Probiotics for Periodontal Health: A Review of the Literature

Allegra Raff, RDH, BS; Lynne Carol Hunt, RDH, MS

This project won 1st place in the ADHA Sigma Phi Alpha Journalism Award Competition, June 2011, under the baccalaureate or degree completion candidate category. Award provided by a generous grant from Johnson & Johnson Healthcare Products, Division of McNEIL PPC, Inc.

Abstract

Purpose: Periodontal disease is common among U.S. adults, and the practice of dental hygiene can be improved by new treatments to control periodontal inflammation and destruction. Probiotics, which are defined as live microbes that confer health benefits to a host when consumed in sufficient quantities, may offer a low–risk, easy–to–use treatment option for periodontal diseases. Experimental probiotic treatments in–vivo and explorations in–vitro published from 2005 to 2010 characterize the effects of specific probiotic strains on factors in periodontal health. Data considered includes clinical parameters such as gingival index, plaque index, periodontal probing depths and bleeding on probing, inhibition versus colonization of known periodontal pathogens and markers of the host immune response. Results of these studies suggest that probiotics may benefit periodontal health. Some of the most promising results occurred when the probiotic treatment was delivered in the form of a lozenge and combined with the traditional treatment of scaling and root planing. Existing commercial probiotic products for periodontal health refer to some of these data. Dosage may also play a role in probiotic efficacy for the periodontium. More research is needed to define the optimal strain or strains, therapeutic dosage, delivery mechanism and patient profile for periodontal probiotics.

Keywords: Probiotics, periodontal disease, gingivitis, Lactobacillus, Lactobacillus reuteri, Streptococci, lozenge, chewing gum

This study supports the NDHRA priority area, **Clinical Dental Hygiene:** Assess the use of evidence–based treatment recommendations in dental hygiene practice.

Introduction

Taking measures to help patients prevent and manage periodontal diseases is a major component of dental hygiene practice. Prevalence of periodontal diseases is difficult to determine accurately, but the most recently released estimate by the Centers for Disease Control and Prevention (CDC) indicates that 8.51% of U.S. adults aged 20 to 64 have periodontal disease defined as at least 1 periodontal probing depth of 4 mm or greater, with 3 mm or more of attachment loss.[1,2] Subsequent examination of this data has suggested that the actual figure may be much higher.[3] It has been reported that more than half of U.S. adults have gingivitis.[4] A combination of specific bacterial activity and the patient's immune response is implicated in pathogenesis of periodontal diseases, causing tissue destruction which can lead to recession, mobility and eventual tooth loss.[5] This article is a review of laboratory and clinical research conducted for the purpose of exploring an emerging treatment option – probiotic therapy to support periodontal health.

A need for new and improved periodontal therapies exists. Dental hygiene practitioners will be familiar with the ubiquity of scaling and root planing as a treatment option for their periodontal patients. Some of the shortcomings of scaling and root planing may be characterized as such: following mechanical removal, periodontal pathogens repopulate pockets within months, compelling continuous and economically burdensome retreatment.[6] There is substantial evidence that complementing scaling and root planing with antimicrobial chemotherapies, such as antibiotics or Chlorhexidine, improves periodontal healing.[7,8] However, the CDC has identified antibiotic resistance as a growing problem and a direct result of antibiotic use, and reports that "almost every type of bacteria has become stronger and less responsive to antibiotic treatment."[9] Additionally, the

possibility of adverse or allergic drug reaction could make antibiotic therapy for periodontal disease an undesirable option for some patients. Allergy to antibiotics appears to be uncommon, but has been shown to rise in incidence with increasing age and use.[10] A recent review of medical records in San Diego Country revealed that out of over 411,000 outpatients given antibiotics in 2007, between 0.5 to 1.1% of men and 1 to 1.5% of women reported adverse reactions, possibly allergic, to non–sulfonamide antimicrobials such as the tetracyclines, macrolides, quinolones and penicillin derivatives sometimes used in treating difficult periodontal cases.[10,11] Chlorhexidine, an antimicrobial agent, has been associated, in some cases, with adverse events in those with poorly controlled diabetes.[12] Thus the search for effective treatment options that offer long–term benefits and pose minimal risk continues. As an alternative, probiotic treatments may not be risk–free — some reports of secondary infection in patients with systemic disease require further analysis — but side effects are considered mild and unlikely. Probiotics have a long history of use in health promotion and are generally considered safe.[13,14]

