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Diagnosis and anti-infective therapy of periodontitis

Hans-Peter Horz[†] and Georg Conrads

Periodontal diseases (gingivitis and periodontitis) are chronic bacterial infections with a remarkably high prevalence and morbidity. Periodontitis, in contrast to gingivitis, is not reversible, is associated with certain bacterial species and affects all of the soft tissue and bone that support teeth. Among the periodontal pathogens, species, such as *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, and several forms of uncultivable spirochetes play the major role in the pathogenesis. In severe chronic, recurrent and especially aggressive forms of periodontitis, diagnosis of the species involved and, whenever possible, an optimized evidence-based antimicrobial treatment is indicated. In order to monitor alarming bacterial changes in the periodontal pocket, several techniques, namely microscopy, culture, immunoassays, enzyme tests and DNA-based techniques, have been established and the methods are described in the first part of this review. In the second part, the selection and use of locally delivered (topical) and systemic antibiotics used adjunctively in periodontal therapy are discussed.

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Impact of periodontal diseases

Periodontal diseases (gingivitis and periodontitis) are chronic bacterial infections with a remarkably high prevalence and morbidity. For an overview of a current classification system for periodontal diseases and conditions, a rather complicated topic that cannot be addressed in its entire complexity in this review, the reader is referred to the paper by Armitage [1].

Almost 100% of adults, but also 90% of children at school age, are periodically or occasionally affected by gingival bleeding, the most objective sign of early gingivitis. The mean percentages of maximum community periodontal index (CPI) among 35–44 year-olds are higher in the WHO region of the Americas and the European region compared with, for example, developing countries [2,3]. Gingivitis, with some exceptions, is a polymicrobial infection with no single associated bacterial agent and is reversible by adequate oral hygiene. By contrast, periodontitis is moderately to rapidly progressive and is clinically diagnosed on the basis of gingival inflammation, pocket formation, loss of gingiva attachment, bone resorption and the number of teeth involved. Advanced chronic or aggressive periodontitis are forms associated with certain bacterial species (called periodontal pathogens or marker bacteria) and affects all of the soft tissue and bone-supporting teeth. An estimated 70% of the US adult population is affected by a kind of periodontitis with prevalence rates and severity greater among men than women and among blacks than whites. Among those affected, between 3 and 15% are susceptible to a rapid and advanced loss of periodontal attachment. They may develop aggressive forms of periodontitis, which cause severe problems. These individuals require prosthetic treatment within a short period of time. In addition, as periodontal diseases disturb the integrity of oral mucous membranes, periodontal pathogens can frequently be detected in blood cultures. These frequent bacterial attacks, together with the host's inflammatory reaction, may not only cause bacteremia but also (under some circumstances) septicemia, organ abscesses or endocarditis, as well as cardiovascular disorders or low birth

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weight when occurring during pregnancy, and the risk of serious health conditions, such as coronary artery diseases, cerebrovascular diseases and stroke, can be increased [4–10].

The direct financial burden of therapy in the USA resulting from periodontal diseases is estimated to be as high as US$5–6 billion annually (total dental costs: $70.3 billion) with increasing tendency [11]. In a relatively small country, such as Germany, with high standards in dentistry, patients and health insurance companies spend $5 billion each year with $20 as a minimum annual cost to treat the inflamed periodontium of just one tooth [12]. Furthermore, in a 2-year retrospective examination of a large insurance company database, Albert *et al*. revealed a possible association between periodontal treatment and increased medical costs per member per month for associated systemic disorders [13].

There is no doubt that periodontal diseases are a major burden and oral health is a WHO priority topic in the 21st Century [2,14].

Etiology of periodontal diseases

Chronic inflammation of the periodontium is a multifactorial disease including several factors with triggering or enhancing potential, such as underlying systemic risk factors, genetic susceptibility, negative (dys-) stress, tobacco use, suboptimal and/or high sugar diet, poor oral hygiene and inadequate restorative procedures. However, most forms of gingivitis and periodontitis finally result from the accumulation of tooth- and/or gingiva-adherent microorganisms in plaque. As documented by numerous publications, periodontal diseases are associated with a shift in the periodontal bacterial flora, from the healthy to the diseased state. The list of designated periodontal pathogens might be long; however, according to the current state of knowledge, species, such as *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, *Campylobacter rectus*, *Eikenella corrodens*, *Filifactor alocis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* and spirochetes appear to play a major role in the pathogenesis (for a recent overview of pathogen clusters and their virulence factors associated with disease, see [15–18]). It is important to note that our current picture of the microbial etiology of periodontal disease is rather incomplete; in particular, as the microflora involved is highly complex consisting of a large number of uncultivable species, with as yet unknown functions at the diseased sites. For instance, some of the recently discovered microbes in periodontal pockets are not even bacteria, but belong to archaea, a distinct domain of life previously believed to be unimportant for human disease [19–21]. In addition, the possible role of viruses (especially various forms of *Herpesviridae*) for periodontal disease has also been the focus of recent research [22–25].

For diagnosis of the activity of the different forms of periodontitis, clinical symptoms (pocket formation, attachment loss and alveolar bone loss) alone may not be sufficient, as they provide a historical record only or have low predictive value, such as 'bleeding on probing'. But predictions of recurrence of disease and prognosis for the patient can be improved significantly when the presence or absence of periodontal pathogens is monitored concurrently.

Recommendations for microbial diagnostics in periodontitis

To monitor periodontal pathogens or shifts in the bacterial community in the gingival sulcus or the periodontal pocket, several techniques have been introduced. These procedures were only available at university institutions 20 years ago but currently, especially in Europe, most of the techniques are widely marketed and principally available for every dental practice.

Therefore, the first goal of this review is to provide an overview of conventional (traditional) and molecular biological techniques for testing patients for periodontal infections. For detecting periodontopathogens, microscopy, culture, immunoassays, enzyme tests and DNA-based techniques have been introduced. Since chair-side (point-of-care) tests are in current development with increasing relevance in future, these will also be discussed. The second goal will be the presentation of the current concepts of anti-infective drug therapy for supporting the treatment of periodontal diseases. For the following techniques – some personnel and cost intensive – a representative sample is the essential precondition for an appropriate diagnosis. As the sampling (i.e., where, how and when) is another complex topic that can hardly be addressed here, we would like to direct the reader to publications in more clinically orientated journals, for example Beikler *et al.* [26].

Microscopy

The microscopic picture of native subgingival plaque from healthy and diseased sites is strikingly different. Whereas healthy plaque consists of mainly coccoid cells or small-to-large rods with almost no motility, the plaque of diseased sites presents cells of high motility and mainly consists of rods, spirochetes or long filaments. The higher the motility of bacteria in plaque (i.e., the bacteria are more invasive/aggressive), the greater the likelihood of further progression of the disease. Nevertheless, microscopic examination of freshly sampled plaque is not particularly practical for routine diagnosis. This technique requires special equipment, is time-consuming and, ultimately, gives results for only a small portion of the microbial ecological system. This is why in the past chair-side microscopic assessments as a diagnostic strategy in periodontal diseases have been mainly applied [27]; however, the principal value of microscopy to provide preliminary insights in the microbial community should not be ignored. In particular, technical advancements, such as fluorescence *in situ* hybridization linked with confocal laser microscopy (CLM), with which bacterial species or groups are specifically 'stained', enable a 3D pathogen-specific microscopic analysis and, thus, have led to a renaissance of microscopic techniques [28].

Culture methods

For routine diagnosis in medical microbiology, approved culture methods are still the gold standard for detecting and characterizing human pathogens. However, most periodontal pathogens

are strictly anaerobic and quite fastidious, and some are still uncultivable [29]. In some specialized laboratories, however, the cultivation of fastidious periodontal pathogens is well established. For culture procedures of anaerobic periodontal pathogens, it is extremely important to bear in mind that these bacteria are killed by oxygen very rapidly. As a consequence, rapid transportation of the samples from the dental practice to the diagnostic laboratory is most important. Cultivation needs time for growing and isolating the bacteria, and for biochemical differentiation of the dominant species. Therefore, the results require 10 days or longer before reaching the dentist. Nonetheless, the cultivation approach is important since resistance testing of pathogens is possible, which might become increasingly important with the growing number of reported penicillin- and metronidazole-resistant anaerobes [30,31], and rare species or those of secondary importance (e.g. *Eubacterium* spp., *Peptostreptococcus micros*, *Streptococcus constellatus*, enteric rods or pseudomonades [32]), usually not covered by molecular approaches, can also be recovered if present in dominating proportions at the diseased sites. Furthermore, culture analysis will always be an important reference approach or complementation for advanced molecular techniques [33–39], which are addressed later in this review.

