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Future Treatment and Diagnostic Strategies for Periodontal Diseases: What's Around the Corner?

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INTRODUCTION

The ultimate goal of periodontal therapy has always been to regenerate lost periodontal tissues, something that's proving to be far more complex than ever predicted. Accordingly, dental clinicians have had to reconcile themselves to the notion that for now, treatments that lead to disease cessation and healing if not outright regeneration provide the most achievable outcomes. Nevertheless, there are newer treatment strategies that are evolving, which over time could lead to regeneration of the periodontium, or at least limited regeneration. Although research continues to focus on the development of regenerative approaches to periodontal disease therapy other equally as novel, and perhaps currently more effective approaches to disease control are also being pursued.¹⁻⁸ Of course prior to considering treatment for any disease, one must be sure that an appropriate diagnosis has been made. As with the concept of regeneration, diagnosis of periodontal diseases are also not as straightforward as one might imagine, requiring research and development of original diagnostic tools that will expectantly demonstrate ever-increasing sensitivity and specificity.⁹⁻¹¹ More sensitive and specific tests should permit us to determine whether a patient has active periodontitis, and what sort of attachment loss might be expected if the patient were not treated.

It is noteworthy that in developing newer treatment strategies and diagnostic tests, it has been essential to become more knowledgeable about the pathophysiological mechanisms underlying periodontitis as a first step. And that with this information the physiological and pathophysiological continuum that connects tissues in the oral cavity to other remote organ systems in both health and disease has also been defined and recognized with increasing clarity. This concept also presages a more biological approach being taken by dentists regarding diagnosis and management of oral diseases.

TRYING TO UNDERSTAND AND DIAGNOSE THE DISEASE

Periodontal diseases are probably one of the most common bacterial infections in humans. Only a few of the several hundred species of microorganisms that have been identified within the gingival crevice and the periodontal pocket are thought to play a significant role in initiation and progression of the disease.^{10,12} So-called putative periodontal pathogenic bacteria are also thought to be necessary but not sufficient to trigger periodontitis, underscoring the complex nature of this "infection." Indeed, such pathogens at low levels should be considered as part of the normal oral flora, a concept that is consistent with the idea that there is a threshold pathogenic bacterial load, "critical mass" which when breached will permit and/or trigger the development of periodontitis.¹³ This critical mass of bacteria probably provide triggers that upregulate inflammatory and degradative processes associated with chronic periodontitis leading to tissue destruction, possibly by way of three different pathways:

1. Pathogens may release their own proteolytic enzymes that could degrade periodontal structures directly.
2. Pathogens may elaborate products (e.g. lipopolysaccharide) that could subsequently trigger host cell populations to express degradative enzymes.
3. Pathogens may stimulate an immune response resulting in release of proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor¹⁴ that indirectly induce increases in levels of degradative enzymes.

Enzymes in this category include matrix metalloproteinases such as collagenase (MMP-8), and elastase, both of which target the principal connective tissue proteins of the periodontium and which, interestingly, might play an equally important role in the pathogenesis of other diseases processes including those associated with congestive heart failure¹⁵ and destabilization of atheromatous plaques, which could lead to acute myocardial infarction.¹⁶

In light of the foregoing then, reliable diagnostic test development has focused in three main areas:

1. To determine the presence and proportions of pathogens found in diseased sites or in susceptible patients.
2. To identify factors that indicate and initiate the first steps of disease activity in apparently healthy sites that otherwise might appear normal from a clinical perspective.
3. Identify patients (hosts) who are unable to balance pathogen aggressiveness and resultant inflammation and perhaps even to be able to identify the degree of genetic predisposition at an early age when periodontal destruction has not yet developed.¹⁷

Microbiologic testing

Periodontal diseases are considered a mixed infection. It has never been possible to prove that specific bacteria directly cause periodontal disease (i.e. according to Koch's postulates). At this point in time, tests have not been developed that can show that the presence of a single causative agent of the disease can be identified that would predict the development of bacterially induced periodontitis. This is likely because there are several criteria that must be fulfilled as follows. Periodontal disease causing bacteria should:¹⁸

1. Occur at higher numbers in disease-active lesions compared with healthy or disease-inactive sites.
2. Their elimination should lead to arrest of disease progression.
3. They should express virulence factors relevant to the disease process.
4. They should evoke a specific immune host response.
5. They should be able to induce similar periodontal destruction in relevant animal models.

