DNA Probes and Primers in Dental Practice

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In clinical microbiology, molecular genetic techniques are increasingly being used to detect and/or differentiate uncultivable, anaerobic, or fastidious microorganisms. During the past decade, DNA probe hybridization and in vitro amplification by polymerase chain reaction have also been introduced to detect oral pathogens. The present review describes experiences with methods and commercial test systems for the detection of pathogens in periodontitis and caries.

Molecular microbiology has revolutionized modern medicine. Especially in medical microbiology, a broad spectrum of techniques are used to detect and/or differentiate uncultivable, anaerobic, or fastidious microorganisms. The 2 basic methods are (1) hybridization that uses either genomic or oligonucleotide probes and (2) PCR, by which repeated cycles of oligonucleotide (primer)–directed DNA synthesis of target “signature sequences” are carried out in vitro.

Hybridization involves the denaturation of double-stranded DNA into single strands and detection of single-stranded DNA (ssDNA) with a labeled, complementary ssDNA probe. For research, probe hybridization can be used to demonstrate genetic relatedness in the DNA (e.g., ribosomal genes) of different organisms to construct phylogenetic taxonomic schemes. In the diagnostic laboratory, DNA probes are being used for detection of uncultivable or fastidious organisms directly in clinical specimen or for culture confirmation. Commercially available probe tests include a number of bacterial pathogens, such as Campylobacter species, Chlamydia trachomatis, Enterococcus species, Gardnerella vaginalis, Haemophilus influenzae, Legionella pneumophila, Listeria monocytogenes, Mycobacterium species, Neisseria gonorrhoeae, Streptococcus agalactiae, and Streptococcus pyogenes, and pathogenic fungi, protozoa, and some viruses. Direct probe methods are much less sensitive (limit, 10^3–10^6 cells) when used without DNA amplification procedures because the number of target cells may fall below the sensitivity of the assay.

However, by testing cell-rich oral specimens (e.g., samples from mixed anaerobic infected periodontal pockets or plaque of initial carious lesions), this threshold is met and probes can be used for most cases directly, without preamplification. Thus, additional targets for DNA probe detection can be found in the dental practice. They are the periodontopathogens Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, and caries agents—namely, Streptococcus mutans–group species. In fact, because of the high prevalence of periodontal diseases and caries, oral infections are a main indication for DNA probe application [1–5].

PCR is a highly sensitive technique by which minute quantities of specific DNA (or RNA after reverse transcription) can be enzymatically amplified. The technique can be used to detect very small amounts of bacterial, fungal, or viral nucleic acid in clinical specimens. PCR and other amplification techniques (especially nested and multiplex variants, transcription-based amplification, strand displacement amplification, and ligase-chain reaction) have a high variety of applications in the clinical laboratory. PCR is used for direct detection or identification of microorganisms,
Toxoplasma gondii, as well as fungi (Enterocytozoon bieneusi, Encephalitozoon hellem, Plasmodium spp., Toxoplasma gondii, and Trypanosoma cruzi), and almost every virus known to be pathogenic in humans.

But again, oral microbiology is a very important field for PCR application. PCR-based tests are for research and are commercially available to detect the periodontopathogens as well as cariogenic agents [6–9]; PCR is referred to as the new diagnostic standard for these common infections. The following paragraphs describe some test systems available in the United States and/or in Europe, especially those in daily use.

APPLICATION OF DNA PROBES AND PRIMERS IN PERIODONTAL DISEASES

Periodontal diseases (gingivitis and periodontitis) are chronic mixed anaerobic infections with a remarkably high prevalence and morbidity. Whereas gingivitis, with some exceptions, is a reversible polymicrobial infection with no single associated bacterial agent, periodontitis is moderately to rapidly progressive and is associated with facultative or obligate anaerobic pathogens. The species A. actinomycetemcomitans, B. forsythus, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, P. gingivalis, P. intermedia, and several forms of uncultivable spirochetes play major roles in the pathogenesis [10–12]. Moderate forms of periodontitis are demonstrated by 50%–98% of adults, with regional and age-based differences. Between 3% and 13% of the population may develop aggressive forms of periodontitis, such as rapidly progressive, juvenile, or refractory periodontitis. These individuals require prosthetic treatment within a short period of time. Periodontal pathogens not only destroy the integrity of the dentition or of oral mucous membranes but can frequently be detected in the blood cultures of patients, causing not only bacteremia but also, under some circumstances, septicemia, organ abscesses, endocarditis, or cardiovascular disorders [13–17].