Enzymatic activity

A rapid but less precise diagnosis can sometimes be preferable to a diagnosis that might be highly accurate but time-consuming. Taking this as a rationale, chair-side tests were developed based on the enzymatic activity of periodontal pathogens. Pathogens, such as *Treponema denticola* (one of the few cultivable spirochetes), *P. gingivalis* or *T. forsythensis*, produce trypsin-like proteases. If these enzymes are present in the paper-immobilized plaque tested, special substrates (benzoyl-DL-arginine-β-naphthylamide [BANA]) are hydrolyzed, leading to a color reaction. According to the first publication by Loesche *et al.*, the sensitivity of this technique lies between 90 and 96% and specificity between 83 and 92% [40]. Note that the terms sensitivity and specificity here and in the following text refer to other diagnostic methods, especially culture methods, and not to disease progression.

However, one of the major pathogens, *A. actinomycetemcomitans*, is negative for the trypsin protease reaction, which means that important cases must be diagnosed differently. Another disadvantage of this method is its insensitivity for diagnosis of the disease at an early stage, such as early onset in childhood and puberty [41]. However, until now it is the only practical and easy-to-use chair-side (point-of-care) test available for studying, for example, the effect of antimicrobials on specific (proteolytic) microbes [42,43].

As an alternative, markers involved in inflammation and tissue destruction, such as matrix metalloproteinases (MMPs; especially MMP-8, including collagenases), are becoming widely used for predicting periodontitis but also other oral diseases using immunoassays [44–48]. These approaches could be integrated in miniaturized point-of-care systems.

Immunoassays & serological tests

For the detection of periodontal pathogens, polyclonal and monoclonal antibodies are available. Conjugated with fluorescent reporter molecules, these antibodies can enhance the specificity and sensitivity of light microscopic methods (see CLM, previously). Additional immunological methods, such as ELISA or latex-agglutination tests, were also designed for detecting periodontal pathogens in plaque [49]. By combining immunoassays with chromatography in minicolumns, a chair-side technology is currently in development in Germany. With a requirement of approximately 10^4–10^5 bacterial cells per sample, the sensitivity of these tests is generally low. However, immunological tests may become a considerable tool in the future for diagnosing periodontal diseases, yet specific antigenic molecules for each marker pathogen still have to be identified [50,51].

Nucleic acid-based analysis

It has become widely recognized that a high proportion of microbes (at least 50% of the microflora in humans) cannot be cultivated under state-of-the-art laboratory conditions and that those that can be grown in the laboratory are not necessarily the most relevant species. As mentioned previously, this difficulty applies, in particular, to periodontal pathogens. Therefore, they were one of the first candidates used as a target for nucleic acid-based identification techniques in the field of medical microbiology. Two different main strategies have been pursued, based on hybridization of genomic DNA of single bacterial species used as targets to total genomic DNA obtained from clinical samples, and hybridization of short oligonucleotides of 18–35 bp in length to the homologous region of specific genes from individual bacterial species. While the former strategy, so-called 'checkerboard hybridization', has been applied in very specialized laboratories and more frequently in research than in routine diagnosis [52,53], the use of the latter approach is common and widespread in both fields. The reason for this is that oligonucleotides are synthetically produced, short, stable molecules and can be introduced easily into automated systems, the future trend of diagnostics. In addition, with the development of the PCR and sequence technology and recognition of the *16S rRNA* gene as an outstanding phylogenetic marker gene, specific probes and primers at almost every taxonomic level have been designed and used for detection and phylogenetic characterization of known and novel human pathogens [54]. Since then, *16S rRNA* gene databases have been growing constantly and becoming increasingly robust, leading in turn to an increased usefulness and attractiveness of the *16S rRNA* gene, which had become the most common target for broad-ranged or species-specific microbial identification. Meanwhile, numerous oligonucleotide-based test systems for different periodontal pathogens have been developed [15,34,36,38,39,55–60] and different detection formats have been introduced into the European market. Two approaches are of particular interest. First, single pathogens or the whole bacterial load in periodontal pockets can be accurately quantified by use of real-time quantitative PCR (abbreviated inconsistently in the literature as 'RTQ-PCR' or

'qPCR'). This approach enables not only identification but also determination of the proportion of individual species relative to the total microflora, an important aspect for assessing the role any given species might play in the disease. The second promising approach is multiplex species identification and (semi)quantification using microarray technology. Microarrays were originally introduced for differential expression profiling in both eukaryotic and prokaryotic cells. They have also been applied to support bacterial identification, especially in polymicrobial infections [61–64]. An example of a commercially available microarray is the ParoCheck® chip for the rapid detection of ten or 20 different periodontal marker species [33].

Despite intriguing advantages for species identification, the nucleic acid-based approach also has an important shortcoming when polymicrobial infections are to be analyzed. This is because the DNA extraction procedure (lysis conditions), as well as the PCR-based techniques (access of primer to template sequences), might lead to a biased retrieval of amplicons [65]. Consequently, some bacterial species might be discriminated against others. Such biases can be crucial since knowledge regarding the proportion of individual pathogens in a polymicrobial infection is important for deciding the most adequate antibiotic therapy.

In future, diagnostic systems will be available for chair-side testing by targeting either microbial DNA or enzymes involved in infection/inflammation with a time requirement of probably 1 h or less [42,44,45,60,66,67]. However, proper interpretation of the onsite findings and the measures to be taken can vary considerably from patient to patient so that, at least in some cases, the consultation of a microbiologist will still be indispensable.

From species identification to antimicrobial therapy

Severe chronic or aggressive forms of periodontitis often cannot be controlled by instrumental treatment (scaling and root planing [SRP]) alone. In addition, refractory subjects or non-responding sites are also a problem. Recurrence is mainly related to the persistence of pathogens in the pocket after treatment or to the production of specific bacterial virulence factors (leukotoxin and encapsulation) interfering with the host defense. It could also be due to the recolonization of treated sites from oral reservoirs, such as the deep sites of mucous membranes [68]. In this context, it is evident that local or systemic treatment with an antimicrobial agent is a valuable adjunctive to mechanical therapy.

In the early 1980s, systemically applied antimicrobials were first introduced for the treatment of periodontitis. However, concern emerged regarding the risks of hypersensitivity, gastrointestinal disturbances and bacterial resistance, and regarding the problem of reaching an adequate concentration at the periodontal site for a sufficiently long period of time [69]. As a consequence, locally (topically) applicable formulations (e.g., slow-release matrices) of antimicrobial agents were developed. Such formulations are of particular use in cases where systemic drug application seems inappropriate, such as localized periodontitis [70].

Locally delivered anti-infective drugs

For the treatment of periodontitis, several locally applied products for the slow release of antimicrobials have been approved within the last few years. The concept that local delivery of an antibiotic into the periodontal pocket always adjunctive to mechanical debridement achieves a greater and, thus, more potent concentration than systemic delivery has some striking advantages. The amount of drug delivered often creates sulcular medication concentrations exceeding the equivalent of 1000 µg/ml. This high level is bactericidal for the majority of bacteria and will cover some species otherwise not affected by the lower systemically delivered concentrations. On the other hand, the physiological flora (e.g., of gut, skin and vagina) is not affected by the immobilized drug in the periodontal pocket.

Local tetracyclines

Each of the three prominent tetracyclines, doxycycline, minocycline and tetracycline itself, which all inhibit bacterial protein synthesis at the 30S ribosomal subunit, are commercially available in form of local delivery devices. However, as the application procedure is not only time-consuming and relatively expensive but has also led, in some cases, to suboptimal results, it did not gain very much popularity among dentists. Nevertheless, all three are major tetracycline preparations for local delivery: 12.7 mg tetracycline-HCl in an ethylene/vinyl acetate copolymer fiber (known as Actisite®), 10% doxycycline hyclate in a gel delivery system (known as Atridox®), and the more lipophilic minocycline-HCl microspheres (known as Arestin®), confer a statistically significant improvement of clinical and microbial parameters when compared with mechanical SRP alone [71–76]. Page has recently shown that Atridox and Arestin (as well as the device PerioChip® [see chlorhexidine (CHX) section]) enabled a significantly greater reduction of periodontal pocket depth and increase in clinical periodontal attachment level (~0.8 mm) than sole mechanical treatment. However, the increase of clinical attachment level was on average larger in deeper than in shallower pockets [77]. The effect of tetracycline fibers was recently reproven [78]. However, as a matter of fact, these fibers produced in the USA (ALZA Corp.) did not prevail on most markets (including the German market), probably because of the time-consuming placement and replacement procedure. Two further tetracycline alternatives, Atridox gel and Arestin microspheres, exist but the marketing strategy is in a constant state of flux. For instance, the Atridox gel was retracted from the European market in 2006 and is currently almost exclusively available for US and Canadian dentists (for background information, the delivery platform for Atridox is Atrigel®, a QLT Inc. product and licensed to CollaGenex Pharmaceuticals in the USA and to PharmaScience Inc. in Canada). On the other hand, the Arestin microsphere product (OraPharma Inc.) was announced for marketing in Europe by 2005–2006 but has not been launched in this area so far. Meanwhile, both products, where available, have become important in periodontal

therapy, especially in cases of locally expressed recurring or refractory periodontitis and especially in patients with systemic risk factors.

