The Brown

PerioDontaLetter

PDL tm

I. Stephen Brown, D.D.S., *Periodontics & Implant Dentistry*

Summer

From Our Office to Yours...

Most forms of periodontal disease are primarily the result of a specific infection, usually by one or more anaerobic bacteria.

Today there are a variety of advanced microbiologic, biochemical, and genetic tests, available to identify the specific bacteria causing the infection. In certain difficult cases, these new tests represent important components of diagnosis and especially the selection of therapeutic regimen.

This current issue of ***The PerioDontaLetter*** *addresses the emerging periodontal microbiologic diagnostic tools which can provide information about the future progression or periodontitis, the identification of patients at risk and criteria for an individualized recall program.*

As always, we welcome your comments and suggestions as we continue to seek the most advanced diagnosis and care for our mutual patients.

Microbial Testing for Periodontal Diagnosis

Periodontal probing has generally been the standard for measuring periodontal disease. Probing, however, measures previous disease progression and cannot tell us if tissue infection is currently present. To know attachment loss has actually occurred requires a depth change greater than one millimeter.

Microbial tests which can assist our diagnosis and treatment of periodontal disease may be able to predict increased risk of attachment loss and can indicate which causative agents are present and which have been eliminated.

In a 2014 *Journal of Periodontology* commentary, Niklaus Lang pointed out that "**bacteria play a critical role in the etiology of periodontal disease."**

Jorgen Slots in a 2012 *Perio 2000* article stated that **"The underlying concept of the periodontal therapy proposed here is that periodontal disease is an infectious disease caused by specific pathogenic bacteria and herpes viruses, and that a treatment that identifies and markedly reduces or eradicates the pathogens is able to arrest progression of the disease."**

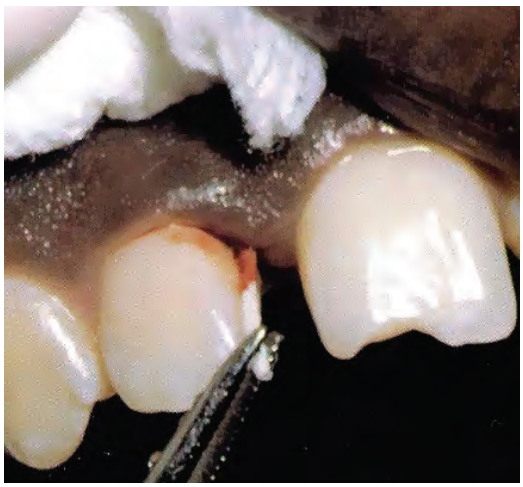

Figure 1. A paper point is placed as close to the base of the pocket as possible. The supragingival bacteria is first wiped away and the gingiva is isolated and dried. The paper point is then placed into the media.

I. Stephen Brown, D.D.S. *220 South 16th Street, Suite 300 • Philadelphia, PA 19102 • (215)* ***735-3660***

Columbo and Socransky reported in 2012 that 17 patients with refractory periodontitis and 32 patients who responded well to treatment were followed for 15 months and analyzed for the presence of 300 species. The persistence of distinct periodontal pathogens similar to those detected by culture, as well as a low prevalence of beneficial species, was associated with refractory periodontitis. They concluded **"a more intense regimen of professional mechanical and/or chemical plaque control and different antimicrobial combinations should be developed to reduce the pathogenic microorganisms, to increase host-compatible species, and achieve long-term clinical stability."**

Using a microbial test, Wennstrom showed that no clinically significant loss of attachment occurred during a one-year observation period in the absence of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and in patients with less than five percent of Prevotella intermedia. By contrast, in patients with one or more of these indicator bacteria, 20 percent of the sites exhibited clinical attachment loss of more than 2mm within one year.

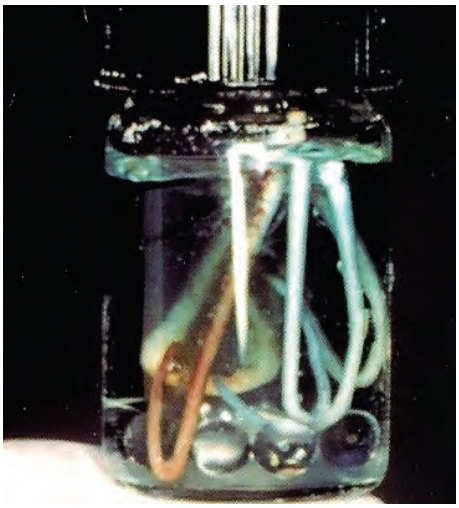

Figure 2. Paper points are placed in a special media that keeps oxygen away from the anaerobic bacteria that may be present. Most bacteria survive up to three days in this media for testing.

