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Microbial colonization of the periodontal pocket and its significance for periodontal therapy

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The cardinal symptoms of periodontal disease are loss of periodontal tissue attachment, loss of alveolar bone and pocket formation. To prevent and treat this disease, it is key to recognize the pathogenic role of bacteria that accumulate in the periodontal pocket. In fact, clinical trials have repeatedly demonstrated that scaling and root planing, a procedure that aims to remove subgingival bacterial deposits by scraping on the tooth surface within the pocket, is quite effective in reducing probing pocket depths (106). As mechanical cleaning alone cannot completely eliminate all bacteria involved in periodontal disease, adjunctive antimicrobial protocols have been devised and tested. They have been the subject of several reviews published in *Periodontology 2000* (20, 23, 25, 34, 60, 81, 96, 101, 107). Numerous trials have assessed the benefits of systemic antibiotics, locally delivered antimicrobial agents and antiseptic rinses. More recently it has also been shown that an antimicrobial effect of some substances can be activated in periodontal pockets using the principle of photodynamic therapy (116). The aim of this review was to reassess strategies for periodontal therapy from the perspective of the disease being a consequence of microbial colonization of the periodontal pocket environment.

Microbial colonization of the gingival crevice and its consequences

A groundbreaking experiment in humans suggested a cause–effect relationship between the aggregation of bacterial deposits in the area of the gingival crevice and gingival inflammation (51). After having been instructed not to clean their teeth any more, young volunteers with healthy gingiva were rechecked at varying time intervals. 'As soon as inflammatory changes were observed (. . .), the patients were given detailed instructions in oral hygiene methods using brush and wood massage sticks' (51). As a consequence of the absence of oral hygiene, bacteria multiplied to form macroscopically visible deposits on the teeth. Within 9–21 days, clinical signs of gingivitis appeared. When the bacterial deposits were removed and tooth cleaning was resumed, gingivitis subsided. The composition of these microbial deposits was assessed microscopically (104): early they consisted mainly of gram-positive cocci and rods; later they also contained fusiform and filamentous organisms; and, after some days, spirochetes were also detected. However, when this experiment was repeated in the same institution and in a similar cohort two decades later (18), associations between plaque and gingivitis were

not statistically significant (no statistical analysis had been carried out in the original trial). 'During phases of both plaque accumulation and thorough oral hygiene, sites were found to convert from non-inflamed to inflamed status concurrently, as in the reverse direction' (18).

Interindividual variation in gingival inflammation had already been noted in the first trial. This variation was thought to be related to quantitative differences in plaque mass or differences in its microbial composition. Interindividual and local differences in the newly formed crevicular microbiota, after cessation of oral hygiene, have indeed been shown. In one experiment, after 4 days of undisturbed plaque accumulation, there was significant interindividual variation in the proportions of cocci, nonmotile rods and fusiform organisms (67). Samples obtained from different locations also varied: bacterial counts were augmented at a more posterior location, and samples from interdental locations contained more bacteria than did samples from midbuccal sites. However, interindividual variation in gingival inflammation has also been observed in the absence of notable quantitative or qualitative differences in plaque accumulation (1) and in studies on naturally occurring gingivitis (73). To identify microbial changes associated with the development of puberty gingivitis, the composition of the crevicular microbiota was longitudinally monitored in 22 boys and 20 girls passing through puberty (39). The analysis showed that some microbiological changes, such as an increase in the number of *Capnocytophaga* spp., preceded the clinical onset of gingivitis, whereas others, such as an increase in the number of *Prevotella* spp., appeared to be a consequence of ecological changes associated with increased bleeding (64).

The individual and local expression of signs of gingival inflammation also depend on host-derived factors modulating the inflammatory response to microbial colonization. This is reflected in an inconstant local and systemic expression of inflammatory mediators. The amounts of inflammatory myeloid-related protein, MRP8/14, also called calprotectin, and its subunits MRP8 and MRP14, were assessed in the gingival crevicular fluid from 15 healthy nonsmoking young adults during experimental gingivitis (84). The amounts of these proteins increased with plaque accumulation in one-half of the participants and decreased in the other half. The levels recorded 11 days before the trial could predict the inflammatory rections observed after 10 days of plaque accumulation to a large extent. A distinct topographic pattern of calprotectin expression emerged, with a significant tendency for higher levels in more posterior locations.