Dental hygienists are in a position to communicate oral health discoveries to patients, and may encounter questions about probiotics for oral health. A probiotic is defined by the Food and Agriculture Organization of the United Nations and the World Health Organization as a live microbe that confers health benefits to its host, when consumed in sufficient quantity.[15] In recent decades, the targeted use of probiotics to treat diseases has gained popular as well as medical interest. A 2007 review states that since 2000, publications about probiotics increased almost exponentially.[15] A PubMed search yields 559 U.S. based articles about probiotics published in the last 5 years, while a Google search of "probiotics for sale" yields 571,000 results. Commercial probiotic use to promote health is on the public radar.

The efficacious use of probiotics to treat gastrointestinal conditions has been well established.[16] The scientific basis for periodontal application is still emerging, as evidenced by the small body of publications on this topic, but commercial products marketed for periodontal health promotion exist nonetheless. EvoraPlus® products (from Oragenics Inc™, a Biopharmaceutical company based in Tampa, FL) are an example, featuring 3 trademarked strains of Streptococcus species.[17] The Swedish biotechnology company BioGaia® (Stockholm, Sweden) is a commercial manufacturer of L. reuteri probiotic supplements ("ProDentis®") marketed for oral health promotion.[18] ProDentis® is distributed in the United States under the name "Periobalance®" by Sunstar Americas, Inc./G.U.M (Chicago, IL).[19]

Since current research in probiotics may lead to new options for maintaining oral health, the purpose of this review is to evaluate the scientific literature regarding probiotic treatment of periodontal diseases.

Methods and Materials

Articles were selected for this review from multiple Medline (PubMed) searches, many of which combined the phrases "periodontal disease" or "periodontal diseases" and "probiotics" with other descriptive terms, but also some that substituted specific bacterial names associated with probiotic use and periodontal pathogens in order to maximize the yield of related research. Some articles were selected from the bibliographies of other qualifying and non–qualifying sources. Only articles classified as "clinical trials" (in–vitro or in–vivo, with humans or animal subjects) were included. Other published reviews are not reviewed, but in some cases are referenced for background and supporting information. Peer–reviewed publications between 2000 and 2011 were a criterion for inclusion. Only articles published in English with full–texts available were considered.

The bacteria examined for probiotic use in the articles reviewed were selected from Lactobacilli, Streptococci, Bifidobacterium and Bacilli species.[7,20–27] Some of the researchers in these articles examined specific bacterial strains, in accordance with the FAO/WHO guidelines. For example, Teughels et al studied Streptococcus mitis BMS, Streptococcus sanguis ACTCC 49297, Streptococcus salivarius TOVE.7 Mayanagi et al and Shimauchi et al studied Lactobacillus salivarius–WB21.[21,22] Twetman et al looked at Lactobacillus reuteri DSM17938, ATCC PTA 5289 and ATCC 55730.[23] Staab et al examined Lactobacillus casei Sharota.[26]

Lactobacilli species were frequently chosen because of their existing uses in targeted probiotic therapy for humans (mainly gastrointestinal) and their otherwise common and often beneficial presence in normal human flora.[20–23] The L. reuteri WB21 strain studied by Mayanagi et al and Shimauchi et al, in particular, has been cultivated to survive stomach acids.[21,22] Some researchers point to evidence of Lactobacilli's anti–inflammatory effects in the gastrointestinal tract via mechanisms which could conceivably function in the periodontium as well.[23] Krasse et al were inspired to study L. reuteri by anecdotal observations suggesting oral benefits.[24] Teughels et al selected 4 species of Streptococci, which are part of the normal oral flora and had previously been shown to possess anti–cariogenic properties, to

examine for periodontal benefits.[7] Zhu et al chose to experiment with a set of strains that they had cultured from commercial yogurt, thus testing the periodontal relevance of bacteria that patients may encounter through usual dietary practices.[25] There is a clear concentration of interest in the available literature around the Lactobacilli, and L. reuteri in particular, with 3 out of 10 studies included focusing on that species.[23,24,27]