For diagnosis of the activity of the different forms of periodontitis, clinical symptoms alone may not be sufficient, because they provide a historical record only (pocket formation, attachment loss, and alveolar bone loss) or have low predictive value (bleeding during probing). But predictions of recurrence of disease and prognosis for the patient can be significantly improved when the presence or absence of periodontal pathogens is monitored as well [12].

To monitor bacterial changes or shifts in the gingival sulcus or the periodontal pocket, several molecular genetic test systems have been introduced within the past decade, and millions of samples (e.g., subgingival plaque) have been analyzed with DNA probes and primers on a routine basis. In the next section, some experiences from both the dentists and the laboratories involved in these test procedures will be summarized.

Two different molecular genetic strategies have been introduced for the detection of periodontal pathogens in subgingival plaque, based on (1) genomic or oligonucleotide DNA probes or (2) PCR. Murray and French [18] were the first investigators who could detect P. intermedia and P. gingivalis with the aid of purified DNA fragments labeled with 32P or biotin–11-dUTP by nick translation. The sensitivity of these genomic probes was increased, requiring only 10^2–10^3 bacteria. However, the same procedure was found to be very difficult for the major periodontal pathogen A. actinomycetemcomitans (A.a); thus, the authors had to clone specific fragments of the genome into plasmids, and these recombinant DNA probes were then used further for diagnosis. With this procedure, cross-hybridization between the A.a. probe and other species of the genus Pasteurella were reduced to the minimum of 1%. Other pathogens were added into this system, including T. denticola (genomic probe) and F. nucleatum, E. corrodens, C. rectus, and B. forsythus (recombinant DNA probes). A similar testing system was marketed by the US company OmniGene Laboratory Services and is frequently used in some European countries (under the name DMDx/PATHOTEK). The samples are centrally analyzed at ANAWA Laboratories. In the year 2000, ~16,000 of these tests were performed in Europe, with 14,000 tests sent from German dental practices, 600 from Switzerland, and the remaining tests from practices in the rest of Europe.

In contrast to genomic probes, oligonucleotides are synthetically produced, short, stable molecules and can easily be introduced in automated systems, the future of diagnostics.

Chuba et al. [19] were the first to establish oligonucleotide probes directed against species-specific sequences of the 16S rRNA to detect the periodontopathogens P. gingivalis, P. intermedia, and A. actinomycetemcomitans. By way of background, the 16S rRNA is a part of the small subunit of bacterial ribosomes. This amazing molecule consists of both highly conserved regions, appropriate as primer targets for amplification, and species-specific sequences, which are of major interest to molecular taxonomists and, so far, an ideal target for deducing specific oligonucleotide-probes able to distinguish between very closely related species, as, for example, P. intermedia and P. nigrescens are (figure 1). When pure cultures are used, the specificity of oligonucleotides can be noted as 100%, but this might be reduced in detecting bacteria in complex samples such as subgingival plaque. The sensitivity of oligonucleotide probes depends on the labeling system but can be maximized to detect...
Figure 1. Representative dot-blot hybridization for the detection of Prevotella intermedia and Prevotella nigrescens. Lane A, ATCC 25611 (10^5, 10^4, and 10^3 cells); lane B, ATCC 33563 (10^5, 10^4, and 10^3 cells); lane C, L1440, LR22, L583, and LR15; lane D, LR78, LR53, and PK71; lane E, PK35, L621, LR100, LR119, and PK65; and lane F, PK80, L610, LR20, and ACJ5. The cell concentrations in lanes C–F are between 10^3 (strain L583) and 10^6 (strain LR119). In blot A, the Pi-probe was used, and in blot B, the Pn-probe was used. No cross-reactions appeared when 65°C was used as the hybridization temperature.