Discovering Bacteria with Pathogenic Potential

Since over 700 bacterial types have been identified in periodontal pockets, it is important to discover those bacteria which have the highest pathogenic potential.

In a position paper published by the American Academy of Periodontology, bacterial types were categorized by their association with periodontal disease. (See table at top of page 4.) Most of these organisms are gram negative anaerobic or faculative rods and vary considerably in their sensitivity to antibiotics.

A unique study by Dahlen concludes why these organisms are considered to have disease-producing potential. In a five-year study, Dahlen investigated the association between the recurrence of periodontal pathogens and recurrent attachment loss. Reappearance of A. actinomycetemcomitans, P. gingivalis and P. intermedia was observed in nine of 13 patients. In six of these nine patients, further periodontal attachment loss was observed. Patients without detectable subgingival A. actinomycetemcomitans, P. gingivalis, or P. intermedia during the maintenance period of five years remained clinically stable.

Eliminating Specific Pathogens

Since it has been shown that A. actinomycetemcomitans is difficult to eliminate by mechanical means alone, the practicality of antibiotics in the treatment of A. actinomycetemcomitans periodontitis may be inferred. In this respect, it is important to determine which bacteria are present and the need for adjunctive antibiotic therapy as indicated by specific bacterial sensitivity.

After microbial testing to identify periodontal pathogens, Dutch researchers Winkel and van Winkelhof demonstrated that therapy aimed at specific pathogens can eliminate them for three years. Using a combination of amoxicillin and metronidazole for seven days, they were able to eradicate A. actinomycetemcomitans for three years 95 per cent of the time and P. gingivalis 90 per cent of the time. These two pathogens are considered exogenous.

Predictably, they were not able to reduce P. intermedia long term. P. intermedia is indigenous to the oral cavity and becomes a pathogen only at higher concentrations.

Dr. Tom Rams reported in a 2014 *Journal of Periodontology* article that in 400 cultures of deep pockets, 74% showed resistance to one or more antibiotics. Strains of Prevotella, Fusobacterium and Streptococcus constellatus were most commonly resistant. Resistance was present to doxycycline 55 per cent of the time, amoxicillin 43 per cent of the time, metronidazole 30 per cent of the time and clindamycin 27 per cent of the time. Fifteen per cent of the cultures were entirely resistant to the combination of amoxicillin and metronidazole. He stated ***"this wide variability should concern clinicians empirically selecting antibiotic regimens."*** (See Laboratory Report on page 4.)

Antibiotics for Specific Conditions

A 2004 position paper by the American Academy of Periodontology states that since periodontal disease is an infectious disease, the use of antibiotics in some instances is not only desirable but warranted. In severe infections, it may include antimicrobial testing.

It is extremely important that clinicians not employ antibiotics indiscriminately, but reserve their use for specific conditions, medically-compromised patients and those patients who do not respond to non-surgical and/or surgical treatment.

Slots laid out the rationale for culturing and the use of antibiotics in 2004: pockets harbor many different pathogens with diverse susceptibility profiles. ***Eradication of many subgingival pathogens is not predictable by scaling or surgical therapy. Systemic delivery has the***

advantage of reaching deep pockets and furcations, into gingival tissue, and reaching other oral sites.

Feres et al in a January 2015 *Perio 2000* article further emphasized the need for systemic antibiotics. ***"A less well known factor is that even shallow pockets can be highly colonized with several periodontal pathogens, a very unfortunate finding since we currently treat mostly the deep pockets***."

They added, "The presence of periodontal pathogens on all the other oral surfaces has also been shown. ***These data suggest that not only deep pockets but also shallow pockets and all the other oral surfaces demand anti-infective treatment."***

A systematic review of the effectiveness of systemic antimicrobial therapy in combination with scaling and root planing was published in the March, 2015 *Journal of the American Dental Association* by Canas et al. They concluded that antibiotics may provide benefits especially in cases of aggressive periodontitis and in deep pockets greater than 6mm. They added that the clinician should make the decision on the basis of the specific needs of the patient.