Each of the in–vivo clinical studies was randomized, double–blinded and controlled, with the exception of one which was not double–blinded.[26] The human sample sizes are generally small, ranging from 30 to 66 individuals. All had a high rate of completion, and no adverse events attributable to the test products were reported in any of the studies.[21–24,27] All of the human subjects were healthy adults. One study collected samples from human subjects, but performed all subsequent experimentation in–vitro and one was completely in–vitro.[20,25] Those performed with human subjects selected participants who were considered healthy apart from some degree of periodontal infection, who were not undergoing active dental treatment (except when scaling and root planing were included in the study design), who did not have concurrent probiotic supplementation, who could tolerate dairy products and who were not undergoing treatment with antibiotics.[20–24,26,27] None of the studies were formulated to specifically examine a smoking population. However, data specific to the subjects who were smokers were considered in 2 studies.[22,26] One study actively excluded smokers.[27] The inclusion of professional prophylaxis and oral hygiene instruction varies from study to study, as does severity of disease in the subjects and sample size. This heterogeneous collection of studies has been reviewed together because of the limited number of sources available for comparison.

The significant findings of these articles are organized within this review by the 3 major categories of results that emerged:

- The clinically observable responses of periodontal tissue to probiotic exposure
- Changes in periodontal pathogen populations in the presence of probiotic bacteria, due to competitive displacement
- Measurable changes in host immune response to probiotic treatment

Results

Probiotic effects on clinical signs of periodontal diseases: Periodontal diseases are characterized by the clinical signs of gingival inflammation and deepened periodontal probing depths, and generally associated with plaque biofilm formation. The human studies concerning periodontal probiotic treatments invariably collected some data on these parameters.

Krasse et al found that 2 different L. reuteri formulations (LR–1 and LR–2, respectively) significantly improved plaque index (PI) scores in subjects with moderate to severe gingivitis compared to similar subjects taking a placebo ($p<0.05$ for LR–1, $p<0.01$ for LR–2). The LR–1 formulation also significantly improved gingival index (GI) scores compared to a placebo ($p<0.0001$). The test products were formulated in a chewing gum containing $1x10^8$ colony forming units (CFU) of L. reuteri.[24] Subjects chewed the designated product twice a day after brushing for 2 weeks, and the significant results were recorded at the end of this 14 day test period. The positive effects of both were observed with the use of L. reuteri chewing gum on GI and PI, and surpassed the improvements observed in subjects who only received an initial professional prophylaxis and OHI, which all subjects received at the start of the study. Twetman et al, who also tested the effects of chewing gum containing L. reuteri strains and recorded PI or GI scores from their periodontally diseased subjects, also measured bleeding on probing.[23] At the 2 week evaluation of these subjects, bleeding was significantly reduced in both test groups but not in the placebo group ($p<0.05$).

Two research teams at Tohoku University in Japan, Mayanago et al and Shimauchi et al, evaluated the probiotic effect of 1 specific bacterial strain, Lactobacillus salivarius–WB21, on the periodontal pathogens in a group of subjects with mild to moderate periodontal disease.[21,22] Sixty–six adult participants were divided into treatment and control groups, statistically similar at baseline. No patients with severe periodontal disease were included, defined as 1 or more periodontal pocket depths of 6 mm or greater (on one of the patient's 6 teeth selected), pathologic mobility or abscess. The test product was a xylitol–based tablet formulated with $6.7x10^8$ CFU of L. salivarius–WB21. Participants were instructed to let the tablet dissolve in their mouths 3 times a day for 8 weeks, but not to alter their usual oral hygiene habits. Shimauchi et al reported that both test groups showed improvements in the clinical indices at 4 and 8 week evaluations, and there were no significant improvements of the test group compared to the placebo group taken as a whole.[22] However, when the non–smokers were ignored and only the smokers from the 2 groups were compared, the test group smokers did show significantly greater improvements in probing depths (PPD) and plaque indices than the placebo–

group smokers at 4 weeks ($p<0.05$ PPD, $p<0.01$ plaque indices) and at 8 weeks ($p<0.05$ for PPD, $p<0.05$ plaque indices) of using the experimental treatment.[22]