In addition, environmental factors influence inflammatory responses to microbial colonization. The amounts of interleukin-1beta, interleukin-4 and interleukin-8 were assessed in gingival crevicular fluid from smokers and nonsmokers with experimental gingivitis (32). Although no differences were noted with regard to plaque accumulation at day 10, the clinical signs of gingivitis (gingival index and bleeding on probing) were significantly less pronounced in smokers than in nonsmokers. Throughout the experiment, nonsmokers showed higher total amounts of interleukin-4 but lower amounts of interleukin-8 than smokers. The total amounts of interleukin-1beta and interleukin-8 increased significantly during plaque accumulation in both groups. The amount of interleukin-4 was unchanged in the smoker group but decreased in the nonsmoker group.

In conclusion, accumulation of bacterial deposits at the gingival crevice induces inflammation of the gingiva. The considerable intra- and inter-individual variation in gingival inflammation is viewed as a consequence of dissimilarities in the quantity and composition of these deposits, differences in systemic and local inflammatory responses, and environmental factors.

Microbial colonization and periodontal pocket formation

The periodontal and microbiological status of the participants of the previously mentioned puberty gingivitis study (39, 64) was reassessed 6 years after puberty (68). Individuals with a marked and sustained increase in mean papillary bleeding scores during puberty differed 6 years later from individuals without pronounced puberty gingivitis in the following aspects: a significantly higher gingival bleeding tendency; an increased number of sites with more than 3 mm of attachment loss; and the presence of spirochetes in subgingival samples. Individuals without pronounced puberty gingivitis had very low anaerobic cultivable counts 6 years later.

The postulate that subgingival microbial aggregates cause periodontitis is an extrapolation of the finding that bacterial deposits at the gingival crevice cause gingivitis. As a result of gingival swelling and attachment loss, pocket depth increases and an anaerobic subgingival microbiota concomitantly evolves. It is assumed that gingivitis converts to periodontitis when complex bacterial interactions overload local

host resistance (49, 50). As the development of a complex subgingival microbiota depends on ecological factors, local environmental changes that favor the growth of pathogens or trigger the expression of virulence factors (82) may be viewed as the underlying cause for periodontal tissue destruction. Cases of refractory peri-implant infections caused by the persistence of excess luting cement (70, 117) illustrate how mixed anaerobic infections can be triggered and sustained by a foreign body (54, 62). Even though bacteria cause suppuration and loss of bone, antimicrobial agents alone cannot resolve these problems. On the other hand, studies have shown that suspected periodontal pathogens prevail in oral sites other than the periodontal pocket if the micro-environmental conditions are favorable. For example, in subjects free of periodontal disease, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* and other suspected periodontal pathogens were recovered from deep pericoronal sites of third molars under normal eruption (58). In another study, in patients treated for cleft lip, alveolus and palate (57), *Fusobacterium* spp., and *Prevotella* spp. were isolated in samples from residual clefts or pronounced soft-tissue grooves.

Over the last 15 years it has become clear that the overall diversity of the periodontitis-associated microbiota is very broad, with potential involvement of several hundred different species and subspecies (47, 78). These organisms may aggregate in various configurations, some of which have been associated with distinctive patterns of cytokine expression, as measured in gingival crevicular fluid. Subjects with aggressive periodontitis were characterized by a higher interleukin-1beta/interleukin-10 ratio than were periodontally healthy subjects, suggesting an imbalance between pro- and anti-inflammatory cytokines in aggressive periodontitis (102, 103). Only a few individual species show a unique association with disease. *A. actinomycetemcomitans* and *Porphyromonas gingivalis* have been suspected to be of particular importance in the disease process as a result of their pathogenic potential demonstrated in animal models and an association with disease progression and clinical response to therapy, as found in prospective and retrospective clinical trials (7, 11, 13, 17, 37, 40, 41, 85, 115). *A. actinomycetemcomitans* displays a broad genetic and phenotypic diversity and is heterogeneously distributed in various populations and cohorts worldwide (46). In an extensive prospective study (42), only one subpopulation of *A. actinomycetemcomitans*, the 'JP2 clone' (105), showed a degree of association that one would expect from a