Bacterial Assessment

Several types of bacterial assessment are currently available, each with advantages and disadvantages.

Culturing techniques in the dental office have been used to select an antibiotic of choice in those cases where clinical parameters have indicated antibiotics should be considered. Plaque samples are placed on culture plates (see figures below) to identify those groups of bacteria susceptible to the antibiotics. ***Commercially available culture and sensitivity reports for 16 major pathogens are available from laboratories at Temple University*** (See Laboratory Report on page 4) ***and the University of Southern California .***

DNA probe analysis uses DNA probes prepared by creating a strand of DNA from known bacteria. Sample bacteria are broken by hydrolysis and the DNA strands which match the known bacteria combine with them. The known bacteria sample has been radioisotope-labeled and thus can be counted with an auto-radiographic plate.

A DNA analysis of saliva (Oral DNA) for periodontal bacteria is now available for the 11 most common periodontal pathogens. DNA analysis of paper point samples is also available (microlDent, Hahn Diagnostics). These tests have the advantage of not requiring viable organisms and quicker turnaround times.

A third method is immunofluorescent assay, which involves reacting the bacterial sample with known antibodies.

Phase contrast microscopy has the advantage of being a rapid, low cost, in-office screening test which can be used before and after therapy to indicate the presence or absence of anaerobic infection. It can be used to identify uncultivable spirochetes, motile rods and protozoa such as amoeba and trichomonads. Spirochetes can also be used as indicator organisms for the aggressive non-motile pathogen, P. gingivalis, since they are usually found together.

Newer techniques such as Human Oral Microbe using Next Generation Sequencing (HOMINGS) that identify nearly 600 oral bacteria have been developed by the Forsyth Institute in Boston. Others are using 16S rRNA pyrosequencing to identify over 900 oral species.

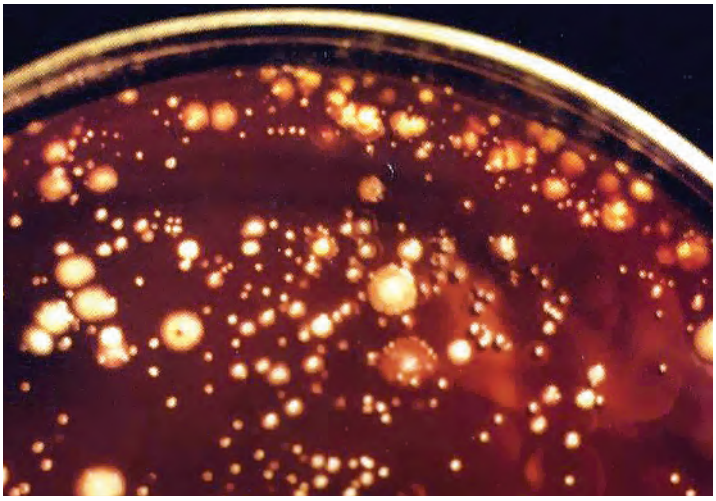

Figure 3. The white colonies in this culture are Aggregatibacter actinomycetemcomitans, the primary pathogen in aggressive periodontitis which occurs in the localized form in younger people and the generalized form in adults.

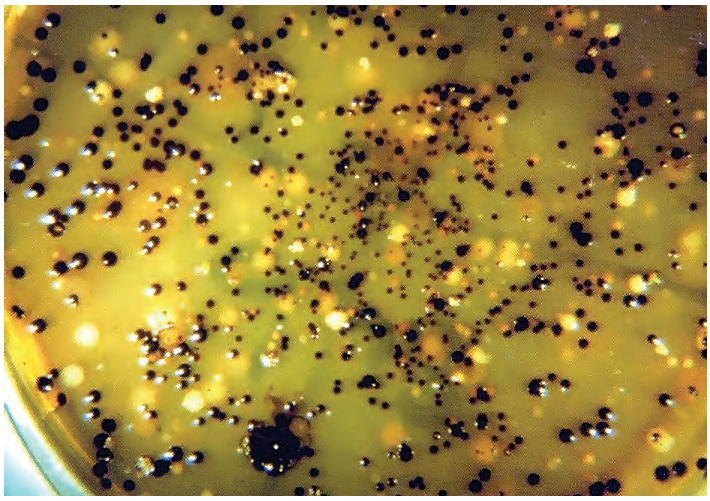

Figure 4. The dark-colored colonies are Prevotella intermedia. The tan colored colonies are Porphyromonas gingivalis. Both of these bacteria are important pathogens in periodontal diseases. Notice that this patient has a different infection from the one on the left and may require different antibiotics to help treat.