Although several types of microbiologic tests have been developed, some available on the market, their reliability is questionable because of various confounding variables. For example, even if a test can accurately identify periodontal pathogenic bacteria when present in a sample, variations in sampling techniques can seriously alter the results. In this regard, in comparing

only two methods used to assess/collect subgingival plaque samples: curettes and paper points great variability has been found. It is possible to remove 60% to 90% of the bacteria populating a diseased pocket with the use of a curette, whereas only about 6% to 41% of bacteria are sampled with the use of a paper point.^{19,20} And using paper point sampling might only pick up bacteria from the outer layers of the subgingival biofilm, which is a critical weakness since the inner layers of plaque biofilms probably harbour the more pathogenic species.²¹

Thus, when interpreting microbiological test data it must be recognized that experimental or clinical variations could be due to sampling errors alone and not necessarily to fluctuations in the true levels of whatever bacterial species are being assayed. Nevertheless, various microbiologic tests have been developed that are used in clinical and research settings and include the following:

1. Cultures

Bacterial culture is still considered the "gold standard" against which other microbiologic identification methods must be compared. It is a quantitative method and most cultivable microorganisms can be identified. Nevertheless, the technique has limitations such as (1) the inability to detect noncultivable organisms such as spirochetes; (2) high cost; (3) the short time required for transportation to the culture laboratory before cells die and cannot be cultured (24–48 hours); and (4) a prolonged period before results are obtained.

2. Microscopic identification

This method is limited to the determination of the relative proportion of coccid and the more-pathogenic, filamentous-shaped bacteria.²² This technique cannot be used to help in selection of an antimicrobial therapeutic agent if desired or to predict recurrence of the disease. In fact, bacteria thought to be periodontal pathogens cannot be identified or distinguished by microscopic assessment alone. Hence, as a chairside diagnostic system, the cost-to-benefit ratio is essentially near or below 1.

3. Assays of enzymes produced by bacteria

Although enzymatic assays permit detection of bacteria that possess trypsin-like enzymes, other pathogenic bacteria that do not produce such enzymes are not detected. Two tests have been developed: the BANA test (PerioScan, Oral B) [23] and the PerioCheck test (Sunstar).²³⁻²⁶ The presence of specific hydrolytic enzymes allows for the measurement of a surrogate outcome that supposedly correlates with pathogenic bacterial load. Interpretation of these tests is also based on the supposition that non-pathogenic bacteria do not produce such enzymes. It must also be recognized that the tests cannot account for the fact that other potential pathogens might not produce trypsin-like enzymes. Thus, there is still ongoing controversy as to the predictive value of these types of tests, not only because they identify bacteria indirectly, and the mere presence of bacteria also does not necessarily predict disease activity (double-surrogate measures?).

4. Immunoassays

Detection of immunoglobulin against bacterial antigens present in serum by the use of immunoassays (ELISA, agglutination assays, immunofluorescence) requires the development of polyclonal or monoclonal antibodies that recognize specific lymphocyte epitopes.²⁷⁻³⁰ This then is an even more indirect way to assess bacterial load than assessment of trypsin-like enzyme activity. Unfortunately these tests are not simple to administer in part because of several factors including the fact that local sampling cannot be done and therefore site-specific disease identification is not possible. Moreover, immunoassays cannot be used to determine bacterial virulence.

5. Nucleic acid probes

DNA extracts from samples of pocket-derived bacteria can be hybridized with so-called “anti-sense DNA probes.”³¹ When these probes are also labeled with an enzyme such as alkaline phosphatase, they can be detected using enzyme-staining assays, thus indicating the presence of DNA from specific bacteria.³² Nucleic acid probes have other interesting advantages including easy sampling and transport (viability of bacteria is not a requirement), and can be used to accurately identify a

wide spectrum of bacterial species including noncultivable organisms. Despite these strengths, currently available molecular techniques cannot be used to assess antibiotic sensitivity or bacterial virulence. However the development of so-called “checkerboard” DNA-DNA hybridization analyses does permit some differentiation as to pathogenicity and antimicrobial susceptibility.^{33,34} Polymerase chain reaction methods (PCR) allow for the synthesis of a vast number of copies derived from even the smallest samples of bacterial DNA,³⁴ so these tests are highly sensitive. Unfortunately, such increases in sensitivity can also be accompanied by unacceptable reductions in specificity (that is, indicating that disease is present when it is not).

