Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Implantology and Prosthetic Dentistry, Iaşi, Romania,
[3]"Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Oral Medicine, Iaşi, Romania,
[4] Phd student "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania,
[5] Student "Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Dental Medicine, Iaşi, Romania,

Coresponding author: Pasarin Liliana*: liliana.pasarin@yahoo.com

#All authors had equal contributions with the first author

Abstract:
Periodontal diseases are arguably among the most ancient and common infectious diseases affecting humans, leading to permanent destruction of the supporting structures of the dentition and ultimately tooth loss. It has been established that dental biofilm (''plaque''), consisting of many microbial species and their products, is an etiological agent of periodontal disease. It is widely accepted that immunological and inflammatory responses to dental plaque via hostparasite interaction are manifested by clinical signs 40 Dentino et al. and symptoms of periodontal diseases.
Keyword: *periodontal disease, etiology, pathogenesis and risk modifiers.*

Introduction

Etiology. Periodontal diseases are arguably among the most ancient and common infectious diseases affecting humans [1]. It is accepted that dental plaque microorganisms existing in the form of biofilms are primary etiological agents of periodontal diseases. Biofilms are matrix-enclosed bacterial communities that adhere to each other and to surfaces or interfaces. Enormous advances in biology and technology have provided ever more sophisticated tools for the investigation of dental biofilms. Introduction of the GasPak system [2] and the anaerobic glove box [3] allowed discovery of numerous fastidious anaerobic planktonic species in the oral cavity [4,5] Due to this advancement in anaerobiosis, coupled with epidemiological data, it was possible to associate a population shift toward certain gram-negative anaerobic species in dental plaque biofilms with the initiation and progression of periodontal diseases. However, it was the application of DNA-based assays, polymerase chain reaction and confocal microscopy [6,7] that deepened our understanding of the formation, maturation and ecology of dental plaque biofilms.

Bacterial cells in a biofilm are held together by a matrix composed of extracellular polysaccharides, proteins, and other compounds. Biofilm development occurs in response to extracellular signals, both environmental and self-produced.

Biofilms protect bacteria from a wide array of insults as diverse as antibiotics, predators, and the human immune system.

The biofilm shown in the figure was grown for 3 days on a human enamel sliver worn by a healthy volunteer in the region of the maxillary premolars and molars. The surface to be imaged was facing towards the natural teeth to simulate retention areas. The biofilm was stained using fluorescent in situ hybridization (FISH) to show streptococci (yellow) and all bacteria (red). Reflected imaging (blue) was used to show the surface of the plaque and the slime matrix between the bacteria in the biofilm. The plaque biofilm was composed of dense clusters of streptococci interspersed with clusters formed by other bacteria.

The conditions for bacteria to initiate successful colonization vary greatly depending on tissue type, location, and exposure to external shear forces. The gingival sulcus, and especially the col region, which forms the bridge between adjacent gingival papillae, offer protected niches that favor bacterial settling. Pioneer colonizers include oral species of the genera *Streptococcus, Veillonella, Prevotella, Neisseria, Gemella, Actinomyces* and others [8]. During biofilm maturation, bacteria interact with each other within and between species via surface-associated structures (co-aggregation), leading to a unique spatial organization[9].

As part of a sophisticated ecological system, biofilm residents communicate through exchange of genetic information and quorum sensing, a mechanism that allows coordination of their gene expression according to population density [10].

Molecular detection of the microflora in the oral cavity has led to identification of approximately 700 bacterial species or phylotypes [11]. Approximately 50–60 species can be identified in a typical plaque sample when 16S rRNA probes are used [12]. 16S rRNA is a highly conserved gene sequence that permits estimation of evolutionary distance and relatedness of organisms[13]. Species compositions of dental biofilms vary greatly from sample to sample and are site-specific[14].

From initial colonization to formation of mature and potentially pathogenic supra- and subgingival communities, dental biofilms pass through several stages, including colonization, growth of commensal bacteria, and integration and invasion of pathogenic species [15].

The bacterial composition of mature biofilms sampled in the gingival sulcus of periodontally healthy subjects over an extended time period shows a high level of temporal stability. In contrast, in subjects whose clinical status changes from health to disease or vice versa, many bacterial species disappear or emerge [16,17]. Likewise, periodontal diseases are considered to be opportunistic polymicrobial infections.

Putative bacterial pathogens associated with periodontal diseases have been identified in subgingival biofilms. These include *A. actinomycetemcomitans, P. gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum, Eikenella corrodens, Campylobacter rectus, Parvimonas micra (previously Peptostreptococcus micros) and Streptococcus intermedius*. Three species, *P. gingivalis, T. forsythia and T. denticola,* have been designated as the red complex

[18], and are implicated in progression of chronic periodontitis.