Local metronidazole

In Europe, but not in the USA, an injectable lipid-like vehicle based on glycerol monooleate and sesame oil containing 25% metronidazole benzoate (Elyzol®; Dumex-Alpharma, Copenhagen, Denmark) is used frequently with apparent evidence of efficacy. After being syringed into the pocket, the gel first becomes more liquid owing to body temperature but after contact with sulcus fluid, the carrier turns into highly viscous liquid crystals and immobilizes (based on ambient responsive liquid crystal technology, Camurus®; Lund, Sweden). Metronidazole benzoate gradually disintegrates into metronidazole and the drug is released into the periodontal pocket for approximately 24–32 h after placement. Normally, two such applications, 1 week apart, are recommended. High levels (100–1000 µg/ml) of metronidazole have been initially measured in the sulcus fluid for the first 8 h and therapeutic dosages (5–20 µg/ml) have been reported for another 24 h. Given its spectrum against anaerobic bacteria, which are by far the most common of all periodontopathogens, metronidazole gel in combination with SRP appears to be more effective in terms of both clinical and microbiological improvements compared with pure mechanical treatments. However, controversial results do exist [79,80], which might be due to three facts:

- The sometimes rapid, burst-like, release of metronidazole together with rapid elimination from the pocket, for instance, when the sulcus fluid rate is increased in inflamed pockets;
- The primary evaluation of chronic periodontitis patients responding well to SRP alone without the need for adjunctive antibiotics;
- The presence of tissue-invasive anaerobes, especially spirochetes, which can hardly be reached by topical antibiotics.

However, the gel formulation enables minimal amounts of drug to achieve high concentrations, alleviating many adverse reactions and unpleasant side effects, as is often the case with systemic administration.

In conclusion, after meta-analyzing a high number of pre- and contra studies, it is still difficult to ascertain whether local delivery of metronidazole as an adjunct to SRP conveys a significant clinical advantage over SRP alone.

Chlorhexidine

The use of CHX as an auxiliary antibacterial (and antifungal) agent in dentistry has a long tradition and is well documented. Its mode of action relates to disintegrating the microbial cell wall, increasing microbial cytoplasmatic membrane permeability and, ultimately, leading to cell lysis and alteration of bacterial adherence to the pellicle-covered teeth. As a highly kationic disinfectant, CHX exhibits high substantivity, which means that it remains on oral surfaces for a prolonged period after a single usage. It has been applied primarily for controlling dental plaque in order to reduce the risk of caries or gingivitis. When used during nonsurgical and surgical periodontal treatment, CHX offers three recognized advantages:

- A bacteriostatic effect
- Improved wound healing
- General plaque control as an alternative when proper tooth-cleaning is difficult or painful

Furthermore, a number of studies have indicated that CHX is also applicable for direct anti-infective treatment of periodontitis but concentration and exposure time need to be adjusted. While simple intracrevicular irrigation has only a short-term effect on the sulcus or pocket flora, long-term efficacy of CHX on the periodontal microflora increases with duration of exposure. In order to reduce periodontal pathogens significantly, a slow-release device might be advisable [81,82]. Cosyn and Sabzevar summarized the results of eight studies in which gel vehicles with 0.2–2.0% CHX were used [83]. Although some evidence of temporary reduction of bleeding on probing was found, the use of the adjunctive medicament did not have a beneficial effect on the overall treatment outcome. The matrix of CHX gel in its current form seems not to be appropriate to further support the substantivity of CHX.

A second-generation slow-release device, such as a bioabsorbable chip containing 2.5 mg CHX in a cross-linked hydrolyzed gelatine matrix, was developed recently (PerioChip, AstraZeneca). While the chip is degraded, CHX is gradually released for approximately 7–10 days with concentrations approaching, on average, 125 µg/ml within the gingival crevicular fluid. A systematic review performed by Cosyn and Wyn has listed discrepant results using CHX chips [84]. Although earlier multicenter studies indicated significantly higher pocket reduction and clinical attachment gains, some more recent studies failed to confirm the value of CHX chips. However, while chip administration itself is a standardized aspect of treatment procedure, the application intervals or further aspects might have varied among these studies. For instance, by choosing a regime of 3-month intervals of CHX chip administration, the overall clinical outcome might be better than with SRP alone [85]. Of course, some side effects of CHX, such as staining of teeth, taste disturbances, increase in calculus accumulation and the additional costs of chip production, are aspects that have to be taken into account. Finally, it should be noted (although well understood and self-evident), that for an optimum beneficial effect of (every) adjunctive chemotherapy, a proper disruption of the biofilm is indispensable.

Other local anti-infective drugs

Povidone–iodine (polyvinylpyrrolidone–iodine complex [PVP–iodine]) might constitute a valuable adjunct to current periodontal therapy because of its broad-spectrum antimicrobial activity, low potential for developing resistance and adverse reactions, broad availability, ease of use and low financial cost. Hoang *et al.* concluded that addition of subgingival

PVP–iodine irrigation to conventional mechanical therapy is a cost-effective means of reducing periodontal pathogens and helping to control disease [86]. However, in chronic periodontitis, no effect was seen by Zanatta *et al.* [87] and by testing cases of class II furcation involvements, again no adjunctive effect was seen in another study [88]. Finally, some 'alternative' approaches formulating medicinal herbs or green tea catechin in the form of biodegradable chips or strips for subgingival application have recently been reported [89,90], and the development of various further local formulations can be expected in the future.

Systemic administration

Systemic tetracyclines

Since the early 1980s, tetracycline has been recognized as a drug with elevated gingival crevicular fluid levels inhibitory for periodontal pathogens [81,91,92]. Several smaller clinical trials using various designs have been conducted evaluating the efficacy of tetracycline adjunctive to SRP in the treatment of the then-called 'adult periodontitis'. In these studies, probing depth and attachment level were slightly improved in the tetracycline group but were rarely significant (reviewed in [81]). By contrast, double-blind clinical studies enrolling patients with refractory or recurrent periodontitis demonstrated that adjunctive systemic tetracyclines, especially doxycycline, significantly reduced major clinical parameters relative to SRP and placebo, yet failed to prevent further disease progression as was shown in other studies. Profound clinical studies on the then-called 'localized aggressive (juvenile) periodontitis' led to similar results [93–95]. Temporary improvements were probably due to the repression of *A. actinomycetemcomitans* at the infected site(s) but a complete elimination of this marker bacterium could not be achieved using doxycycline or minocycline [96,97]. For a recent metareview see [98] and for predicting changes in antibiotic susceptibility, see [99].

In summary, systemic administration of tetracyclines as adjunct to SRP may yield some benefits in certain clinical conditions, such as localized aggressive and refractory periodontitis where *A. actinomycetemcomitans* is the principal agent.

Tetracyclines have a 'second nature' as they are not only inhibitors of microbial growth but also inhibitors of MMPs, a family of enzymes that degrade extracellular matrix molecules, such as collagen [100]. When periodontal disease is present, increased secretion of MMP-8 and -9 occurs by infiltrating polymorphonuclear leukocytes, leading to digestion of collagen, a main structural component of the periodontal ligament. A subantimicrobial (or low) dose of doxycycline (abbreviated inconsistently as SDD or LDD, 20 mg twice daily, Periostat®) downregulates the collagenase activity in inflamed periodontal tissues (and also in inflamed skin under acne or rosacea conditions) by a mechanism unrelated to its antimicrobial properties [101,102]. A number of double-blind, placebo-controlled clinical trials have demonstrated clinical improvement while the subgingival flora was stable and an increase in antibiotic resistance was not found [82,102–105]. As found frequently in further studies, the greatest improvement following LDD treatment was observed at the most severely diseased sites [47,98,104,106–108]. The lack of any detectable effect on the (physiological) bacterial flora and on antibiotic resistance seems evident not only for the oral cavity but also for the intestinal and vaginal tract, as well as the skin [81,109–111]. The reason why LDD treatment might not affect the physiological flora is that 20 mg twice daily leads to serum concentrations of 0.7–0.8 µg/ml and to steady state concentrations of around 0.4 µg/ml. This concentration of doxycycline is significantly lower than most MICs of periodontal species, especially when drug diffusion is additionally inhibited by the subgingival biofilm [81]. However, although microbial resistance appears to not be induced under prolonged therapy with LDD treatment, the potential of any inverse development should be kept in mind and further examined in future studies.

In summary, as new diagnostic systems will soon be able (and available) to measure the destructive MMPs chair side and as LDD has a worldwide recognized anti-MMP effect, a new concept for diagnosing and treating periodontal disease may be emerging.