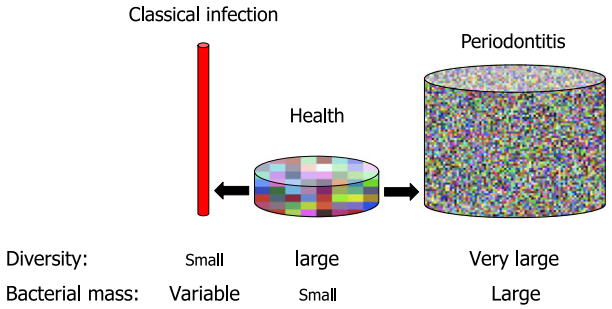

Fig. 1. In classic bacterial infections the diversity of the microbiota decreases as the disease develops. In most cases of periodontitis, however, the diversity of the flora increases as the disease progresses.

true pathogen. Although most bacteria are thought to harm tissues only if present in high numbers over prolonged periods of time, at relatively low numbers organisms such as strain JP2 may cause damage in susceptible individuals.

In classic bacterial infections the diversity of the microbiota actually decreases as the disease develops, and therefore the likely causative agent is easily recognizable, for instance *Staphylococcus aureus* or *Pseudomonas aeruginosa* in a purulent infection. In most cases of periodontitis, however, the diversity of the flora increases as the disease develops (Fig. 1). In an attempt to understand the microbiological events occurring in noma (cancrum oris), an extremely aggressive gangrenous disease affecting the maxillofacial region of young children in developing countries, especially in Western Africa, we characterized the gingival flora of lesions with acute noma and acute necrotizing gingivitis and compared them with healthy gingiva of control subjects with the same geographical and social backgrounds using phylogenetic low-density microarrays targeting the 16S rRNA gene (44). Compared with healthy controls, lower bacterial diversity was found in acute necrotizing gingivitis and even lower bacterial diversity was recorded in noma samples. Organisms typically associated with periodontal disease – *A. actinomycetemcomitans*, *Capnocytophaga* spp., *Porphyromonas* spp. and Fusobacteriales – were more abundant in healthy controls. The overall loss of bacterial diversity observed in noma samples, as well as its homology to that of acute necrotizing gingivitis microbiota, supports the hypothesis that acute necrotizing gingivitis might be the immediate step preceding noma. Using the same technology, another group determined the microbial diversity in saliva from HIV-seronegative and HIV-seropositive subjects, and in the latter before and 6 months after highly active antiretroviral therapy (HAART). The prevalence of several species of microorganisms that have regularly been associated with

periodontal disease (98), such as *Fusobacterium*, *Campylobacter*, *Prevotella*, *Capnocytophaga*, *Selenomonas* and *Actinomyces*, actually increased after HAART, and seven genera, including *Capnocytophaga*, *Porphyromonas* and *Peptostreptococcaceae*, were detected only in HIV-negative samples (48). Taken together, recent findings support the hypothesis that the complex microbiota associated with common periodontitis is the result of a slow, continuous process, taking place in a habitat with favorable ecological conditions, whereas the microbiota in highly active lesions, or in subjects with severe medical conditions, evolves under ecologic pressure and thus has a low diversity. The periodontal pocket flora may be compared to a forest growing in a natural reserve, whereas the conditions in an acute necrotizing lesion may rather resemble those of a heavily used football field.