Staab et al found that the practice of consuming a daily probiotic milk drink containing the Shirota strain of Lactobacillus casei did not reduce overall plaque. The product was tested without other modifications over the course of 8 weeks. Unsurprisingly, plaque levels increased even more after a 4 day experimental gingivitis period, during which the subjects ceased plaque removal practices but continued consumption of the drink. The test subjects showed a greater plaque increase than subjects who consumed none of the probiotic drink, possibly due to the carbohydrate content of the test product. Papillary bleeding on probing, however, increased for both groups but remained statistically similar between groups at all data points.[26]

Vivekananda et al found that scaling and root planing a L. reuteri lozenge treatment showed significant improvement in all clinical parameters, including clinical attachment, plaque levels, gingivitis (as measured by GI) and bleeding ($p=0.001$).[27] Scaling and root planing combined with the L. reuteri strains was more effective than either treatment alone. This study used a split–mouth design in the test group in addition to a placebo–controlled comparison group. Both the test and placebo groups received scaling and root planing treatment in only half of the mouth and none in the other half. Thus, each subject served as his or her own control. Even in the halves of the mouths that were not treated with scaling and root planing, the active–lozenge group showed a significantly lower plaque index than the placebo group ($p<0.001$). Meanwhile, the un–scaled quadrants of the placebo group did not show a significant improvement compared with baseline values for these sites. The largest reduction in pocket depth, by 1.31 mm, was also found among the sites that received combined treatment of scaling and root planing plus the L. reuteri strains. However, neither scaling and root planing nor L. reuteri treatment alone provided even half of the combined improvement.[27] The 30 adult subjects were considered to have chronic periodontitis based on clinically evident gingivitis, 5 to 7 mm probing depths and radiographic bone loss. The test group's lozenges contained $1x10^8$ CFU of each of the L. reuteri strains DSM17938 and ATCC PTA 5289. Subjects in this study waited 3 weeks after scaling and root planing to begin using the lozenges, and continued to use the lozenges twice daily for 3 more weeks.

Of the studies that evaluated clinical parameters, subjects who received lozenge and chewing gum delivery systems showed significant improvements, especially when the treatment was combined with traditional mechanical therapies.

Periodontal pathogens: Many of the studies reviewed examined the potential probiotics' interaction with specific periodontal pathogens. Pathogens in these examinations included Actinobacillus actinomycetemcomitans, Aggregatibacter actinomycetemcomitans, Aggregibacter actinomycetemcomitans [sic], Prevotella intermedia, Prevotella nigrescens, Porphyromonas gingivalis, Porphyromonas gulae, Porphyromonas circumdentaria, Treponema denticola, Tannerella forsythia, Campylobacter rectus, Fusobacterium nucleatum, Peptostreptococcus anaerobius and Bacteriodes fragilis.[7,20,22,25,27]

Mayanagi et al, looking for periodontal benefits from L. salivarius WB21 consumption, isolated 5 periodontal pathogens from the supragingival and subgingival plaque of all subjects and then evaluated quantitative changes in pathogen colonies over the 8 weeks of the study.[21] No adjunctive treatments, such as scaling and root planing or oral hygiene instruction, were provided. The 5 pathogens identified, using DNA amplification, were Aggregatibacter actinomycetemcomitans, P. intermedia, P. gingivalis, T. denticola and T. forsythia. By the fourth week of using the L. salivarius WB21 tablets, plaque from the test group yielded significantly reduced total bacterial levels of the 5 pathogens ($p=0.012$). However, when bacterial counts of each pathogen were examined individually and differences among patients, such as baseline bacterial presence, plaque levels and smoking status, were considered, only counts of T. forsythia were significantly different between the test and placebo–controlled group ($p<0.001$ at 4 weeks, $p=0.006$ at 8 weeks), with lower subgingival T. forsythia counts in the test group.[21]

In a canine model, Teughels et al examined whether the introduction of 3 Streptococcus species, S. salivarius, S. sanguis and S. mitis, could inhibit re–infection of periodontal pockets after scaling and root planing. The 3 infectious pathogens considered were P. intermedia, a known human periodontal pathogen, Porphyromonas gulae, which has been considered a canine equivalent of P. gingivalis and C. rectus. Microbial composition was compared in artificially created periodontal 5 mm pocketing. Only when the scaling and root planing treatment was followed by 3 separate insertions of a pellet containing live Streptococci probiotics directly into the periodontal pocket, 1 or 2 weeks apart, were lowered pathogen levels maintained