Association with Periodontal Diseases

Very Strong	Strong	Moderate	Initial Evidence
Aggregatibacter actinomycetemcomitans	Bacteriodes forsythus	Streptococcus intermedius	Enteric Rods
Porphyromonas gingivalis	Prevotella intermedia	Prevotella nigrescens	Pseudomonas
	Eubacterium nodatum	Parvimonas micra	Staphlococcus
	Treponema denticola	Fusobacterium nucleatum	Selenemonas
	Spirochetes in ANUG	Campylobacter rectus	Yeasts
			Eikenella corrodens

Where Microbial Diagnosis Can Help

Van Winklehof and Winkel in a 2005 *Perio 2000* article spelled out the aims of a microbial diagnosis:

- Discriminate between different microbial types of periodontal infections.
- Select patients most likely to benefit from adjunctive systemic antimicrobial therapy.
- Assist in selecting the most appropriate antibiotic treatment in accordance with the composition of the subgingival microflora.
- Contribute to minimizing antimicrobial resistance.
- Screen horizontal and vertical transmission of periodontal pathogens among family members.
- Help to determine the endpoint of active periodontal treatment and to establish the recall interval for periodontal maintenance care.
- Help select patients in need for periodontal treatment before inserting implants in partially edentulous subjects. This may especially be indicated in subjects with a history of periodontitis.

Conclusion

In light of the strong evidence supporting microbial testing, it is increasingly important for the clinician to undertake such testing. Microbial testing can be conducted before and after treatment to determine the specific bacterial cause of periodontitis and peri-implantitis. Doing so will assist the clinician in reducing or eliminating the bacterial pathogens associated with periodontal disease.

This testing is especially beneficial for periodontal conditions which are aggressively destroying bone support in areas of tissue breakdown with minimal evidence of local etiology to explain the amount of attachment loss, and for periodontal conditions which have been resistant to standard treatment.

PDL

Laboratory Report Showing Antibiotic Resistance

Putative Periodontal Pathogens Presumptive Identification (critical % threshold level)	% Cultivable Microbiota	Antibiotic Resistance Testing S = 100% in vitro inhibition at threshold value R = resistant Doxycycline (4 jug/ml)	Amoxicillin (8 jug/ml)	Metronidazole (16 jug/ml)	Clindamycin (4 jug/ml)
Aggregatibacter actinomycetemcomitans (0.01%)	0.0				
Red Complex Species:					
Porphyromonas gingivalis (0.1%)	0.0				
Tannerella forsythia (1%)	0.0				
Orange Complex Species:					
Prevotella intermedia (2.5 %)	8.3	R	R	S	R
Fusobacterium nucleatum (10%)	1.7	S	S	S	S
Parvimonas micra (P. micros) (3%)	13.3	S	S	S	S
Campylobacter rectus (2%)	0.8	S	S	S	S
Streptococcus constellatus (2.5%)	20.8	S	S	R	S
Other Opportunistic Species:					
Streptococcus intermedius (5%)	0.0				
Enteric gram negative rods (5%)	33.3	R	R	R	R
Enterococcus faecalis	0.0				
Staphylococcus aureus	0.0				
Candida species (yeast)	0.0				

In this patient's culture, Prevotella intermedia was not sensitive to penicillin because it often produces beta-lactimase which inactivates penicillin. Some P. intermedia species are also resistant to metronidazole necessitating the use of Augmentin which contains clavulanic acid which inhibits beta-lactimase. This patient also had high levels of Enteric gram negative rods which are not sensitive to the common empirical choice of amoxicillin and metronidazole and required the additional use of ciprofloxacin.

I. Stephen Brown, D.D.S. *220 South 16th Street, Suite 300 • Philadelphia, PA 19102 • (215)* ***735-3660***