10^2–10^3 cells per analysis. In the important publication by Dix et al. [20], highly species-specific and 16S rRNA–directed DNA probes of 24 bp length and ^32P labeled were used for A. actinomycetemcomitans, B. forsythus, P. gingivalis, P. intermedia, E. corrodens, F. nucleatum, and C. rectus. The authors found that the sensitivity and specificity of the probes was not reduced when subgingival plaque was used for the samples, but this might be true only under ideal conditions, which could be problematic for routine testing. Meanwhile, oligonucleotide probe–based test systems for periodontal pathogens are available in different formats on the European market, including the IAI Pado Test 4.5 system (Institute for Applied Immunology), which is available in some European countries, Switzerland, Germany, and The Netherlands. Whereas the IAI-Test system uses radioactively labeled DNA probes, the new LCL system (LCL Biokem) uses chemiluminescence-generating oligonucleotide probes. Both companies perform ~10,000 tests per year in Europe. In the case of the LCL system, it is known that the demand for such tests is rapidly growing among practitioners.

To further enhance specificity, PCR and combinatory molecular genetic techniques were also introduced for the routine diagnosis of periodontal pathogens. The extended sensitivity can be used to demonstrate P. intermedia or B. forsythus in plaque and in oral mucous membranes (figure 2) [6].

After amplification of the 16S rRNA gene, specific DNA probes used in a reversed hybridization procedure are able to quantify the bacteria in the amplicon. This 2-step testing system may also be very sensitive, but it seems questionable whether the ratio of different bacteria, a result that is an important factor in reaching therapeutic decisions (e.g., the most appropriate antibiotic therapy), is still the same when obtained by in vitro amplification before hybridization. However, a testing system distributed by the company HAIN Diagnostic is frequently used in Germany. As an advantage, the samples are not analyzed only in a central laboratory—the entire test system is distributed to local medical laboratories, making specimen transport easier.

In the future, mini-molecular laboratories will be available for chair-side DNA probe testing in ≤1 h (Periodontal Microbial Identification Test; Saigene Corp.) [21]. However, the advice of a microbiologist might still sometimes be necessary to avoid problems that may occur in the diagnostic process. Some of these problems will be reviewed in the next section, below.
EXPERIENCES IN DENTAL PRACTICE

As was mentioned above, German dentists are confronted with various genetic test systems for periodontal pathogens. With this in mind, we performed a survey among practitioners. Among the ~50,000 dentists practicing in Germany, 3%–6% (only) specialize in the diagnosis and treatment of periodontitis and were thus chosen to report their experiences. The 100 most experienced practices (according to data from LCL Biokey) were contacted for a statement, and 32 responses were received. More details about this survey, especially representative answers and categories, have been published elsewhere [22].

In general, these dentists seemed to be very satisfied with the current test systems. However, the information I got from numerous lengthy discussions with dentists in a more private setting is somewhat different. The problems “under the surface” are interesting and are as follows. (1) Periodontal diseases are chronic infections and “healing” is almost impossible, even with the most sophisticated diagnostic and treatment strategies. The prolonged treatment is definitely very time consuming and costly (health insurance covers only a portion of the expenses) and can be frustrating for both dentist and patient. As a consequence, some practitioners might give up on these cases—unfortunately, sometimes too early. (2) Some practitioners, highly encouraged, use different test formats over time (sometimes for 1 patient) to select a favorite. To a certain extent, they get different results from the different tests. Some of the discrepancies are minor (e.g., different cell counts of an individual species) but still confusing, and others are major (different species detected because of different formats, e.g., some P. intermedia-directed tests do still include P. nigrescens, and others do or do not include T. denticola. As a further problem for comparison, the detection limits vary among the test systems, and some laboratories report real cell counts, whereas others report percentages of the total flora). More validation is needed for microbial test systems in periodontal diseases before one can improve the acceptance of these useful tests.

EXPERIENCES FROM LABORATORIES

In our survey, we also asked the managers of laboratories about their experiences with dentists (the 6 “major players” were contacted, with 4 responding). Most of the laboratories are very satisfied with the collaboration and are very optimistic about the future of their testing systems for periodontal pathogens. However, the present review article tries to concentrate on problems in the communication that can occur between dentists and microbiologists, some of which are as follows.

Some dentists are overenthusiastic at the beginning and send samples from almost every patient. Testing periodontal pathogens should, for several reasons, be concentrated on a small spectrum of diseases and patients. Patients for whom microbial testing is indicated include

1. patients with advanced attachment and bone loss before age 25 years (juvenile periodontitis or prepubertal periodontitis);
2. patients, usually aged 25–35 years, with rapid destruction of attachment and bone in a relatively short period of time (rapidly progressive periodontitis); and
3. patients who continue to lose attachment despite adequate treatment (refractory periodontitis).