Numerous virulence factors, which can initiate and modulate pathways of the host response, were identified and characterized from these species [19,20]. *P. gingivalis* (previously Bacteroides gingivalis), a gram-negative black-pigmented, immotile obligate anaerobe, is a late or secondary colonizer that depends on the presence of other biofilm species [21]. Its virulence potential is characterized by: (1) adhesion and co-aggregation mediated by fimbriae, vesicles, several hemagglutinins and outer membrane proteins, (2) evasion of host responses mediated by capsule lipopolysaccharides, and immunoglobulin and complement proteases, and (3) tissue damage mediated by a large number of peptidases that enable tissue invasion and destruction, and production of toxic metabolic end-products [21].

The invasion is enhanced in the presence of *P. gingivalis* [22]. In addition, *T. forsythia* possesses a unique surface structure called a surface (S-) layer that is involved in hemagglutination and adherence ⁄ invasion of epithelial cells (335). [23]. *T. denticola* is a gram-negative, aerotolerant anaerobic spirochete. It is a late colonizer, and adheres readily to other bacteria, such as *P. gingivalis, T. forsythia and F. nucleatum*, using various adhesins. T. denticola also possesses numerous proteinases and peptidases. The functions of selected virulence factors identified from these periodontal pathogens have been validated by construction of isogenic mutants and animal studies [24].

Pathogenesis

The characteristic gingival and periodontal lesions are the result of biofilm-induced, orchestrated inflammatory responses involving the innate and adaptive arms of the immune system. Inflammation that remains limited to the gingiva is the outcome of a well-balanced symbiosis between biofilms and the host tissues, while periodontitis is the result of breakdown of this symbiosis. [25]

Moreover, it may be speculated that even the most common forms of periodontal diseases are merely analogous phenotypes of different pathogenetic pathways, initiated by biofilm products, of which only a few lead to tissue destruction in susceptible hosts. Much progress has been made at all levels of inquiry towards understanding these pathways [26].

Considering biofilms a nuisance, without any benefit to the host, may be a big mistake. Not only do biofilms affect the host, there is mounting evidence that the hosts responses similarly influence the metabolism and composition of biofilms. In a healthy person, host defense and biofilms co-exist in a mutually beneficial symbiotic state [27].

Bacteria are released continuously from dental biofilms, and to a large extent are eliminated before they elicit any host response. Significant bacterial invasion is not observed in subjects with clinically healthy periodontal tissues. Various physiological mechanisms are in place to maintain tissue integrity; bacterial products are rinsed off by the continuous saliva flow, crevicular fluid flushes the gingival sulcus, and the high turnover of the junctional and gingival epithelia eliminates bacteria-loaded superficial cells. In addition to the mechanical cleansing action, highly potent first-line

antimicrobial defense systems can sense and destroy intruders. These cells constantly migrate towards the gingival sulcus, responding to a gradient of interleukin-8 that is expressed in the junctional and sulcus epithelium [28].

In addition to exhibiting excellent antibacterial properies, defensins can activate the complement cascade, up-regulate production of the chemokine interleukin-8 in epithelial cells, and attract immune cells [29].

Recently, Toll-like receptors, a subgroup of the signaling family of PRRs, were identified in periodontal tissues [30]. Interaction between a PRR and its PAMP leads to a rapid cascade of events, including formation of a PRR–ligand complex that can be internalized, activation of Toll-like receptors and NFjB, and transcriptional activation resulting in the synthesis of reactive oxygen species, reactive nitrogen species, cytokines (interleukin-1, interleukin-12 and tumor necrosis factor-a), and chemokines (interleukin-8, monocyte chemoattractant protein-1, regulated on activation, normal T cell expressed and secreted (RANTES)). Finally, Toll-like receptors instruct dendritic cells to initiate a highly differentiated, specific T-cell response [31].

Thus, defensins and PRRs not only neutralize microbial components, they also constitute an important link between innate and adaptive immune responses. Increased vascular leakage and activation of serum protein systems potentiate the local acute inflammatory response. Gingival crevicular fluid flows at an increased rate [32]. These effectors do not discriminate between host and bacteria, resulting in collateral damage to gingival connective tissue. A successful inflammatory response eliminates the infectious agent and initiates tissue repair.

However, if the infection prevails, as a result of persisting inflammation and instructed by macrophages and dendritic cells, cells of the adaptive immune system appear, and the lesion takes on chronic traits. T-cells and B-cells start to accumulate, and ultimately dominate the lesion.

Their proportions are determined by the type of immune response elicited by the antigens and the presence of modulating cytokines.

The local physiological defense mechanisms are very robust and exhibit substantial redundancy.