A polymicrobial infectious disease model for periodontitis has been proposed in which interactions between herpesviruses and bacteria are essential (93). Active herpesvirus infection induces local immune suppression, which may result in the up-growth of periodontopathic bacteria, leading to periodontal disease progression (95). A number of publications report findings in favor of the hypothesis that herpesviruses may be involved in the onset or exacerbation of periodontitis. They include association studies documenting the presence of human cytomegalovirus, Epstein–Barr virus and herpes simplex virus in gingival tissue, gingival crevicular fluid and subgingival plaque of periodontitis lesions (95), immunological research on virus pathogenicity (16, 53) and results suggesting a beneficial effect of intervention with antiviral drugs (100). It has been reasoned that mechanical debridement targeting subgingival bacterial aggregates also reduces subgingival herpesviruses (36) and that antiseptics such as povidone-iodine and sodium hypochlorite are effective against both bacteria and viruses (94). Like any other hypothesis on the etiology of periodontitis, concerns exist regarding viral sampling, viral detection methods and inferring causality from observational data (10).

Conclusion

In contrast to classic bacterial infections, in most cases of periodontitis the diversity of the microbiota increases as the disease develops. Most incriminating bacteria are thought to harm tissues only if present in high numbers over prolonged periods of time. One notable exception is the clone JP2 of *A. actinomycetemcomitans* that may be regarded as a true pathogen in susceptible individuals.

Interventions to limit the microbial occupation and their consequences

The importance of removing supragingival plaque for resolution of gingivitis is undisputed and no therapies of periodontal disease have shown continued efficacy without adjunctive supragingival plaque control. It is generally believed that supragingival plaque control alone has little effect on the subgingival microflora of deep periodontal pockets. Nevertheless, for moderately deep pockets (4–5 mm), which may represent a pathological state between gingivitis and marginal periodontitis, professional tooth cleaning, three times a week for 12 weeks, led to significant changes in the composition of the subgingival microbiota (55).

As mentioned in the Introduction, mechanical removal of calculus and biofilm from the tooth surface is a reasonably effective method for treating periodontal disease (Fig. 2). Adjunctive systemic antibiotics may further 'eliminate or markedly suppress specific microorganisms with the potential of causing breakdown of periodontal attachment in susceptible patients' (107). As a comprehensive review of all possible systemic antimicrobial regimes goes beyond the scope of this paper, in the following we will focus on the most important protocols and recent advances. The clinical effectiveness of antimicrobials, especially of the combined administration of amoxicillin and metronidazole, is well documented (88, 90). As a result of its proven capacity to suppress *A. actinomycetemcomitans* (5, 19, 28, 33, 69, 79, 80, 108, 109), this combination has been recommended specifically for the treatment of advanced/aggressive

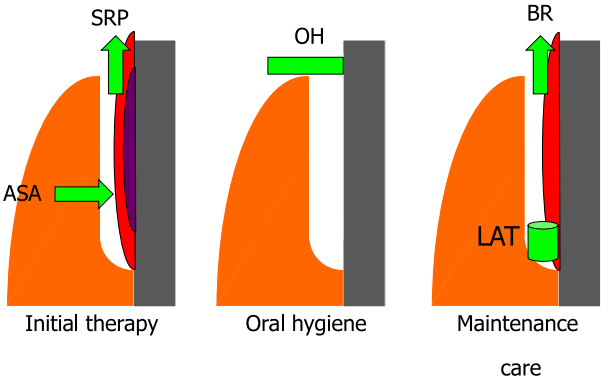

Fig. 2. Clinical significance of microbial pocket colonization for initial cause-related periodontal treatment (left), immediately thereafter (middle) and during maintenance (right). ASA, adjunctive systemic antibiotics; BR, biofilm removal; OH, oral hygiene to interfere with recolonization; LAT: local antimicrobial treatment; SRP, scaling and root planing to remove calculus (purple) and biofilm (red).