To receive useful information, an appropriate mechanical treatment and an optimized oral hygiene regimen are essential preconditions for subsequent testing. Reducing the biofilm is necessary both for access to the important, base-located, subgingival plaque for testing and for supporting the diffusion of antimicrobial agents.

A fraction of the dentists seem to accept only “positive” results from the tests to support their (predetermined) antibiotic therapy. But even in a patient with serious clinical symptoms, the periodontal disease has nonactive periods, when microbiological testing is “truly negative.” Therefore, diagnosis needs both clinical and microbiological data [23–25].

A diagnostic result can only be as representative as the sample that was analyzed. This is true for medical microbiology in general but is especially true for periodontal diseases. According to Mombelli et al. [26], the deepest periodontal pocket with the highest tendency for bleeding must be chosen from each quadrant for sampling. The dentist must get an overview of the destructive processes in 1 session before sampling in a second session, to avoid provocation of bleeding. It is extremely important that sampling is not performed in a bleeding periodontal pocket because the paper points, the sampling device in most cases, can absorb only ~20 μL (1 drop) of fluid, and if this volume is mainly blood or saliva or supragingival plaque, not much is left for the pathogen-containing deep fluid. Several paper points should be used for sampling and either tested separately or “pooled” (more cost-efficient) [27].

To get the highest benefit from testing marker bacteria in periodontal diseases, the education of the staff involved must be improved. It is very important that clinical microbiologists learn more about oral and anaerobic microbiology, dentistry, and periodontology and that periodontologists keep in touch with microbiology, antibiotics, and the principles of modern diagnostic systems, especially their predictive values and shortcomings.

APPLICATION OF DNA PROBES AND PRIMERS IN CARIES PREVENTION

Dental caries is a slow decomposition of teeth that results from the loss of hydroxyapatite crystals. The mineralized matrix dis-
solution reduces the structural integrity of the teeth. The bacterial nature of this process may result in a chronic infection of the tooth and eventual loss of teeth and supporting alveolar bone. Current understanding supports the “plaque-host-substrate” theory, whereby caries has a bacterial etiology interdependent on (1) the host defense system (e.g., tooth brushing and buffer capacity of saliva), (2) dietary factors, and (3) time. Caries occurs when all these factors are operating together. Depending on the bacterial composition, plaque can be aggressive or not toward the enamel surface. The most consistently found bacterial organisms in (human) initial dental lesions are Streptococcus mutans–group species (S. mutans, S. sobrinus, S. cricetus, and S. rattus) [28–32]. They ferment sugars (especially sucrose) to lactic acid, and, in parallel, they polymerize insoluble extracellular dextran (called mutan), which allows bacteria to stick to the tooth surface. As a consequence, the tooth surface–located acid production derives the demineralization of enamel in vivo. A single enzyme, glucosyltransferase, is responsible for the first step in acid and dextran production by S. mutans [33, 34].

For more than a decade, culture strips have been available to monitor S. mutans cell count in saliva and plaque to predict the individual patients (especially children’s) risk for future demineralization [35]. However, this procedure is sometimes inaccurate, time-consuming, and laborious. DNA probes and PCR primers could probably overcome these limitations. Targets chosen for DNA probe hybridization and/or in vitro amplification are the genes encoding for glucosyltransferase (gtfB), fructosyltransferase (fft), surface proteins (wapA and spaP), dextranase (dex) [2, 36, 37], and specific regions of the 16S rRNA.

In addition, typing of S. mutans and S. sobrinus by arbitrarily primed PCR fingerprinting has been introduced [8]. Routine screening for S. mutans by DNA probe hybridization and glucosyltransferase-directed PCR in saliva and plaque is available for private practices (LCL Biokey).

Of course, the use of DNA probes and PCR reaction for risk assessment in caries is “high-end” medicine and might be reserved for countries with high standards in dentistry. However, because the costs for molecular techniques are constantly dropping, this could be a future standard worldwide in industrialized countries.

CONCLUSION

Microbiological testing that uses DNA probes and primers in advanced forms of periodontitis is a very promising tool to determine active disease and predict future attachment loss, ultimately improving treatment prognosis. In addition, molecular screening for S. mutans could help prevent caries, the most frequently found and cost-intensive infectious disease.

References

26. Mombelli A, McNabb H, Lang NP. Distribution patterns of black-