Systemic clindamycin

Clindamycin (lincosamide) inhibits the bacterial protein synthesis by binding to the 50S ribosomal subunit. Depending on the local drug concentration and the susceptibility of bacteria, clindamycin has either a bacteriostatic or bactericidal effect. While this drug is active against anaerobes associated with periodontitis, it is not active against aerobic Gram-negative periodontal pathogens, such as *E. corrodens* and, unfortunately, *A. actinomycetemcomitans*. However, the orally administrated clindamycin-HCl has been shown to penetrate into the gingival crevicular fluid and to achieve and maintain high and effective concentrations [112].

Owing to its acidic nature and to its effect on the Gram-negative intestinal bacteria, minor adverse effects, such as diarrhea, abdominal cramping, esophagitis and stomach irritation, are not uncommon. A severe adverse effect, the pseudomembranous colitis has also been attributed to administration of this drug, however, more frequently in the form of clindamycin phosphate than clindamycin-HCl [81].

Gordon *et al.* demonstrated that 11 subjects out of a total of 13 tested experienced no further loss of clinical attachment after clindamycin therapy and the number of active sites decreased significantly [113,114]. Other studies confirmed these results, demonstrating that clinical improvement was associated with a reduction of the Gram-negative periodontopathic flora [81,98,115,116], which fortunately seem not to become resistant to clindamycin over time [117].

However, as *A. actinomycetemcomitans* is intrinsically resistant to clindamycin, prior to initiating therapy, microbial testing is strongly recommended to screen for the presence of *A. actinomycetemcomitans* and, with minor impact, *E. corrodens*. If these species are present in high numbers, clindamycin is contraindicated. If present in relatively low numbers, and if other anti-infective options (e.g., owing to allergy against penicillins)

do not exist, clindamycin could, however, still be prescribed since its second mode of action, the stimulation of granulocyte activity [118] could indirectly help in eradicating *A. actinomycetemcomitans* and *E. corrodens*. However, successful elimination of these pathogens should be confirmed by subsequent diagnostic tests. In addition, due to the potential serious adverse effects, although relatively rare with clindamycin-HCl, this drug should be reserved for aggressive and/or refractory periodontitis patients.

Systemic penicillins

Penicillins are a broad class of bactericidal antibiotics that inhibit the enzymatic activity of transpeptidases (also referred to as penicillin-binding proteins), which are essential for bacterial cell wall (murein) synthesis. All penicillins consist of a β-lactam ring, a thiazolidine ring, and an acyl side with varying substitutions yielding penicillin derivates with improved qualities, including stability against gastric acid, absorption, serum concentrations and the antimicrobial spectrum. In particular, amoxicillin, a semisynthetic penicillin, has excellent activity against oral bacteria, is absorbed well following oral administration and reaches high levels in sulcus fluid or the periodontal pocket. However, two main problems associated with the administration of amoxicillin are allergic reactions (as are common for all penicillins and, to some extent, for related cephalosporins as well) and its high susceptibility to bacterial β-lactamases, which inactivate the antibiotic through hydrolyzation of the β-lactam ring. The β-lactamases are relatively common in periodontal pockets and correlate positively with age of patient and depth of pocket [119], reducing the overall drug efficacy. The efficacy of Augmentin® (the combination of amoxicillin with the β-lactamase inhibitor clavulanic acid) is theoretically higher than that of amoxicillin alone, as has been tested in a few clinical trials, but the results are still conflicting [120–122]. In summary, clinical studies do not support the use of Augmentin as a particularly effective adjunctive antibiotic in advanced periodontitis. It may provide some benefit over mechanical therapy for certain patients but other amoxicillin-containing combinations (see later) appear more effective.

Systemic metronidazole

Metronidazole, a 5-nitroimidazole, specifically targets anaerobic microorganisms including anaerobic bacteria, anaerobic protozoa, such as trichomonades or anaerobic parasites. In the oxygen-free cytoplasma of these organisms, the highly oxidized (nitrogroup) drug causes a radical chain reaction as the short-lived free radicals interact with bacterial DNA and, possibly, other macromolecules, resulting in cell death. Although resistance to metronidazole occurs in some anaerobic bacteria, for example, intestinal *Bacteroides fragilis* and related species [123,124] but also oral *Fusobacterium*, *Porphyromonas* and *Prevotella* spp. [30,31], resistance among anaerobic periodontopathogens is (still) relatively rare but should (or probably must) be monitored in future. High levels of metronidazole can be achieved in the periodontal pockets [125,126]. A number of common side effects with metronidazole exist, including gastrointestinal disorder, vomiting, headache, anorexia, drowsiness, depression, skin rashes and vaginal or urethral burning [81]. Alcohol ingestion under therapy is strictly contraindicated as metronidazole affects the hepatic enzyme alcohol dehydrogenase, causing accumulation of acetaldehyde in the blood. Furthermore, metronidazole is strictly contraindicated for nursing mothers or during pregnancy and, principally, as a single drug in cases of periodontitis in which aerobic pathogens, such as *A. actinomycetemcomitans* and *E. corrodens*, play a key role [127].

In summary, the adjunctive use of metronidazole results in significant reduction of anaerobic periodontal pathogens, including *P. intermedia, P. gingivalis, T. forsythensis* and spirochetes. Clinical improvement has been reported to be better in deep pockets (>5 mm) than in moderate sites (≤5 mm) [128,129].

Other systemic monotherapies

Fluoroquinolones are active and bactericidal by inhibiting the bacterial topoisomerases class II (gyrase), which interferes with bacterial DNA packaging, transcription and replication. Older quinolones, such as ciprofloxacin, are only recommended as part of combined therapies (see later). However, newer fluoroquinolones (moxifloxacin and levofloxacin) have an extended spectrum of activity, including against Gram-negative (aerobic and anaerobic) periodontopathogens [127,130,131]. However, as they serve as 'reserve antibiotics' for intensive care patients, their regular prescription – even in cases of aggressive periodontitis – cannot be recommended.

Amoxicillin plus metronidazole

The combination of amoxicillin plus metronidazole (also known as the 'van Winkelhoff combination' after the first describer [132]) in conjunction with SRP in periodontal therapy provides a substantial benefit over SRP alone. Clinical improvement and pathogen reduction have been reported in patients with periodontitis associated with *A. actinomycetemcomitans* [133–136] but also with other severe periodontitis cases. In the study by Winkel *et al.*, in which 49 adult periodontitis patients were included, the antibiotic treatment group demonstrated significantly greater improvement in bleeding, probing pocket depth and clinical attachment level as *P. gingivalis*, *P. intermedia* and *T. forsythensis* were reduced [136]. Similar results were found in a placebo-controlled clinical trial by Rooney *et al.* [137]: reduction in bleeding, suppuration and pocket depth, as well as a gain in attachment level, could be improved best in the metronidazole/amoxicillin group followed by the metronidazole/SRP and amoxicillin/SRP groups with these clinical parameters being significantly different compared with the placebo/SRP group. In addition, recent data from Guerrero *et al.* indicate that a 7-day adjunctive course of systemic metronidazole and amoxicillin significantly improves the short-term clinical outcomes of full-mouth nonsurgical periodontal debridement in subjects with generalized aggressive periodontitis [138]. In addition, data from Slots and Ting suggest that metronidazole/amoxicillin is an appropriate choice for

approximately 70% of advanced periodontitis patients [93]. However, given the increasing number of patients allergic to penicillins, microbial diagnosis should be performed prior to administration of metronidazole/amoxicillin and, in the absence of *A. actinomycetemcomitans*, alternative antibiotic drugs should be considered.

In summary, a therapy of metronidazole/amoxicillin in conjunction with SRP appears to be the treatment of choice for generalized aggressive periodontitis and for any other forms of periodontitis associated with *A. actinomycetemcomitans*. However, a few exceptions exist, in particular when periodontal lesions are associated with pseudomonades, Gram-negative enteric rods or *E. corrodens*, which are intrinsically resistant against metronidazole and also, to a large extent, to aminopenicillins [32,81,139–141].

Other systemic combinations

The combination of ciprofloxacin/metronidazole has been suggested as adjunctive therapy for periodontal infections when enteric rods, pseudomonads or *A. actinomycetemcomitans* are present or in cases of penicillin allergy [139,142].

This combination is useful since ciprofloxacin has an excellent activity against a wide range of Gram-negative aerobic and facultative anaerobic bacteria with the gap in the spectrum of Gram-negative anaerobes being filled by metronidazole. Drug-related side effects with ciprofloxacin are generally mild and consist primarily of photosensitivity, headache, dizziness, lightheadedness, nausea, abdominal discomfort or epigastric upset. As with almost all antibiotics, ciprofloxacin is contraindicated during pregnancy and lactation.