12 weeks after the initial treatment ($p<0.001$ for black–pigmented species and $p=0.002$ for anaerobic species). In comparison, animals who received scaling and root planing but no probiotics showed significantly reduced black–pigmented pathogen levels after treatment, but the reduction was not sustained over time ($p>0.001$).[7]

In their study of the L. reuteri lozenge, Vivekananda et al also collected subgingival plaque samples for examination of the microbial composition.[27] From these samples, Aggregibacter actinomycetemcomitans [sic], P. gingivalis and P. intermedia were cultured. Significant reductions in the levels of all 3 red–complex periodontal pathogens occurred only in the active–lozenge group, and the reduction of each pathogen was 10–fold in these instances (p values ranging from <0.01 to <0.005). Similarly to the Teughels et al canine study, only sites treated with the probiotic showed significantly reduced pathogen levels in pooled subgingival plaque over an extended period after initial treatment, while sites treated with scaling and root planing alone did not. The combined treatment of scaling and root planing plus ProDentis® showed a more significant reduction of A. actinomycetemcomitans, P. gingivalis and P. intermedia significant than the scaling and root planing plus placebo treatment (A. a. $p<0.005$, P. g. $p<0.005$, P. i. $p<0.05$).[27]

Krasse et al did not measure pathogen displacement, but did find that their test groups treated with L. reuteri experienced a large increase in L. reuteri presence in the saliva. Fewer than 10% of test patients in this study were found to be colonized with salivary L. reuteri at baseline, but by the end of the 2 week study 65% of subjects receiving the LR–1 formulation and 95% of subjects receiving LR–2 were colonized. In both groups, L. reuteri made up close to half of the final bacterial presence in saliva while the placebo group had no L. reuteri colonization at any point.[24]

Zhu et al were the only group to examine probiotic inhibition of pathogens in comparison to an accepted antimicrobial chemotherapy, though the study was performed only in–vitro.[25] Chlorhexidine was used as a control for the experimental competition of periodontal pathogens with microorganisms found in yogurt. From a commercial brand of yogurt available in China, they isolated and confirmed 4 live strains of bacteria: Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium. The researchers then tested inhibition of F. nucleatum, P. gingivalis, A. actinomycetemcomitans, P. intermedia, P. nigrescens, P. anaerobius, B. fragilis and P. circumdentaria against the yogurt cultures by diffusing yogurt through BHI agar that had been seeded with the selected periodontal pathogens. Since the low pH of yogurt has some potential for inhibiting periodontal pathogens, the researchers controlled for pH by preparing another diffusion using yogurt that had been heat–treated to reduce the microbial content to almost zero. Different combinations of bacteria were inoculated onto the same plates, in controlled chronological variations. Zhu et al showed that active yogurt inhibits all 8 pathogens better than the heated yogurt, but not as well as 0.2% Chlorhexidine, which was used as a control. The pathogens tended to be inhibited when the yogurt cultures were introduced to the medium first. When the periodontal pathogens were introduced first, the yogurt cultures and pathogens grew side–by–side with no inhibition with the exception of P. intermedia, which was able to inhibit growth of yogurt cultures Bifidobacterium and S. thermophilus. When inoculated simultaneously, S. thermophilus inhibited the pathogen P. nigrescens.[25] Though it is impossible to tell from an in–vitro study how these organisms would behave in a clinical trial, this evidence suggests that rapid inoculation with probiotics in an environment free of periodontal pathogens could act preventatively in the growth, or re–growth, of pathogens. The dominance of P. intermedia in this instance reminds us that certain pathogens may have the ability to break through the protective colonization of probiotics. The clinical narrative that would result from these inhibitory wins and losses cannot be described from the non–clinical data.