6. Biosensors

Periodontal pathogenic bacteria produce various metabolites (e.g. volatile sulfur compounds) that can be detected biochemically.^{35,36} Putative periodontal pathogens including *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Tannerella forsythia*, are capable of reducing sulphates. This activity leads to the production of measurable levels of S, HS, H₂S, and CH₃SH caused by degradation of serum proteins, cysteine, and methionine. A sulfide sensor, Perio 2000, developed by Diamond General Corp. can measure levels of these compounds and report them as scores ranging from 0 to 5 in increments of 0.5. A score of 0 represents undetectable sulfide levels, whereas a score of 5 represents a concentration of 0.1 M sulfide. This test is, however, nonspecific but could still theoretically help to predict what sites are infected with periodontal pathogenic bacteria and are hence at risk for ongoing tissue breakdown.³⁶

Analysis of Disease Activity

1. Enzymes found in gingival crevicular fluid

Salivary and gingival crevicular fluid enzymes and other proteins have the potential to be useful markers of disease progression and could be considered more direct indicators of actual disease as opposed to bacterial markers that are at best only surrogate markers for disease risk. Presently, more than 65 gingival crevicular fluid components have been examined and identified as potential markers of disease progression and they fall into three general categories:

1. Host-derived enzymes such as MMPs and their inhibitors
2. Inflammatory mediators and host response modifiers such as cytokines
3. Tissue breakdown products such as glycosaminoglycans, osteonectin, osteopontin, and laminin

A major problem with measurement of enzymes is that it is often difficult to distinguish those associated with gingivitis and periodontitis sites from active and inactive disease sites. Enzymes like the collagenases (MMP-1, MMP-3, MMP-8, and MMP-13), elastase, and gelatinases (MMP-2 and MMP-9) may be significantly elevated in the presence of existing disease, but measurement of their levels to predict future destruction remains unclear.^{37,38} Despite the belief that these biomarkers of disease activity should be useful for direct identification of ongoing disease activity, many enzyme tests evaluated to date have demonstrated fairly high rates of false-positive findings (i.e. although a test is “positive,” there may still not be any disease activity and therefore no disease progression). The same can be said about assays for inflammatory mediators. The most promising gingival crevicular fluid markers of disease progression/connective tissue destruction are probably host breakdown products (as opposed to the enzymes that break down host tissues). This is especially true of markers that represent bone destruction and include chondroitin-4-sulfate,^{39,40} pyridinoline cross-links of the carboxyterminal telopeptide of type I collagen, and RankL (receptor activator for NF- κ B ligand).^{41,42} Other components of gingival crevicular fluid include cellular

elements, polymorphonuclear neutrophils (PMNs) being the most important. Several studies have suggested that measurement of the levels and/or activities of these cells and this particular approach to diagnosis have been highlighted in another article in this series of publications (See Glogauer and McCulloch. An Overview: Introduction to the RCDSO Symposium – Oral Health: A Window to Systemic Disease. Dispatch Magazine Spring 2005).

2. Genetic analyses

The etiology of periodontal disease is multifactorial and there is no doubt that some individuals are more predisposed to this disease than others suggesting that there are heritable factors involved that govern the host's interrelationships with the external environment.⁴³ Classically this was demonstrated by identifying a defective gene on chromosome 11 responsible for cathepsin C activity⁴⁴ in a disorder known as palmar-plantar dyskeratosis (Papillon-Lefevre syndrome), a disease associated with complete exfoliation of teeth over time. Similar modifications in that gene on chromosome 11 have been demonstrated in patients with severe chronic periodontitis.⁴⁵ Other studies have focused on IL-1 gene polymorphism leading to the development of the periodontitis susceptibility trait test,^{46,47} the only commercially available genetic test for periodontitis risk. However even this test is generally only done when it's already known that a patient is susceptible to periodontitis so its impact on diagnosis and more importantly disease management is not clear.

PERIODONTAL DISEASES; NOVEL THERAPEUTICS

Regenerative treatments

As stated at the outset, and from an historical perspective, regeneration of periodontal tissues lost as a result of periodontitis has been an elusive goal despite the development of widely available regenerative surgical techniques.