Another combination could be the use of metronidazole with amoxicillin plus clavulanic acid (Augmentin, see previously); however, no real advantage over metronidazole/amoxicillin was observed in the vast majority of periodontal cases. In addition, the clavulanate component is strongly acidic and, for some patients, difficult to tolerate, so the only indication is given when penicillin-resistant β-lactamase-producing *E. corrodens* is involved. This pathogen is susceptible to amoxicillin/clavulanate and the metronidazole component would cover the anaerobic periodontopathogens.

Expert commentary

Most severe periodontal diseases occur and progress due to the destructive activity of opportunistic pathogenic microorganisms that overgrow and infect the subgingival area. Several so-called periodontopathogens have been characterized but still a considerable proportion of microbes, including life forms other than bacteria, such as methane-producing archaea, parasites and viruses, await detection and elucidation of the role they might play in this disease. Understanding and treating periodontal disease is particularly challenging since the microflora involved are not only different from patient to patient but change constantly within a patient along with other rapidly shifting parameters, such as pH, redox potential and gingival crevicular fluid rates. To deal with such an intractable polymicrobial disease, we are equipped with a wide range of modern diagnostic techniques, including microarrays, able to detect simultaneously a growing number of periodontopathogenic species. While complete elimination of most virulent microorganisms might be impossible, a significant reduction in bacterial cell number by adequate antimicrobial therapy in conjunction with SRP results, at least, in an improvement in periodontal health. Since improvement has been consistently reported to be better when treating sites with deeper pockets, the use of an adjunctive antimicrobial treatment to support SRP in mild or moderate cases should be considered carefully, especially in light of adverse side effects and development of drug resistance.

Five-year view

Oral microbiology is an emerging research area owing to its importance not only for dentistry but for the entire body and general medicine. In the next few years, new oral microbial species and genera and, presumably, even novel divisions will be characterized. On the other hand, known oral taxa will be reclassified and renamed, as has for instance occurred most recently with *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*. This means that the etiological picture of periodontal diseases becomes more and more complex. For diagnosis, chair-side (point-of-care) tests will be available and used by some specialized practices while others will send samples to laboratories that have a variety of diagnostic tools to quantify putative pathogens and/or monitor host-specific markers associated with genetic predisposition, inflammation and tissue destruction. Concern will arise regarding the complexity of incoming data and its clinical handling, especially as only a few new drugs will become available and most of them as locally deliverables.

Some periodontologists will appreciate the new evidence-based dentistry using selected diagnostic and treatment options, while others might prefer to rely on clinical diagnosis solely and will probably still use nothing but mechanical debridement to treat periodontitis. An ongoing discussion of the controversial findings reviewed in this article is needed and more guidelines should be established by the dental societies and directed to the individual practitioner. However, every case or patient has an individual form of periodontitis with an individual mixture of underlying risk factors. This means that the dentist, while facing exponentially growing information, still has to find individual solutions.

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Key issues

- Although our perspective on the complexity of the microflora associated with the multifactorial disease periodontitis has improved enormously in the past few decades, we are facing a constantly growing number of newly identified periodontal species with as yet unknown function at the infected sites.
- Since species-directed antibiosis can be crucial for successful treatment of periodontitis, a variety of approaches aimed at pathogen identification, including real-time quantitative PCR, microarrays and miniaturized enzyme tests, have been established and are commercially available as high-end diagnostic systems.
- Besides nucleic acid-based detection methods, chair-side diagnosis of metalloproteinase (collagenase) activity and therapeutic inhibition of this enzymatic activity by administration of low (subantimicrobial) doses of doxycycline appears to be a rising and promising new concept.
- For periodontal therapy both, local and systemic, no general consensus worldwide and even not within Europe exists regarding the choice of the anti-infective drug, indicating the lack of a global gold standard for treating periodontal diseases.
- For treatment of generalized aggressive periodontitis and for cases in which the prominent marker pathogen *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* is involved, however, general agreement exists that the drug combination metronidazole plus amoxicillin in conjunction with scaling and root planing is the treatment of choice.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Armitage GC. Development of a classification system for periodontal diseases and conditions. *Northwest Dent.* 79(6), 31–35 (2000).

2 Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. *Bull. World Health Organ.* 83(9), 661–669 (2005).

3 Petersen PE, Ogawa H. Strengthening the prevention of periodontal disease: the WHO approach. *J. Periodontol.* 76(12), 2187–2193 (2005).

4 Willershausen B, Krahwinkel T, Valentinis L, Todt M. Correlation between inflammatory periodontal diseases and cardiovascular diseases. *Eur. J. Med. Res.* 8(11), 499–504 (2003).

5 Persson GR. What has ageing to do with periodontal health and disease? *Int. Dent. J.* 56(4 Suppl. 4), 240–249 (2006).

6 Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 366(9499), 1809–1820 (2005).

7 Renvert S, Pettersson T, Ohlsson O, Persson GR. Bacterial profile and burden of periodontal infection in subjects with a diagnosis of acute coronary syndrome. *J. Periodontol.* 77(7), 1110–1119 (2006).

8 Dumitrescu AL. Occurrence of self-reported systemic medical conditions in patients with periodontal disease. *Rom. J. Intern. Med.* 44(1), 35–48 (2006).

9 D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. *Am. Heart J.* 151(5), 977–984 (2006).

10 Dortbudak O, Eberhardt R, Ulm M, Persson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. *J. Clin. Periodontol.* 32(1), 45–52 (2005).

11 Page RC, Martin JA, Loeb CF. The Oral Health Information Suite (OHIS): its use in the management of periodontal disease. *J. Dent. Educ.* 69(5), 509–520 (2005).

12 Conrads G. Testing for marker bacteria in progressive periodontitis: the European experience. *Infect. Dis. Clin. Pract.* 10, 481–487 (2001).

13 Albert DA, Sadowsky D, Papapanou P, Conicella ML, Ward A. An examination of periodontal treatment and per member per month (PMPM) medical costs in an insured population. *BMC Health Serv. Res.* 6, 103 (2006).

14 Petersen PE. Priorities for research for oral health in the 21st Century – the approach of the WHO Global Oral Health Programme. *Community Dent. Health* 22(2), 71–74 (2005).

15 Ledder RG, Gilbert P, Huws SA *et al.* Molecular analysis of the subgingival microbiota in health and disease. *Appl. Environ. Microbiol.* 73(2), 516–523 (2007).

16 Kumar PS, Leys EJ, Bryk JM, Martinez FJ, Moeschberger ML, Griffen AL. Changes in periodontal health status are associated with bacterial community shifts as assessed by quantitative 16S cloning and sequencing. *J. Clin. Microbiol.* 44(10), 3665–3673 (2006).

17 Moter A, Riep B, Haban V *et al.* Molecular epidemiology of oral treponemes in patients with periodontitis and in periodontitis-resistant subjects. *J. Clin. Microbiol.* 44(9), 3078–3085 (2006).

18 Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J. Clin. Periodontol.* 25(2), 134–144 (1998).

•• **Introduction of microbial complexes, especially the red complex, with striking etiological importance to periodontology.**

19 Lepp PW, Brinig MM, Ouverney CC, Palm K, Armitage GC, Relman DA. Methanogenic Archaea and human periodontal disease. *Proc. Natl Acad. Sci. USA* 101(16), 6176–6181 (2004).

•• **Series of three basic studies** ([19–21]) **about the role of archaea, the third lineage of life, in medical microbiology.**

20 Vianna ME, Conrads G, Gomes BP, Horz HP. Identification and quantification of archaea involved in primary endodontic infections 1. *J. Clin. Microbiol.* 44(4), 1274–1282 (2006).

21 Robichaux M, Howell M, Boopathy R. Methanogenic activity in human periodontal pocket. *Curr. Microbiol.* 46(1), 53–58 (2003).

22 Yapar M, Saygun I, Ozdemir A, Kubar A, Sahin S. Prevalence of human herpesviruses in patients with aggressive periodontitis. *J. Periodontol.* 74(11), 1634–1640 (2003).

23 Ling LJ, Ho CC, Wu CY, Chen YT, Hung SL. Association between human herpesviruses and the severity of periodontitis. *J. Periodontol.* 75(11), 1479–1485 (2004).

24 Kubar A, Saygun I, Ozdemir A, Yapar M, Slots J. Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein–Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. *J. Periodontal Res.* 40(2), 97–104 (2005).

25 Santangelo R, D'Ercole S, Graffeo R *et al.* Bacterial and viral DNA in periodontal disease: a study using multiplex PCR. *New Microbiol.* 27(2), 133–137 (2004).

26 Beikler T, Schnitzer S, Abdeen G, Ehmke B, Eisenacher M, Flemmig TF. Sampling strategy for intraoral detection of periodontal pathogens before and following periodontal therapy. *J. Periodontol.* 77(8), 1323–1332 (2006).

27 Greenstein G, Polson A. Microscopic monitoring of pathogens associated with periodontal diseases. A review. *J. Periodontol.* 56(12), 740–747 (1985).

28 Stender H. PNA FISH: an intelligent stain for rapid diagnosis of infectious diseases. *Expert Rev. Mol. Diagn.* 3(5), 649–655 (2003).