In–vitro experimentation by Kõll–Klais et al suggests that the difference between the normal, non–pathogenic flora of periodontal patients versus healthy patients can be characterized by mode of carbohydrate fermentation.[20] In both healthy and diseased patients sampled, the majority of the flora was comprised of Lactobacilli species, which are not considered pathogenic. Known oral pathogens, including Streptococcus mutans, A. actinomycetemcomitans, P. intermedia and P. gingivalis were also cultivated from the diseased patients. Some of these Lactobacilli species were homofermentative, which refers to their metabolic production of a single by–product, lactic acid. Others were heterofermentative, a categorization that refers to their multiple metabolic by–products. Overall, facultative heterofermentatives (homofermentative bacteria that can alter their metabolism to resemble heterofermentation under certain conditions) were present in higher numbers in the periodontitis patients than in the healthy patients, while the obligate homofermentatives (those that must metabolize using homofermentation, with a limited by–product) were relatively low. Lactobacillus gasseri, an obli-

gate homofermentative, was not only among the most prevalent in healthy subjects, but was also much less prevalent in the diseased patients. This reviewer noted from the data that Lactobacillus acidophilus, a mainstay of commercial probiotic dairy products, made up less than 10% of the obligate homofermentatives in healthy subjects and 0% in the diseased.

When different combinations of the Lactobacilli and oral pathogens collected from the subjects' gingival crevicular fluid were cultured and grown together under appropriate conditions, patterns of inhibition could be observed. When grown together, obligate homofermentative and facultative heterofermentative Lactobacilli demonstrated the greatest ability to inhibit the pathogens.[20] The value of this study to the development of probiotic treatments appears mainly to be in understanding the roles of existing flora, without treatment.

Probiotic effect on host immune response: In response to evidence that some of the health benefits of probiotics are due to immunomodulatory effects, Shimauchi et al, Twetman et al and Staab et al measured host response to potential periodontal probiotics by way of inflammatory markers.[22,23,26]

Twetman et al primarily evaluated the inflammatory markers present in GCF of otherwise healthy adult periodontal patients.[23] Of 3 treatment groups, which differed by number of probiotic active (A) pieces of gum chewed versus number of placebo (P) pieces chewed, only the A/A group showed a significant reduction in 3 of the inflammatory markers evaluated in this study. TNF–alpha and IL–8 showed reduction at weeks 1 and 2 during treatment, respectively, and IL–6 was reduced 2 weeks after the treatment was ceased ($p<0.05$). Every piece of active gum contained 2 live strains of Lactobacillus reuteri, ATCC 55730 and PTA 5289, in the quantity of $1x10^8$ CFU each. Subjects in this study were all given oral hygiene instruction. One possible limitation of the site selection was that none of the sites tested were molar sites and all were buccal. Results showed a decrease in BOP and amount of GCF in all subjects after the 2 weeks of chewing the gums, but only the reduction in the experimental groups, A/A and A/P ($p<0.05$) was considered significant.

Shimauchi et al[22] chose to measure levels of salivary lactoferrin (Lf) based on evidence published in 2007 by Komine et al[28] that Lf proteins in whole saliva indicate periodontal inflammation. The test group, participating in L. salivarius WB21 treatment, showed significantly lower salivary Lf levels at 8 weeks, while the placebo group did not ($p<0.01$). As previously mentioned, examination of smokers was not the purpose of any of these studies, but when the data were calculated to separate the subjects who smoked from the non–smokers, the change in Lf levels was most pronounced among the test subjects who had also smoked.[22] Staab et al, in the only other study reviewed, specifically addressed the relevance of smoking to periodontal health and reported a balanced distribution of smokers among their groups such that any effects of smoking would not skew the results.[26]

To study the clinical and immunologic effects of L. casei strain Shirota consumed as a drink, Staab et al measured the inflammatory markers myeloperoxidase (MPO), Polymorphonuclear (PMN) elastase and matrix metalloproteinases (MMP–3), a host enzyme thought to be involved in periodontal destruction. Among the test subjects who consumed the drink every day for 8 weeks, MMP–3 and PMN elastase levels dropped after the 8 week trial, even though plaque increased. In the test group, MPO did increase over the 8 week trial, but then dipped slightly when measured after the 4 "experimental gingivitis" days of ceased plaque removal. In comparison, the control group's MPO levels, as well as MMP–3 and MPO levels, increased at every time point.[26]