However, robust systems are weak when their non-redundant components are attacked [33], resulting in deficiencies in the immune system. Such attacks may have critical consequences for periodontal tissue integrity, as illustrated in patients with (genetic or acquired) immune deficiencies.

In particular, class IV.B cases of periodontal disease have been associated with mutations in the genes encoding for polymorphonuclear leukocyte elastase (severe congenital neutropenia), Chediak–Higashi syndrome 1 protein (Chediak–Higashi syndrome), integrin-b2 (leukocyte adhesion deficiency), cathepsin C (Papillon–Lefe`vre syndrome) and others [34]. Resorption of the alveolar bone is a defining characteristic of many periodontal diseases. Several inflammatory pathways can result in bone destruction [35].

Macrophages, in addition to processing and presenting antigens for activation of the specific immune response, also

produce cytokines and enzymes that induce bone resorption.

RANK is expressed on mature osteoclasts and their precursors, and osteoprotegerin is synthesized by mesenchymal cells. The interaction of RANK and RANKL initiates the differentiation and activation of bone-resorbing osteoclasts, and can be blocked by the decoy ligand osteoprotegerin. RANKL and osteoprotegerin are found in crevicular fluid, and their relative levels appear to predict disease [36].

Risk modifiers

Development and progression of periodontal disease in an individual are personalized by a number of endogenous and exogenous factors. Assessment, knowledge and proper management of these factors facilitate the prevention of disease or its containment in the case of an existing periodontal condition. An intelligent algorithm that estimates the risk for periodontal disease based on easily accessible clinical information was developed, validated and implemented in practice [37,38].

Smoking

An association between smoking and alveolar bone loss was first reported by Waerhaugs group in the late 1950s. This greatly increased risk of smokers experiencing periodontal breakdown was confirmed in many studies [39-41]. The research further estimated that more than 40% of cases of periodontitis among adults can be attributed to current cigarette smoking.

Of major clinical relevance is the observation that smoking impairs wound healing following scaling and root planing [42-44], periodontal surgery [45-48], and guided tissue regeneration procedures [49].Mechanisms for smoking-induced adverse effects have been postulated, but the precise molecular pathways remain to be identified [50,51]. Smoking is unquestionably a major risk modifier for most inflammatory periodontal diseases.

Genetics

It has been well established from twin studies that genetic factors contribute substantially to the risk of chronic periodontitis [52]. A population-based study [53] in more than 10,000 Swedish twin pairs estimated that genetics-attributable contributions to the cummulative risk of periodontal disease amounted to 39% (95% CI 31–47%) and 33% (95% CI 24–42%) in women and men, respectively.

Furthermore, the magnitude of the effect was strongly influenced by age and smoking status, suggesting substantial gene–environment interaction. Based on the currently available evidence, chronic and aggressive forms of periodontitis are not associated with single gene mutations or acquired molecular abnormalities.

However, DNA sequence variations in genes that result from alteration of a single nucleotide can substantially affect the disease phenotype. Single-nucleotide polymorphisms are thought to play a role in periodontal diseases [54]. Examples of single-nucleotide polymorphisms that were considered in such studies include those in interleukin-1, interleukin-6, interleukin-10, interleukin-12RB2, Fc-c, matrix metalloproteinase-9 and tumor necrosis factor-a, to name but a few.

The authors conclusion that more benefits would result if risk-specific treatments were available is very much to the point.

The epigenetic profile is modified by the environment over time, and may have substantial implications for periodontal disease expression [55].

Diabetes

Estimates suggest that approximately 7% of the total population in the USA have diabetes, and the prevalence is increasing [56]. Subjects with a history of type 2 diabetes mellitus have a higher prevalence and severity of periodontitis, as shown in Pima Indians [57]. In another study, 25- to 74-year-old diabetics had significantly increased likelihood of experiencing attachment loss [58].

Subjects with poor glycemic control showed faster recurrence of periodontal pockets after periodontal treatment than non-diabetic controls [59].

There has been increased interest in the question of whether or not treatment of periodontal diseases leads to improved glycemic control in diabetic patients [60]. Nine controlled clinical trials were performed in an attempt to answer this important question, and the results were summarized meta-analytically [61]. The overall results indicated that periodontal treatment led to a statistically significant reduction in the surrogate marker glycosylated hemoglobin (HbA1c).

Obesity

Obesity is a major risk contributor to disease and death worldwide. More than 60% of adults in the USA are overweight, and approximately 30% are obese [62].Close relationships have been established between obesity and diabetes, hypertension, coronary heart disease and stroke, and cancer [63]. Body fat, which is accumulated to excess in obese people, is produced by adipocytes. In addition to producing fat, these cells also release molecules that affect insulin resistance, and secrete hormones and cytokines, leading to a hyperinflammatory state.