29 Wardle HM. The challenge of growing oral spirochaetes. *J. Med. Microbiol.* 46(2), 104–116 (1997).

30 van Winkelhoff AJ, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. *J. Clin. Periodontol.* 32(8), 893–898 (2005).

31 Papaparaskevas J, Pantazatou A, Katsandri A, Legakis NJ, Avlamis A. Multicentre survey of the *in vitro* activity of seven antimicrobial agents, including ertapenem, against recently isolated Gram-negative anaerobic bacteria in Greece. *Clin. Microbiol. Infect.* 11(10), 820–824 (2005).

32 Barbosa FC, Mayer MP, Saba-Chujfi E, Cai S. Subgingival occurrence and antimicrobial susceptibility of enteric rods and pseudomonads from Brazilian periodontitis patients. *Oral Microbiol. Immunol.* 16(5), 306–310 (2001).

33 Vianna ME, Horz HP, Gomes B, Conrads G. Microarrays complement culture methods for identification of bacteria in endodontic infections. *Oral Microbiol. Immunol.* 20, 253–258 (2005).

34 Lau L, Sanz M, Herrera D, Morillo JM, Martin C, Silva A. Quantitative real-time polymerase chain reaction versus culture: a comparison between two methods for the detection and quantification of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythensis* in subgingival plaque samples. *J. Clin. Periodontol.* 31(12), 1061–1069 (2004).

35 Tsai CY, Wolff LF, Germaine G, Hodges J. A rapid DNA probe test compared to culture methods for identification of subgingival plaque bacteria. *J. Clin. Periodontol.* 30(1), 57–62 (2003).

36 Sanz M, Lau L, Herrera D, Morillo JM, Silva A. Methods of detection of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythensis* in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. *J. Clin. Periodontol.* 31(12), 1034–1047 (2004).

37 Boutaga K, van Winkelhoff AJ, Vandenbroucke-Grauls CM, Savelkoul PH. Periodontal pathogens: a quantitative comparison of anaerobic culture and real-time PCR. *FEMS Immunol. Med. Microbiol.* 45(2), 191–199 (2005).

38 Verner C, Lemaitre P, Daniel A, Giumelli B, Lakhssassi N, Sixou M. Carpegen real-time polymerase chain reaction vs. anaerobic culture for periodontal pathogen identification. *Oral Microbiol. Immunol.* 21(6), 341–346 (2006).

39 Boutaga K, van Winkelhoff AJ, Vandenbroucke-Grauls CM, Savelkoul PH. The additional value of real-time PCR in the quantitative detection of periodontal pathogens. *J. Clin. Periodontol.* 33(6), 427–433 (2006).

40 Loesche WJ, Lopatin DE, Giordano J, Alcoforado G, Hujoel P. Comparison of the benzoyl-DL-arginine-naphthylamide (BANA) test, DNA probes, and immunological reagents for ability to detect anaerobic periodontal infections due to *Porphyromonas gingivalis*, *Treponema denticola*, and *Bacteroides forsythus*. *J. Clin. Microbiol.* 30(2), 427–433 (1992).

41 Zuza EP, de Salis AM, de Toledo BE, Mendes AJ. Relationship between gingival clinical parameters and the reactivity of the BANA test in subgingival samples from children. *J. Int. Acad. Periodontol.* 8(3), 78–82 (2006).

42 Apsey DJ, Kaciroti N, Loesche WJ. The diagnosis of periodontal disease in private practice. *J. Periodontol.* 77(9), 1572–1581 (2006).

43 Faveri M, Gursky LC, Feres M, Shibli JA, Salvador SL, de Figueiredo LC. Scaling and root planing and chlorhexidine mouthrinses in the treatment of chronic periodontitis: a randomized, placebo-controlled clinical trial. *J. Clin. Periodontol.* 33(11), 819–828 (2006).

44 Herr AE, Hatch AV, Throckmorton DJ *et al.* Microfluidic immunoassays as rapid saliva-based clinical diagnostics. *Proc. Natl Acad. Sci. USA* 104(13), 5268–5273 (2007).

45 Sorsa T, Tjaderhane L, Konttinen YT *et al.* Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann. Med.* 38(5), 306–321 (2006).

- **Series of four publications** ([45–48]) **about the contribution of matrix metalloproteinases to pathogenesis and diagnosis. Furthermore, see** [100–111] **for the resulting treatment strategy.**

46 Verstappen J, Von den Hoff JW. Tissue inhibitors of metalloproteinases (TIMPs): their biological functions and involvement in oral disease. *J. Dent. Res.* 85(12), 1074–1084 (2006).

47 Gapski R, Barr JL, Sarment DP, Layher MG, Socransky SS, Giannobile WV. Effect of systemic matrix metalloproteinase inhibition on periodontal wound repair: a proof of concept trial. *J. Periodontol.* 75(3), 441–452 (2004).

48 Sorsa T, Tjaderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis.* 10(6), 311–318 (2004).

49 Di Murro C, Paolantonio M, Pedrazzoli V, Lopatin DE, Cattabriga M. Occurrence of *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola* in periodontally healthy and diseased subjects as determined by an ELISA technique. *J. Periodontol.* 68(1), 18–23 (1997).

50 Booth V, Solakoglu O, Bavisha N, Curtis MA. Serum IgG1 and IgG2 antibody responses to *Porphyromonas gingivalis* in patients with periodontitis. *Oral Microbiol. Immunol.* 21(2), 93–99 (2006).

51 Ihara H, Miura T, Kato T *et al.* Detection of *Campylobacter rectus* in periodontitis sites by monoclonal antibodies. *J. Periodontal Res.* 38(1), 64–72 (2003).

52 Dahlen G, Leonhardt A. A new checkerboard panel for testing bacterial markers in periodontal disease. *Oral Microbiol. Immunol.* 21(1), 6–11 (2006).

53 Socransky SS, Haffajee AD, Smith C *et al.* Use of checkerboard DNA–DNA hybridization to study complex microbial ecosystems. *Oral Microbiol. Immunol.* 19(6), 352–362 (2004).

54 Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin. Microbiol. Rev.* 9(1), 18–33 (1996).

▶55 Boutaga K, Savelkoul PH, Winkel EG, van Winkelhoff AJ. Comparison of subgingival bacterial sampling with oral lavage for detection and quantification of periodontal pathogens by real-time polymerase chain reaction. *J. Periodontol.* 78(1), 79–86 (2007).

▶56 Caposio P, Torta AI, Romano F *et al.* Molecular approaches to the identification and treatment monitoring of periodontal pathogens. *New Microbiol.* 26(1), 121–124 (2003).

▶57 Kuboniwa M, Amano A, Kimura KR *et al.* Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with TaqMan probes. *Oral Microbiol. Immunol.* 19(3), 168–176 (2004).

▶58 Nonnenmacher C, Dalpke A, Mutters R, Heeg K. Quantitative detection of periodontopathogens by real-time PCR. *J. Microbiol. Methods* 59(1), 117–125 (2004).

59 Suzuki N, Yoshida A, Nakano Y. Quantitative analysis of multi-species oral biofilms by TaqMan real-time PCR. *Clin. Med. Res.* 3(3), 176–185 (2005).

▶60 Takada K, Sakaguchi Y, Oka C, Hirasawa M. New rapid polymerase chain reaction-immunochromatographic assay for *Porphyromonas gingivalis*. *J. Periodontol.* 76(4), 508–512 (2005).

▶61 Bryant PA, Venter D, Robins-Browne R, Curtis N. Chips with everything: DNA microarrays in infectious diseases. *Lancet Infect. Dis.* 4(2), 100–111 (2004).

▶62 Campbell CJ, Ghazal P. Molecular signatures for diagnosis of infection: application of microarray technology. *J. Appl. Microbiol.* 96(1), 18–23 (2004).

▶63 Chan VL. Bacterial genomes and infectious diseases. *Pediatr. Res.* 54(1), 1–7 (2003).

▶64 Xiang CC, Chen Y. cDNA microarray technology and its applications. *Biotechnol. Adv.* 18(1), 35–46 (2000).

▶65 von Wintzingerode F, Gobel UB, Stackebrandt E. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.* 21(3), 213–229 (1997).

•• **Key publication regarding pitfalls of probably the most important molecular diagnostic technique – PCR.**

▶66 Agan S, Sonmez S, Serdar M. The effect of topical doxycycline usage on gingival crevicular fluid MMP-8 levels of chronic and aggressive periodontitis patients: a pilot study. *Int. J. Dent. Hyg.* 4(3), 114–121 (2006).

▶67 Mantyla P, Stenman M, Kinane D *et al.* Monitoring periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test. *J. Periodontal Res.* 41(6), 503–512 (2006).

