## Current Microbiological Diagnostic Strategies in Periodontitis and Future Trends

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In the treatment of periodontitis, the value of microbiological testing is closely linked to the value of a specific, adjunctive antimicrobial therapy. Surprisingly, there is only little evidence that knowledge of a patient’s oral microbial colonisation results in improved clinical outcomes. Therefore, it has to be proved by further clinical studies that routine microbial testing is really justified. Besides, technical improvements of in vitro diagnostics are desperately needed to prove the benefit of routine microbiology testing convincingly.

**Key words:** periodontal microbiological testing, periodontitis, microbiology, antibiotic therapy

## INTRODUCTION

There is no doubt any more that bacteria play a specific and important role in the pathogenesis of periodontitis. Various oral bacterial species form in sequential order subgingival biofilms, communicate with each other, and cause host inflammatory responses resulting in the destruction of periodontal tissues (Kolenbrander et al, 2002). The human subgingival plaque is colonized by at least 500 different species of which more than 50% are still uncultivable (Paster et al, 2001). Some species, especially *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Tannerella forsythia*, show characteristics of an exogenous infection and their persistence after mechanical periodontal debridement has been associated with poor treatment response and ongoing disease activity (van Winkelhoff and Winkel, 2005). In the past decade, knowledge in oral microbiology has significantly increased; however, the role of microbes in periodontitis is still only partially understood. Therefore, it can be assumed that there will be a great deal of research activity in this field also in the future. Currently, the value of oral microbiological diagnostic testing as a routine is not yet clear. Microbiological testing is not necessary and actually does not help to diagnose periodontitis. The progression of periodontitis is not only determined by chronic polybacterial disease activity but in addition by various other endo- and exogenous factors (for example, smoking behaviour, diabetes mellitus, etc.) of which some still are only poorly understood (for example, host genetics). Therefore, it is still very difficult to make prognostic statements of general validity. Microbiological testing can be helpful in periodontal therapy. However, its value is closely linked to the value of adjunctive antimicrobial therapy in the treatment of periodontitis patients. Although two recent systematic reviews came to the conclusion that adjunctive antimicrobial therapy may provide a clinical benefit for certain periodontitis patients, it is also stated that further comprehensive long-term, controlled clinical studies measuring ‘true’ outcome parameters are needed for convincing evidence (Herrera et al, 2002;
Haffajee et al., 2003). All current recommendations regarding the selection of antibiotic regimes after microbial testing base on in vitro susceptibilities without detailed knowledge of in vivo pharmacokinetic and -dynamic data (Beikler et al., 2004). Finally, there is only weak evidence (Winkel et al., 2001; Ehmke et al. 2005) demonstrating that knowledge of a patient’s oral microbial colonisation and selection of an antibiotic regimen on this basis result in improved clinical outcomes compared to those where an antibiotic was selected empirically, especially under consideration of the limited choice of available antimicrobial agents (Loomer, 2004). For all of the above stated reasons, there currently is only little evidence that routine microbial testing is really justified. Arguments for microbial testing are mainly based on theoretical considerations and it can currently only be assumed that testing
- helps to select an optimal antibiotics regime and thus to achieve improved treatment outcomes,
- helps to exclude patients from systematic antimicrobial therapy who are unlikely to benefit,
- helps to reduce the over- and misuse of antibiotics (less antimicrobial resistance), and
- helps to contribute to a cost-effective treatment (van Winkelhoff and Winkel, 2005).

CURRENT STRATEGIES

Who should be tested? Only a minority of all periodontitis patients (about 10–20%) benefit from initial supportive antibiotic therapy. These are patients suffering from aggressive and severe chronic periodontitis. In addition, patients with ‘refractory periodontitis’ belong to a group that might profit from microbial testing and subsequent antibiotic treatment. The latter can only be diagnosed in the course of periodontal therapy. Furthermore, there are only few occasions, directly after treatment and during maintenance care, where microbial testing might be indicated (reliable data supporting this strategy are not yet available).

When should testing be done? There seems to be growing consensus within the community that systematic antibiotics should be administered once the initial mechanical treatment has been finished, the sub- and supragingival plaque level has been reduced, and the integrity of the biofilm has been destructed. If a diagnosis can already be established microbial testing should be initiated if indicated – before starting the initial treatment. Thus, the results will be available directly after finishing the initial mechanical debridement.