A. actinomycetemcomitans-associated periodontitis (112). However, it has not been proven that the selective suppression of any single member of the pocket microbiota is the key to success of periodontal therapy. We tested the claimed specific benefit of amoxicillin plus metronidazole in *A. actinomycetemcomitans*-positive patients in a specially designed double-blind, placebo-controlled, randomized longitudinal study that included 41 *A. actinomycetemcomitans*-positive and 41 *A. actinomycetemcomitans*-negative participants with moderate-to-advanced periodontitis. With respect to the persistence of pockets that are considered to be in need of further therapy according to common practice (i.e. still deeper than 4 mm and demonstrating bleeding upon probing at re-evaluation), there was no differential outcome for patients testing positive or negative for *A. actinomycetemcomitans* before treatment (61). Patients, irrespective of gender, age or smoking status, benefited from the antibiotics. The only differential advantage of the antibiotics could be identified regarding tooth type, as molars benefited more from the drugs than did nonmolar teeth. From studies with comparable designs in subjects with chronic or aggressive periodontitis (14, 38) it is furthermore clear that there is a beneficial effect of antibiotics in both of these classes of periodontal disease.

Given the large diversity of microbiota associated with all forms of periodontitis, and the complex interactions among members of the flora, a therapeutic concept targeting a single species of bacterium as responsible for periodontitis now looks rather simplistic. Amoxicillin and metronidazole may very well have effects beyond suppressing the selected bacterial species and 'complexes' (99) that were monitored in previous clinical trials by bacterial culture, DNA–DNA hybridization or PCR. The often striking and almost immediate clinical improvements, especially the disappearance of suppuration in previously 'refractory' lesions, suggest that there may be beneficial action beyond that of killing bacteria. There is currently no solid evidence from adequately designed clinical trials to demonstrate that in certain specific, microbiologically distinct forms of periodontitis, treatment with a systemic antibiotic regime other than amoxicillin and metronidazole is superior. This is hard to accept by some (83, 111) and also questions the utility of routine microbiological testing, as offered by commercial laboratories for standard periodontal microorganisms. Future diagnostic protocols should perhaps focus on the unexpected, rather than the expected, attributes of the subgingival microbiome (24, 92, 113).

Oral administration is the most common form of application for antibiotics. If the drug target is present in a clinically demarcated area, as might be the case in a localized periodontal pocket, direct local delivery is an alternative. Local therapy may permit the application of an antimicrobial agent at a concentration that cannot reasonably be achieved through the systemic route and may allow the use of substances (i.e. antiseptics) that would be noxious in other body areas. A few studies have addressed the differential benefits of local or systemic delivery routes. In one investigation, carried out in patients with rapidly progressing periodontitis (6), no significant differences were noted between scaling and root planing supplemented with either systemic amoxicillin/clavulanic acid or application of tetracycline in a local delivery device. In another trial of the same group (45) scaling and root planing plus adjunctive systemic amoxicillin and metronidazole was more efficacious than scaling and root planing plus adjunctive placement of chlorhexidine chips. For patients with adult periodontitis, two studies reported better results of scaling and root planing supplemented with locally applied metronidazole than with adjunctive systemic metronidazole (76, 77). In patients with aggressive periodontitis, the systemic administration of amoxicillin and metronidazole resulted in significantly better results than treatment with antimicrobial photodynamic therapy (2).

Treatment outcomes of scaling and root planing in combination with systemic antibiotics, local antibiotic therapy and/or periodontal surgery were compared over 24 months in a randomized clinical trial (35). Probing depths were reduced by antibiotics (probing-depth reduction = 0.5 mm) and surgery (probing-depth reduction = 0.4 mm). Clinical attachment gain and probing-depth reduction reached a plateau at month 6 that was maintained at 24 months in all groups. Systemic amoxicillin and metronidazole enhanced 24-month attachment level gains by 0.5 mm.

Conclusion

Interventions to limit the microbial occupation of the periodontal pocket using mechanical means are effective. The clinical benefit of adjunctive antibiotics, especially the combination of amoxicillin and metronidazole, is established. Some exponents advocate rationing these drugs for patients with specific microbial profiles (111). However, the evidence for an advantage of bacteriology-assisted clinical protocols is unsatisfactory.