1. Bone grafting

Critical analyses of clinical, histological, and radiographic data, however, suggest that although correction of bone defects can be demonstrated,^{48,49} the regeneration of a new attachment apparatus following bone grafting including new bone, cementum, and a functionally oriented periodontal ligament does not generally occur except at the very base of the periodontal defect. Because bone grafting has been shown to have limited effectiveness with respect to regeneration of lost periodontium, other approaches have been developed that ostensibly would exploit the biologic principles that describe cellular domains.⁵⁰

2. Guided tissue regeneration

A variety of membrane materials have been developed, including some that had to be removed from the surgically treated site weeks or months later and some that were resorbable and could be left in place. Initial reports regarding the use of these membranes (a technique called guided tissue regeneration) were positive, but over the longer-term, it became apparent that apart from the use of guided tissue regeneration used to regenerate bone itself about implants or in other osseous sites requiring 'only' bone augmentation (actually called guided bone regeneration), full regeneration of the periodontium still eludes us.⁴⁹

3. Enamel matrix derivatives

Taking advantage of developmental biologic studies of the periodontal attachment apparatus, it was noted that before development of cementum and new periodontal ligament, enamel matrix proteins are deposited directly onto dentine surfaces.^{51,52} This observation led investigators to hypothesize that enamel matrix proteins might play an important role in the signaling for and recruitment of cells required for production of a normal

tooth attachment apparatus; a biological process that could be used to regenerate lost periodontium. There is now a growing body of evidence based on randomized controlled trials^{4,53} that enamel matrix proteins can be delivered to surgical sites leading to limited regeneration of the periodontium. In addition, further studies have demonstrated that these proteins may prove useful in periodontal plastics and root coverage procedures, thereby reducing or eliminating the need for the harvesting of connective tissues.^{53,54} As the mechanisms underlying enamel matrix derivative effects are understood more precisely, it is possible that other related extracts or even purified proteins will become a part of the routine armamentarium of periodontists in the future when regenerative therapy is required.

Disease Control

1. Bisphosphonates to inhibit bone loss

The bisphosphonates are a class of drugs related to pyrophosphate.⁵⁵ Unlike pyrophosphate, which can be degraded by alkaline phosphatase⁵⁶ and pyrophosphatase, the bisphosphonates are resistant to degradation and have a high affinity for mineralized tissue.⁵⁵ One of the most important uses of bisphosphonates relates to their ability to inhibit bone resorption, presumably by direct or indirect inhibition of osteoclast cell activity.⁵⁵ Despite recent reports that have linked the systematic use of the more potent forms of bisphosphonates to osteonecrosis of the jaws, this property could prove useful in the development of future therapeutic approaches to the prevention of periodontal bone loss^{55,58} and possibly bone-supported implants.^{59,60} For example, it has been shown that local application of bisphosphonates reduces the bone loss that occurs following periodontal flap surgery.⁶¹ Although not reported here, the affinity of these drugs for bone can also be used in bone scans for diagnostic purposes.⁶²

2. Bisphosphonate stimulation of bone formation

In addition to their ability to inhibit bone resorption, it is known that at certain concentrations, bisphosphonates inhibit mineralization,⁶³ a property that has been dealt with by the development of newer drugs⁶⁴ for treatment of osteoporosis. More recently our laboratories have shown that bone matrix (osteoid) formation is inversely

proportional to mineralization.⁶⁵ When mineralization is inhibited using the first-generation bisphosphonate etidronate (HEBP), osteoid formation has been shown to double in vivo and in vitro.^{58,63,66} When HEBP treatment is stopped, however, the previously unmineralized osteoid mineralizes to become bone, a phenomenon leading to absolute increases in the total amount of bone produced as well as the velocity of bone formation. This property of HEBP could be exploited to stimulate new bone formation in the periodontium and possibly for acceleration of osseointegration about implants; much more study is needed to transfer this approach to the clinic.

Antimicrobial therapy

1. Locally delivered antibiotics

It has been well established that most forms of periodontitis are related to chronic infection with periodontal pathogens¹⁷ (usually gram-negative anaerobic species³⁶). As a result, there has been an extensive amount of investigation related to the development of effective antimicrobial regimes for treatment of chronic, refractory, or other forms of periodontitis. Indeed, double-blind placebo-controlled randomized trials have demonstrated that antimicrobial treatment is an extremely useful adjunct for treatment of periodontitis.^{10,67} Before the advent of antimicrobial therapy refractory periodontitis constituted about 20% of all cases (AAP classification system notwithstanding) but is now more in the range of 5% range or lower^{59,68} since the usefulness of antimicrobials has been accepted. That said, the use of systemic antimicrobial medications to treat a local infection has drawbacks including, for example, gastrointestinal side effects such as pseudomembranous colitis, allergic reaction,⁶⁹ superinfection with commensal organisms, or development of resistant organisms. Therefore locally delivered antimicrobials are now being used to treat infected periodontal pockets,⁵⁹ including metronidazole in an ointment form (Elyzol[®]), chlorhexidine (Periochip[®]), and doxycycline in a fiber form (Actisite[®]) or in a polymeric delivery system (Atridox[®]).⁶⁸ These locally delivered antimicrobials, and in particular Atridox[®], have been demonstrated to be efficacious in the treatment of, in particular, localized periodontal