The association between obesity and periodontitis was subsequently substantiated by more extensive studies in various populations [64,65].

Pregnancy

The female body is subject to substantial hormonal fluctuations, especially during pregnancy. The most remarkable hormonal change during pregnancy is the increasing production of estrogens and progesterone, which levels off approximately one month before delivery. These hormones are mostly produced by the placenta, and the levels revert to pre-pregnancy levels within a few days after delivery. An increased prevalence and severity of gingival inflammation were reported in pregnant women [66].

These changes appear to be independent of changes in plaque amount and are reversible [67]. About one pregnant woman in 20 develops a highly vascular, edematous lesion known as pyogenic granuloma. The lesion occurs typically during the first two trimesters, and has a strong tendency to recur if excised during pregnancy. Although hormones are likely to play a significant role in the development of pyogenic granuloma, its etiology remains largely unknown [68].

Medications

Early studies on the effect of oral contraceptives on parameters of periodontal health reported an in creased prevalence and severity of gingivitis in subjects who were taking contraceptives

than in controls [69]. The contraceptives used by study participants contained high doses of estrogen, progestin, or both. The thorough analysis rejected the previously held notion that women on oral contraception are at higher risk of experiencing clinical signs of gingivitis or periodontitis.

Certain anticonvulsants (e.g. phenytoin), immunosuppressants (cyclosporine) and calcium channel blockers (e.g. nifedipine) have been associated with gingival enlargement [70]. The prevalence of this side effect varies from 5% to 50% in adults [71]. The pathogenesis of drug-influenced gingival enlargement remains unresolved, although a multitude of contributing factors appear to be involved, including integrins, cytokines and matrix metalloproteinases.

Nutrition

For many centuries, a deficient diet was considered a cardinal factor in the development of periodontal diseases. The hypothesis was based on anecdotal evidence, and held up to its premise until animal experiments permitted its scientific test. Specifically, the author concluded that a diet deficient in vitamin C resulted in generalized alveolar changes but was not responsible for periodontal pocket formation. Nishida et al. [72] reassessed the relationship between dietary vitamin C intake and periodontal disease using the powerful NHANES III database. They found that persons with low vitamin C intake (180 mg ⁄ day). Diets rich in whole grain have been associated with lower risk of diabetes and cardiovascular disease [73]. This desirable effect is probably the result of improved insulin sensitivity and improved glycemic control [74]. Merchant et al. [75] used information gathered in the Health Professionals Follow-up Study (HPFS) to investigate the relationship between intake of whole grain, refined grain or cereal fiber and risk of periodontitis.

Systemic effects of periodontal disease

After several decades of relative absence from the scientific literature, new reports linking indicators of dental health with an increased risk for a variety of systemic diseases emerged in the 1980s and 1990s. Examples include associations with myocardial infarction [76], stroke [77], cardiovascular disease [78], peripheral vascular disease[79], adverse pregnancy outcomes [80] and pneumonia [81]. The possibility that periodontal and other diseases of the human body could be linked created broad-based excitement among the dental community and beyond, and ushered in the era of periodontal medicine [82]. The foundation for an etiological role of periodontal diseases in general health is based on two assumptions. First, bacteria released from biofilms located in periodontal pockets can enter the blood stream through ulcerations of the pocket epithelium and colonize other body parts, especially in patients with compromised immunity [83]. Second, periodontal pathogens elicit inflammatory reactions in the affected tissues, stimulating the release of proinflammatory cytokines or acute-phase proteins, and contributing to systemic inflammation, possibly atherogenesis, and other pathology ([83,84]. National Institutes of Health-sponsored Periodontitis and Vascular Events (PAVE) study [85]. The trial showed that a beneficial effect of periodontal treatment on the surrogate

marker hs-C-reactive protein was achieved only in non-obese subjects with cardiovascular disease, whereas it was not observed in obese subjects. The investigation confirmed the safety and effectiveness of standard periodontal treatment in pregnant women, but did not detect significant treatment-induced reductions in the rate of pre-term birth or other birth-related out comes. The biological plausibility model of the systemic link assumes that the larger the wound surface area, the greater the chances of bacteremia, and that the large wound surface persists over substantial time periods.

However, the overwhelming majority of patients with chronic periodontitis do not show signs of disease activity in all pockets simultaneously [86], and phases of such activity are typically short-term. Indeed, detection of active periodontal disease is a daunting task in most patients because of its elusive character.

The disease is often present at only a few sites, which could mean that the effective wound size is considerably smaller than previously estimated, possibly just a few square centimeters [87]. Although not impossible, detection of remote effects of such a small wound is a formidable challenge.

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