Microbial recolonization after therapy

Repeated microbiological sampling in treated sites has shown that, over time, a microbiota similar to that present before therapy may re-emerge. Studies mapping the oral distribution of bacteria demonstrate that, in some patients, periodontal bacteria can be distributed throughout the whole mouth (63, 66), including even nondental sites, such as crypts at the dorsum of the tongue or the tonsils (72, 74, 80, 110, 118). These areas may be a source for recontamination of treated sites after periodontal therapy.

In a classical study, microbial samples were repeatedly taken from pockets following treatment with scaling and root planing. Their content was examined by dark-field microscopy (52). If there was no proper oral hygiene after treatment, a subgingival microbiota containing large numbers of spirochetes and motile rods was re-established within 4–8 weeks. In patients rinsing twice daily with a 0.2% solution of chlorhexidine and being seen once every 2 weeks for professional tooth cleaning, a sustained, pronounced reduction in the motile segment of the subgingival microbiota was achieved. In another study (87), the microbial composition, 7 days after a single session of scaling and root planing, was similar to that of periodontally healthy sites. Determined by cultural and dark-field data, differences became apparent at the 21-day sampling point. At 60 days, there was no significant variation in any of the parameters from pretreatment levels. In a more recent study (30), the composition of the subgingival microbiota was monitored in smokers who received scaling and root planing and smoking-cessation counseling. Microbial profiles, determined by terminal restriction fragment length polymorphism, differed significantly between smokers and quitters at 6 and 12 months following smoking cessation. The microbial community in smokers was similar to that at baseline, whereas that of quitters demonstrated significantly divergent profiles.

In patients with multiple deep periodontal lesions, the response to treatment with local antibiotic (subgingival placement of polymeric fibers containing tetracycline for 10 days) depended on the clinical and microbiological conditions of the other teeth in the same mouth (65). In one group of participants, only two lesions were treated with the local delivery device – the rest of the dentition was left untreated. In the other participants, all lesions were treated, the whole dentition was subject to full-mouth scaling and root planing and the patients rinsed with 0.2% chlorhexidine. Although there was a significant reduction in mean pocket depth in all treated sites after 2 and 4 months, the effect was significantly greater in the patients in whom the full dentition was treated. In patients in whom the rest of the dentition had been left untreated there was a tendency for relapse.

The microbiological reaction to scaling and root planing with adjunctive systemic antibiotics was monitored in a number of studies. It was demonstrated, using real-time PCR, that complete eradication of putative periodontal pathogens – *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *P. gingivalis*, *P. intermedia*, *Treponema denticola* and *Tannerella forsythia* – could not be accomplished. In one trial (15), *A. actinomycetemcomitans* was no longer detected in any patient after therapy with scaling and root planing plus systemic amoxicillin and metronidazole. However, despite excellent clinical results, the remainder of these organisms could still be detected in a majority of samples. In another study the subgingival presence of the same microorganisms was monitored over 2 years following scaling and root planing plus amoxicillin and metronidazole (22). Again, with the exception of *A. actinomycetemcomitans*, detection frequencies increased over time. In a trial with an observation period of 1 year (97) reductions in periodontal bacteria achieved with adjunctive antibiotics were retained, whereas after scaling and root planing alone the counts of several species increased.

The intraoral distribution patterns of *P. gingivalis* and *A. actinomycetemcomitans* were recorded in 17 patients with periodontitis after conventional mechanical periodontal therapy (scaling and root planing without antibiotics, followed by flap surgery in areas with persisting pockets greater than 5 mm) by sampling the mesial and distal aspects of every tooth (69). All *A. actinomycetemcomitans*- or *P. gingivalis*-positive teeth were treated with tetracycline fibers. Subgingival microbial samples were again taken 1 month after fiber removal. Eighty-nine per cent of the sites initially positive for *P. gingivalis* were now negative, but 16 sites previously negative for *P. gingivalis* tested positive. Seventy-seven per cent of the sites initially positive for *A. actinomycetemcomitans* were negative, but five previously negative sites tested positive. All *P. gingivalis*- or *A. actinomycetemcomitans*-positive teeth were again treated with the tetracycline fibers. One month later, five subjects still showed culture evidence of *P. gingivalis* at a total of 19 sites, and four subjects were positive for *A. actinomycetemcomitans* in a total of 27 sites. These nine patients were finally