pockets. Full mouth 'decontamination', a concept that has shown promise in controlling periodontal disease⁷⁰ will be technically difficult when using locally delivered antibiotics but might still be achievable.

2. Photodynamic therapy

Other antimicrobial advances relating to treatment of periodontitis have been investigated, including an approach known as photodynamic therapy (PDT). This involves the use of light-activated drugs to kill periodontal pathogens and was developed initially for treatment of malignancy⁷¹ and macular degeneration.⁷² Recently, it has been demonstrated that toluidine blue, when activated by laser or broad-spectrum light, can be used to kill periodontal pathogens.⁷³⁻⁷⁵ Although PDT is now available for use in patients (Periowave[®]), there is a dearth of randomized controlled clinical trials to confirm its clinical impact, although its underlying principles are very sound. Clinical trials could also be focused on decontamination of infected peri-implant tissues.

3. Inhibition of matrix degradation

As discussed previously with respect to diagnostic tests, matrix degradation within the periodontium is a major hallmark and even a predictor of bone and periodontal attachment loss. Hence, there have been major efforts devoted to the development of treatment approaches that downregulate matrix degradation (the bisphosphonates could fit into this category). Most investigation in this area has focused on the use of tetracycline, and its derivatives such as doxycycline, which in addition to their antimicrobial effects also inhibit MMP activity.^{59,68,76,77} Subantimicrobial dosing of doxycycline (Periostat[®]) to inhibit MMP activity has been studied in several randomized controlled clinical trials and shown to effectively prevent attachment loss. In the authors' experience, Periostat[®] has proved to be very helpful in management of periodontitis in a large hospital-based population of young and old patients. Looking farther into the 'future' one might also imagine the development of analogues to or mimics of the protein decorin.⁷⁸ This protein does not inhibit MMP activity directly but might alter the substrates to prevent their degradation by MMPs.

4. Management of periodontal diseases in smokers

It has been demonstrated clearly that individuals who smoke cigarettes are at greater risk for the development of periodontitis, have more severe periodontitis, and do not respond to treatment of periodontitis as well as those who do not smoke.^{8,10,79} This finding was thought to be a lifestyle issue (e.g. poor oral hygiene), compared with nonsmokers, smokers do not necessarily have more bacterial plaque and their plaque is not populated by more periodontal pathogens. Constituents of cigarette smoke such as nicotine⁸⁰⁻⁸² probably trigger or act as cofactors to initiation and progression of periodontitis. Our group has shown that another group of smoke-derived constituents, the aryl hydrocarbon receptor agonists⁸³ including but not limited to benzo-a-pyrene and dimethyl benzanthracene⁸⁴ interfere with bone metabolism. These dioxin-like agents appear to inhibit bone cell differentiation and hence inhibit bone formation. Given these properties, it is conceivable that in addition to nicotine, aryl hydrocarbons also mediate deleterious effects on the periodontium. We have also shown that an agent commonly found in red wine, resveratrol, is an aryl hydrocarbon receptor antagonist that inhibits the effects of aryl hydrocarbons. Hence, it might be possible that in the future agents such as resveratrol (or synthetic analogs) could be used to ameliorate some of the effects of smoking on the periodontium and other tissues. Of course, smoking cessation is the ultimate goal, but this has not proved to be as effective as its proponents would like. Moreover, given the long half-life of aryl hydrocarbons in the human body, it could still be helpful to inhibit their effects, even after a patient has stopped smoking.

SUMMARY

New technologies have been developed or are in development that could be used to enhance the ability to predict, diagnose, and treat periodontitis. Not all of these technologies will bear fruit; however, those that do will provide clinicians of the twenty-first century with more effective means of detection, prevention, and treatment of periodontitis than are currently available. The dentists of the 21st century will approach oral diseases using a more biological model of health, disease and disease management; an approach more befitting physicians of the oral cavity.

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