▶68 Conrads G, Flemmig TF, Seyfarth I, Lampert F, Lutticken R. Simultaneous detection of *Bacteroides forsythus* and *Prevotella intermedia* by 16S rRNA gene-directed multiplex PCR. *J. Clin. Microbiol.* 37(5), 1621–1624 (1999).

▶69 Bollen CM, Quirynen M. Microbiological response to mechanical treatment in combination with adjunctive therapy. A review of the literature. *J. Periodontol.* 67(11), 1143–1158 (1996).

70 Kornman KS. Controlled-release local delivery antimicrobials in periodontics: prospects for the future. *J. Periodontol.* 64(8 Suppl.), 782–791 (1993).

▶71 Drisko CL, Cobb CM, Killoy WJ *et al.* Evaluation of periodontal treatments using controlled-release tetracycline fibers: clinical response. *J. Periodontol.* 66(8), 692–699 (1995).

▶72 Garrett S, Adams DF, Bogle G *et al.* The effect of locally delivered controlled-release doxycycline or scaling and root planing on periodontal maintenance patients over 9 months. *J. Periodontol.* 71(1), 22–30 (2000).

▶73 Garrett S, Johnson L, Drisko CH *et al.* Two multi-center studies evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene, and scaling and root planing in the treatment of periodontitis. *J. Periodontol.* 70(5), 490–503 (1999).

▶74 Newman MG, Kornman KS, Doherty FM. A 6-month multi-center evaluation of adjunctive tetracycline fiber therapy used in conjunction with scaling and root planing in maintenance patients: clinical results. *J. Periodontol.* 65(7), 685–691 (1994).

▶75 Purucker P, Mertes H, Goodson JM, Bernimoulin JP. Local versus systemic adjunctive antibiotic therapy in 28 patients with generalized aggressive periodontitis. *J. Periodontol.* 72(9), 1241–1245 (2001).

76 Radvar M, Pourtaghi N, Kinane DF. Comparison of 3 periodontal local antibiotic therapies in persistent periodontal pockets. *J. Periodontol.* 67(9), 860–865 (1996).

77 Page RC. The microbiological case for adjunctive therapy for periodontitis. *J. Int. Acad. Periodontol.* 6(4 Suppl.), 143–149 (2004).

▶78 Sakellari D, Vouros ID, Aristodemou E, Konstantinidis AB, Socransky S, Goodson M. Tetracycline fibers as an adjunct in the treatment of nifedipine-induced gingival enlargement. *J. Periodontol.* 76(6), 1034–1039 (2005).

▶79 Perinetti G, Paolantonio M, Cordella C, D'Ercole S, Serra E, Piccolomini R. Clinical and microbiological effects of subgingival administration of two active gels on persistent pockets of chronic periodontitis patients. *J. Clin. Periodontol.* 31(4), 273–281 (2004).

▶80 Zee KY, Lee DH, Corbet EF. Repeated oral hygiene instructions alone, or in combination with metronidazole dental gel with or without subgingival scaling in adult periodontitis patients: a one-year clinical study. *J. Int. Acad. Periodontol.* 8(4), 125–135 (2006).

81 Walker CB, Karpinia K, Baehni P. Chemotherapeutics: antibiotics and other antimicrobials. *Periodontol. 2000* 36, 146–165 (2004).

•• **Excellent review article regarding anti-infective therapy in periodontitis.**

▶82 Crout RJ, Lee HM, Schroeder K *et al.* The "cyclic" regimen of low-dose doxycycline for adult periodontitis: a preliminary study. *J. Periodontol.* 67(5), 506–514 (1996).

▶83 Cosyn J, Sabzevar MM. A systematic review on the effects of subgingival chlorhexidine gel administration in the treatment of chronic periodontitis. *J. Periodontol.* 76(11), 1805–1813 (2005).

▶84 Cosyn J, Wyn I. A systematic review on the effects of the chlorhexidine chip when used as an adjunct to scaling and root planing in the treatment of chronic periodontitis. *J. Periodontol.* 77(2), 257–264 (2006).

▶85 Grisi DC, Salvador SL, Figueiredo LC, Souza SL, Novaes AB, Grisi MF. Effect of a controlled-release chlorhexidine chip on clinical and microbiological parameters of periodontal syndrome. *J. Clin. Periodontol.* 29(10), 875–881 (2002).

▶86 Hoang T, Jorgensen MG, Keim RG, Pattison AM, Slots J. Povidone–iodine as a periodontal pocket disinfectant. *J. Periodontal Res.* 38(3), 311–317 (2003).

▶87 Zanatta GM, Bittencourt S, Nociti FH Jr, Sallum EA, Sallum AW, Casati MZ. Periodontal debridement with povidone–iodine in periodontal treatment: short-term clinical and biochemical observations. *J. Periodontol.* 77(3), 498–505 (2006).

88 Del Peloso Ribeiro E, Bittencourt S, Ambrosano GM *et al*. Povidone–iodine used as an adjunct to non-surgical treatment of furcation involvements. *J. Periodontol.* 77(2), 211–217 (2006).

89 Hirasawa M, Takada K, Makimura M, Otake S. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. *J. Periodontal. Res.* 37(6), 433–438 (2002).

90 Sastravaha G, Yotnuengnit P, Booncong P, Sangtherapitikul P. Adjunctive periodontal treatment with *Centella asiatica* and *Punica granatum* extracts. A preliminary study. *J. Int. Acad. Periodontol.* 5(4), 106–115 (2003).

91 Gordon JM, Walker CB, Goodson JM, Socransky SS. Sensitive assay for measuring tetracycline levels in gingival crevice fluid. *Antimicrob. Agents Chemother.* 17(2), 193–198 (1980).

92 Gordon JM, Walker CB, Murphy JC, Goodson JM, Socransky SS. Tetracycline: levels achievable in gingival crevice fluid and *in vitro* effect on subgingival organisms. Part I. Concentrations in crevicular fluid after repeated doses. *J. Periodontol.* 52(10), 609–612 (1981).

93 Slots J, Ting M. Systemic antibiotics in the treatment of periodontal disease. *Periodontol. 2000* 28, 106–176 (2002).

94 Slots J, Rosling BG. Suppression of the periodontopathic microflora in localized juvenile periodontitis by systemic tetracycline. *J. Clin. Periodontol.* 10(5), 465–486 (1983).

95 Novak MJ, Polson AM, Adair SM. Tetracycline therapy in patients with early juvenile periodontitis. *J. Periodontol.* 59(6), 366–372 (1988).

96 Muller HP, Lange DE, Muller RF. Failure of adjunctive minocycline-HCl to eliminate oral *Actinobacillus actinomycetemcomitans*. *J. Clin. Periodontol.* 20(7), 498–504 (1993).

97 Saxen L, Asikainen S, Kanervo A, Kari K, Jousimies-Somer H. The long-term efficacy of systemic doxycycline medication in the treatment of localized juvenile periodontitis. *Arch. Oral Biol.* 35(Suppl.), 227S–229S (1990).

98 Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. *Ann. Periodontol.* 8(1), 115–181 (2003).

99 Buchmann R, Muller RF, Van Dyke TE, Lange DE. Change of antibiotic susceptibility following periodontal therapy. A pilot study in aggressive periodontal disease. *J. Clin. Periodontol.* 30(3), 222–229 (2003).

100 Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J, Sorsa T. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. *Adv. Dent. Res.* 12(2), 12–26 (1998).

101 Golub LM, Lee HM, Greenwald RA *et al*. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm. Res.* 46(8), 310–319 (1997).

102 Golub LM, Sorsa T, Lee HM *et al*. Doxycycline inhibits neutrophil (PMN)-type matrix metalloproteinases in human adult periodontitis gingiva. *J. Clin. Periodontol.* 22(2), 100–109 (1995).

103 Ashley RA. Clinical trials of a matrix metalloproteinase inhibitor in human periodontal disease. SDD Clinical Research Team. *Ann. NY Acad. Sci.* 878, 335–346 (1999).

104 Caton JG, Ciancio SG, Blieden TM *et al*. Subantimicrobial dose doxycycline as an adjunct to scaling and root planing: post-treatment effects. *J. Clin. Periodontol.* 28(8), 782–789 (2001).

105 Caton JG, Ciancio SG, Blieden TM *et al*. Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planing in patients with adult periodontitis. *J. Periodontol.* 71(4), 521–532 (2000).

106 Emingil G, Atilla G, Sorsa T, Luoto H, Kirilmaz L, Baylas H. The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *J. Periodontol.* 75(1), 106–115 (2004).

107 Emingil G, Atilla G, Sorsa T, Savolainen P, Baylas H. Effectiveness of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid laminin-5 γ2 chain levels in chronic periodontitis. *J. Periodontol.* 75(10), 1387–1396 (2004).

108 Lee HM, Ciancio SG, Tuter G, Ryan ME, Komaroff E, Golub LM. Subantimicrobial dose doxycycline efficacy as a matrix metalloproteinase inhibitor in chronic periodontitis patients is enhanced when combined with a non-steroidal anti-inflammatory drug. *J. Periodontol.* 75(3), 453–463 (2004).