treated with systemic amoxicillin and metronidazole. Despite all efforts, *P. gingivalis* was again detected, 3 months later, in isolated sites in three subjects, and *A. actinomycetemcomitans* could be cultivated from one single site. It was concluded that periodontal therapy with tetracycline fibers guided by microbiological diagnosis effectively reduced *P. gingivalis* and *A. actinomycetemcomitans* locally but was unable to eradicate the target organisms completely. Additional systemic antibiotic therapy further reduced the prevalence of *P. gingivalis* and *A. actinomycetemcomitans*. The observed persistence patterns suggest that re-emergence of *A. actinomycetemcomitans* was caused by recolonization, whereas the strikingly reproducible local re-emergence of *P. gingivalis* in some sites indicated failed eradication.

The microbiological and clinical effects of a varnish containing 1% chlorhexidine and 1% thymol, applied to periodontally diseased teeth after scaling and root planing, were studied over 12 weeks (21). The plaque index increased significantly at sites treated with the placebo varnish; however, no similar trend for an increase was seen at test sites. At a microbiological level, no relevant differences could be detected between placebo and test sites during the follow-up period.

The value of microbiological tests on the pocket microbiota after therapy to predict future stability during maintenance is ambiguous. Some studies suggest that the presence of putative pathogens, such as *P. gingivalis*, in plaque after treatment might be indicative of progressive alveolar bone loss (13). A longitudinal study showed a limited potential of microbiological tests, performed after nonsurgical therapy, to predict the clinical outcome 6 months later, but confirmed the importance of good oral hygiene before nonsurgical therapy: in patients still showing multiple sites with visible plaque after the hygiene phase there was an increased tendency for bleeding on probing 6 months after scaling and root planing (8). In another study aiming to identify sites at risk for future progression during 2 years of periodontal maintenance, microbiological parameters reflecting bacterial load proved to be of more value than the presence or absence of individual marker organisms (12).

Conclusion

Treated sites are subject to recolonization with a microbiota similar to that present before therapy. The degree and speed of recolonization depends on the treatment protocol, the distribution patterns of periodontal microorganisms elsewhere in the oral cavity and the quality of the patient's oral hygiene.

Interventions to interfere with microbial colonization of residual pockets

Despite the proven efficiency of subgingival biofilm removal, with or without adjunctive antimicrobials, deep periodontal pockets may not revert rapidly and fully to a sulcus with physiological pocket depth in all instances. As a consequence, regular debridement by professional intervention is necessary to prevent recurrence of disease (Fig. 2). Repeated instrumentation with steel instruments has unwanted effects that may cumulate over time; these effects include gingival recession and loss of tooth substance (29, 119). As subgingival bacterial deposits may not mineralize between two maintenance visits to form hard and firmly attached calculus, methods less aggressive than scaling and root planing may be more appropriate in this situation. In fact, with a specially designed nozzle that can be introduced into a periodontal pocket, it is possible to remove subgingival nonmineralized bacterial deposits with a jet of compressed air containing a lightly abrasive powder. In an initial study, treatment of the first 50 patients with residual pockets using glycine powder showed that 'subgingival air-polishing' appeared to be safe and was well accepted by those treated (56). A follow-up study of 2 months' duration in 20 recall patients confirmed these short-term observations and revealed no relevant differences in clinical or microbiological outcomes in comparison with ultrasonic debridement (114). A subsequent study (27) demonstrated beneficial shifts in the composition of the subgingival microbiota over 3 months in moderate-to-deep periodontal pockets. A randomized clinical trial of 12 months, with a two-arm, within-subject parallel design, evaluated repeated subgingival air-polishing in residual pockets with a new erythritol powder containing 0.3% chlorhexidine (75). In this trial, 50 patients were monitored at 3-month intervals. At months 0, 3, 6 and 9, sites presenting with a pocket depth of > 4 mm were subject to subgingival air-polishing (test) or ultrasonic debridement (control). Subgingival air-polishing and ultrasonic debridement reduced the pocket depth of a similar number of pockets > 4 mm, but subgingival air-polishing induced less pain. The frequencies of six microorganisms were recorded at baseline and month 12; there were no significant differences

between time points for each microorganism at the cut-off points of > 1,000 and > 100,000 cells/ml. At month 12, test sites were less frequently positive for *A. actinomycetemcomitans* at > 1,000 cells/ml compared with controls, and bacterial counts in test sites never exceeded 100,000 cells/ml.