How and where should samples be taken?
There are two methods by which a patient’s subgingival plaque is primarily collected, i.e., either by removal using curets or by adsorption onto endodontic paper points. In both cases supragingival plaque has to be carefully removed at the sites to be sampled (Loomer, 2004). Both methods have their pros and cons. However, sampling might be somewhat more reproducible when using paper points. - Periodontitis is said to be a site-specific disease. This holds especially true for microbiological findings because the various bacteria are not at all equally distributed throughout the affected sites. Therefore, the greater the number of sampled sites, the more representative and valid the analysis, in general will be. As this strategy is not economically feasible, a practical and economical approach for sampling is to pool samples from the deepest pockets of teeth in each quadrant (Mombelli et al., 1991).

How should we test? A plethora of various microbiological test systems are available. Among these, culturing and molecular testing currently are of most widespread use. Mostly, only a few periodontopathogenic bacterial species are currently being looked for. For a long time culture techniques have been used for the detection of oral bacteria. As anaerobic and capnophilic cultivation is time consuming and staff-intensive, and dependent on viable and cultivable bacteria, cultivation has recently been replaced by nucleic-acid based systems – mostly by qualitative in vitro amplification techniques – in many laboratories for initial orientating testing. Currently, there is no molecular system testing for antibiotic resistance. After species identification by using these molecular techniques an antibiotic regimen is chosen on the basis of the empirical data only (rational antibiotic therapy). Therefore, culturing will keep its place in periodontal microbiological testing and serve as basis of evaluation for all new systems ‘gold standard’ and for patients with ‘refractory periodontitis’ after antibiotic treatment. An individual susceptibility testing should be performed for these patients after having obtained pure cultures (specific antibiotic therapy).
How can we use microbiological diagnostic test results for treatment decisions? Several antibiotics have predictable effects on target organisms and initial susceptibility testing therefore is unnecessary. For example, metronidazole is effective against P. gingivalis and most other gram-negative anaerobic rods, whereas a combination of metronidazole plus amoxicillin is needed when A. actinomycetemcomitans has been detected (Beikler et al., 2004; van Winkelhoff and Winkel, 2005). Usually, other antibiotics or combinations are only needed in case of individual contraindications (for example, hypersensitivity against amoxicillin).

FUTURE TRENDS

Because of quality control and assessment constraints for accredited laboratories it can be assumed that homebrew molecular tests that are currently in frequent use will be replaced by commercial in vitro diagnostics (IVD). Several of such IVDs have recently become available (Eick and Pfister, 2002; Vianna et al., 2005). Furthermore, there is no reason to expect that the number of pathogens among the uncultivable oral bacteria is lower compared to the cultivable. Much input regarding this issue can be anticipated from the recently launched and by the National Institute of Dental and Craniofacial Research (NIDCR) funded oral metagenome project that is led by The Institute for Genomic Research (TIGR) and Stanford University. In the future, we will therefore most probably witness an extension of the panel of marker species. From the technical point of view, a microarray is the best platform to achieve such a broadening of tested bacterial species (Vianna et al., 2005). As knowledge will also increase by genomic, proteomic, and functional metagenomic projects, the next generation of IVDs will probably also test for certain and specific bacterial pathogenicity and resistance traits. Elimination and re-colonization trials as well as screenings for horizontal and vertical transmission of periodontal pathogens among family members will benefit from highly reproducible and discriminatory DNA sequence-based bacterial typing methods (Koehler et al., 2003; Mellmann et al., 2006). Finally, because the merely qualitative detection of putative periodontopathogens may not be sufficient as a prognostic or therapeutic marker – some of these bacteria may even be present in healthy people, albeit in low numbers – it has to be expected that future molecular microbial test systems will allow access to subgingival plaque samples by qualitative means. Real-time polymerase chain reaction (PCR) is probably best suited to achieve this goal (Boutaga et al., 2003; Jervoe-Storm et al., 2005).

CONCLUSIONS

Microbiological examinations of subgingival biofilms will continue to be a very active field in periodontal basic research. Currently, there is only little evidence that routine microbiological testing in periodontitis patients is really justified. Arguments for testing are mainly supported by theoretical considerations. If at all, only a small fraction of all periodontitis patients benefits from testing. There is a tendency to replace laborious classical culturing techniques by in vitro amplification-based systems for initial testing. For reasons of quality control homebrew molecular systems will more and more often be exchanged for commercially available IVDs. Comprehensive, long-term, controlled clinical trials measuring true outcomes instead of surrogate parameters and microbiological monitor findings will be needed to allow a definite evaluation of microbial testing in future.

REFERENCES


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