109 Skidmore R, Kovach R, Walker C *et al*. Effects of subantimicrobial-dose doxycycline in the treatment of moderate acne. *Arch. Dermatol.* 139(4), 459–464 (2003).

110 Thomas J, Walker C, Bradshaw M. Long-term use of subantimicrobial dose doxycycline does not lead to changes in antimicrobial susceptibility. *J. Periodontol.* 71(9), 1472–1483 (2000).

111 Thomas JG, Metheny RJ, Karakiozis JM, Wetzel JM, Crout RJ. Long-term sub-antimicrobial doxycycline (Periostat) as adjunctive management in adult periodontitis: effects on subgingival bacterial population dynamics. *Adv. Dent. Res.* 12(2), 32–39 (1998).

112 Walker CB, Gordon JM, Cornwall HA, Murphy JC, Socransky SS. Gingival crevicular fluid levels of clindamycin compared with its minimal inhibitory concentrations for periodontal bacteria. *Antimicrob. Agents Chemother.* 19(5), 867–871 (1981).

113 Gordon J, Walker C, Hovliaras C, Socransky S. Efficacy of clindamycin hydrochloride in refractory periodontitis: 24-month results. *J. Periodontol.* 61(11), 686–691 (1990).

114 Gordon J, Walker C, Lamster I *et al*. Efficacy of clindamycin hydrochloride in refractory periodontitis. 12-month results. *J. Periodontol.* 56(11 Suppl.), 75–80 (1985).

115 Magnusson I, Low SB, McArthur WP *et al*. Treatment of subjects with refractory periodontal disease. *J. Clin. Periodontol.* 21(9), 628–637 (1994).

116 Brook I, Lewis MA, Sandor GK, Jeffcoat M, Samaranayake LP, Vera Rojas J. Clindamycin in dentistry: more than just effective prophylaxis for endocarditis? *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 100(5), 550–558 (2005).

117 Lakhssassi N, Elhajoui N, Lodter JP, Pineill JL, Sixou M. Antimicrobial susceptibility variation of 50 anaerobic periopathogens in aggressive periodontitis: an interindividual variability study. *Oral Microbiol. Immunol.* 20(4), 244–252 (2005).

118 Eick S, Pfister W, Fiedler D, Straube E. Clindamycin promotes phagocytosis and intracellular killing of periodontopathogenic bacteria by crevicular granulocytes: an *in vitro* study. *J. Antimicrob. Chemother.* 46(4), 583–588 (2000).

119 Walker CB, Tyler KZ, Low SB, King CJ. Penicillin-degrading enzymes in sites associated with adult periodontitis. *Oral Microbiol. Immunol.* 2(3), 129–131 (1987).

120 Winkel EG, van Winkelhoff AJ, Barendregt DS, van der Weijden GA, Timmerman MF, van der Velden U. Clinical and microbiological effects of

initial periodontal therapy in conjunction with amoxicillin and clavulanic acid in patients with adult periodontitis. A randomised double-blind, placebo-controlled study. *J. Clin. Periodontol.* 26(7), 461–468 (1999).

121 Bascones-Martinez A, Figuero-Ruiz E. Periodontal diseases as bacterial infection. *Med. Oral Patol. Oral Cir. Bucal.* 9(Suppl.), 100–107 (2004).

122 Liebana J, Castillo AM, Alvarez M. Periodontal diseases: microbiological considerations. *Med. Oral Patol. Oral Cir. Bucal.* 9(Suppl.) 82–91 (2004).

▶123 Snydman DR, Jacobus NV, McDermott LA *et al*. National survey on the susceptibility of *B. fragilis* group: report and analysis of trends for 1997–2004: a US survey. *Antimicrob. Agents Chemother.* 51(5), 1649–1655 (2007).

▶124 Lofmark S, Fang H, Hedberg M, Edlund C. Inducible metronidazole resistance and *nim* genes in clinical *Bacteroides fragilis* group isolates. *Antimicrob. Agents Chemother.* 49(3), 1253–1256 (2005).

▶125 Poulet PP, Duffaut D, Lodter JP. Metronidazole susceptibility testing of anaerobic bacteria associated with periodontal disease. *J. Clin. Periodontol.* 26(4), 261–263 (1999).

▶126 Van Oosten MA, Notten FJ, Mikx FH. Metronidazole concentrations in human plasma, saliva, and gingival crevice fluid after a single dose. *J. Dent. Res.* 65(12), 1420–1423 (1986).

▶127 Muller HP, Holderrieth S, Burkhardt U, Hoffler U. *In vitro* antimicrobial susceptibility of oral strains of *Actinobacillus actinomycetemcomitans* to seven antibiotics. *J. Clin. Periodontol.* 29(8), 736–742 (2002).

▶128 Soder B, Nedlich U, Jin LJ. Longitudinal effect of non-surgical treatment and systemic metronidazole for 1 week in smokers and non-smokers with refractory periodontitis: a 5-year study. *J. Periodontol.* 70(7), 761–771 (1999).

▶129 Carvalho LH, D'Avila GB, Leao A, Haffajee AD, Socransky SS, Feres M. Scaling and root planing, systemic metronidazole and professional plaque removal in the treatment of chronic periodontitis in a Brazilian population. I. Clinical results. *J. Clin. Periodontol.* 31(12), 1070–1076 (2004).

▶130 Sobottka I, Cachovan G, Sturenburg E *et al*. *In vitro* activity of moxifloxacin against bacteria isolated from odontogenic abscesses. *Antimicrob. Agents Chemother.* 46(12), 4019–4021 (2002).

▶131 Eick S, Schmitt A, Sachse S, Schmidt KH, Pfister W. *In vitro* antibacterial activity of fluoroquinolones against *Porphyromonas gingivalis* strains. *J. Antimicrob. Chemother.* 54(2), 553–556 (2004).

▶132 van Winkelhoff AJ, Rodenburg JP, Goene RJ, Abbas F, Winkel EG, de Graaff J. Metronidazole plus amoxycillin in the treatment of *Actinobacillus actinomycetemcomitans* associated periodontitis. *J. Clin. Periodontol.* 16(2), 128–131 (1989).

•• **Introduces probably the most effective antibiotic therapy in *A. actinomycetemcomitans*-associated aggressive periodontitis.**

▶133 Flemmig TF, Milian E, Karch H, Klaiber B. Differential clinical treatment outcome after systemic metronidazole and amoxicillin in patients harboring *Actinobacillus actinomycetemcomitans* and/or *Porphyromonas gingivalis*. *J. Clin. Periodontol.* 25(5), 380–387 (1998).

▶134 Pavicic MJ, van Winkelhoff AJ, Douque NH, Steures RW, de Graaff J. Microbiological and clinical effects of metronidazole and amoxicillin in *Actinobacillus actinomycetemcomitans*-associated periodontitis. A 2-year evaluation. *J. Clin. Periodontol.* 21(2), 107–112 (1994).

▶135 Tinoco EM, Beldi MI, Campedelli F *et al*. Clinical and microbiological effects of adjunctive antibiotics in treatment of localized juvenile periodontitis. A controlled clinical trial. *J. Periodontol.* 69(12), 1355–1363 (1998).

▶136 Winkel EG, Van Winkelhoff AJ, Timmerman MF, Van der Velden U, Van der Weijden GA. Amoxicillin plus metronidazole in the treatment of adult periodontitis patients. A double-blind placebo-controlled study. *J. Clin. Periodontol.* 28(4), 296–305 (2001).

▶137 Rooney J, Wade WG, Sprague SV, Newcombe RG, Addy M. Adjunctive effects to non-surgical periodontal therapy of systemic metronidazole and amoxycillin alone and combined. A placebo controlled study. *J. Clin. Periodontol.* 29(4), 342–350 (2002).

▶138 Guerrero A, Griffiths GS, Nibali L *et al*. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. *J. Clin. Periodontol.* 32(10), 1096–1107 (2005).

▶139 Slots J, Feik D, Rams TE. *In vitro* antimicrobial sensitivity of enteric rods and pseudomonads from advanced adult periodontitis. *Oral Microbiol. Immunol.* 5(5), 298–301 (1990).

140 Walker CB. The acquisition of antibiotic resistance in the periodontal microflora. *Periodontol 2000* 10, 79–88 (1996).

▶141 Lacroix JM, Walker C. Characterization of a β-lactamase found in *Eikenella corrodens*. *Antimicrob. Agents Chemother.* 35(5), 886–891 (1991).

▶142 Slots J, Feik D, Rams TE. Prevalence and antimicrobial susceptibility of Enterobacteriaceae, Pseudomonadaceae and *Acinetobacter* in human periodontitis. *Oral Microbiol. Immunol.* 5(3), 149–154 (1990).

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