Antimicrobial photodynamic therapy may be another beneficial adjunct to mechanical debridement of residual pockets. It is based on the principle that a photoactive substance brought into the pocket can be activated by a light of suitable wavelength to produce free oxygen radicals, which react with bacteria and their products (116). Three systematic reviews have tried to evaluate the benefit of photodynamic therapy on periodontitis, either as single treatment or as an adjunct to mechanical debridement (3, 4, 89). The results were nondefinitive and in part contradictory regarding the clinical and microbiological outcomes. We compared thorough scaling and root planing with short ultrasonic mechanical debridement followed by antimicrobial photodynamic therapy to assess their effects in residual pockets in a clinical trial (9, 31). Pocket depth, bleeding on probing and gingival recession were assessed before treatment and for up to 6 months after treatment. The levels of 13 cytokines and nine acute-phase proteins in gingival crevicular fluid were analyzed using a bead-based multiplexing analysis system. Treatment with both methods led to significant clinical improvements and induced sustained changes in several cytokines and acute-phase proteins. No significant differences were observed between treatment modalities.

A further study by our group (71) assessed the clinical, microbiological and local biological effects of antimicrobial photodynamic therapy, delivered either once or twice in a 1-week interval. After ultrasonic debridement, residual pockets (with pocket depth > 4 mm, clinical attachment loss ≥ 2 mm and bleeding upon probing) were randomly assigned to photodynamic therapy delivered twice within 1 week (group A), photodynamic therapy delivered only once (group B) or sham treatment without activating the laser (group C). Methylene blue was applied with a blunt irrigator tip into the pockets. Sites were irradiated with laser light at a wavelength of 670 nm using a light-diffusing tip introduced into the pocket. Pocket depth was significantly reduced in all groups. Single or double episodes of photodynamic therapy had some additional benefit over ultrasonic instrumentation alone. At month 6, none of the sites in group A had persisting pockets with pocket depth > 4 mm with bleeding upon probing, in contrast to two sites in group B and four sites in group C. Detection frequencies of the microorganisms studied at > 1,000 and > 100,000 cells/ml did not change significantly from baseline to months 3 or 6 in any group. A significant overall decrease was observed from baseline to month 6 for several inflammatory markers in gingival crevicular fluid, namely C-reactive protein, serum amyloid A, fibrinogen, procalcitonin and alpha-2 macroglobulin. When the groups were analyzed separately, the level of C-reactive protein was significantly lower only if the laser was activated twice.

Overall conclusions

In classic bacterial infections the diversity of the microbiota decreases as the disease develops; in most cases of periodontitis, however, the diversity of the flora increases. Given the large diversity and the complex interactions among the members of the microbiota, a therapeutic concept that targets one responsible bacterial species or strain with a highly specific agent appears to be an unrealistic approach. In fact, little evidence supports microbiological testing as an approach to obtain better clinical outcomes (26, 59, 61, 69). At present there exists no protocol with proven superiority, in terms of efficiency (14, 61, 86, 91) or effectiveness (43) over scaling and root planing plus systemic amoxicillin and metronidazole, for the therapy of any form of periodontal disease. Nevertheless, to limit the use and potential overuse of antibiotics, the search for alternatives must continue, and further efforts must be made to find optimal treatment protocols for all possible clinical conditions. Routine prescription of antibiotics for mild-to-moderate periodontitis is not recommended as these conditions in general respond sufficiently well to scaling and root planing alone. Clinical research should investigate novel procedures that are efficient in removing bacteria without inducing trauma, or other harm, even after repeated use in residual pockets.

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