Discussion

The literature reviewed included clinical research since 2001 linking periodontal disease pathogenesis and probiotic treatment. There are many well–documented health benefits of probiotics, including relieving of inflammation and prevention of certain infections and allergies.[29] A 2011 review by Teughels et al provides a more in–depth discussion of the history of probiotic treatments and the mechanistic rationales for applying probiotics to periodontal health.[29] Given the infectious and inflammatory nature of periodontal diseases, combined with the challenges of existing treatments, the search for probiotic periodontal therapy is a reasonable development. In 2002, the FAO and WHO proposed guidelines for regulating probiotics and recommended identifying probiotic candidates by DNA–confirmed strain. The guidelines outline a multiphase empirical approach to establishing a profile of safety, handling and targeted therapeutic use similar to the phases required in drug testing.[16] A review of currently published research indicated that, with regard to treating periodontal diseases, there is room for progress in identifying the most promising bacterial species for probiotic cultivation and the most effective treatment modality. Some commercial probiotic periodontal therapies do already exist. Krasse et al and Vivekananda et al both used products manufactured by BioGaia®.[24,27]

BioGaia® cites the 2010 Vivekananda et al study for support of their product's efficacy.[27,30] Though Vivekananda et al stated no conflict of interest, it should be kept in mind that a publication grant and the test products for this study were donated by the BioGaia® company. At this time, there appears to be no publically available research on probiotic treatments for periodontal diseases conducted in the U.S.

Discussion of Clinical Signs: The most extensively published data on periodontal probiotics to date involves Lactobacilli species. Shimauchi et al, Twetman et al, Krasse et al and Vivekananda et al reported periodontal benefits associated with L. reuteri treatment.[22–24,27] Krasse et al provided evidence that daily, topical L. reuteri treatments (in chewing gum form), adjunctive to professional prophylaxis, could improve gingival health, as measured by plaque and gingival indices.[24] Twetman et al also noted a significant reduction in bleeding on probing in test groups chewing 1 or 2 probiotic–enhanced gums daily containing either the LR–1 or LR–2 strain of L. reuteri, which was not seen in the control group ($p<0.05$).[23] According to Vivekananda et al, an active lozenge containing L. reuteri provided a clear benefit.[27] Even without instrumentation, mouths receiving treatments that included the ProDentis® lozenge showed significant clinical improvement over the placebo group in all clinical aspects measured at 6 weeks except 1 (pocket probing depth reduction) ($p<0.05$ and $p<0.001$).

When Shimauchi et al examined use of a L. salivarius WB21 tablet by patients with mild to moderate periodontal disease, 3 times daily for 8 weeks, only the current smokers showed significant clinical improvements in probing depth and plaque indices ($p<0.05$ and $p<0.01$).[22]

Discussion of Periodontal Pathogen Response: Kõll–Klais et al identified significantly higher levels of homofermentative Lactobacilli, a group that includes L. salivarius, in periodontally healthy subjects than in an otherwise similar group of periodontally diseased subjects ($p<0.05$).[20] However, L. gasseri was the only individual homofermentative that significantly reflected this tendency for greater prevalence in healthy subjects ($p<0.001$). When tested for inhibition of the periodontal pathogens A. actinomycetemcomitans, P. gingivalis and P. intermedia, one of the best performers among the naturally occurring Lactobacilli was L. salivarius, followed by L. crispatus and L. gasseri.

Mayanagi et al and Shimauchi et al selected L. salivarius WB21 as a potential probiotic because Lactobacilli are common oral flora and the species L. salivarius has been shown to reduce salivary levels of black–pigmented bacteria, such as some periodontal pathogens.[21,22] Mayanagi et al discuss that previous research demonstrates a synergistic relationship between T. forsythia and P. gingivalis.[21] Therefore, suppression of T. forsythia could conceivably help to undermine the pathogenesis of P. gingivalis. The authors are encouraged by their finding of T. forsythia reduction, along with the 2008 data reported by Shimauchi et al from the same participants, that the WB21 strain could have a future in periodontal disease management and prevention.[21,22] A potential conflict of interest exists in both the Mayanagi et al and Shimauchi et al studies in that the research was funded by Wakamoto Pharmaceutical Co., the same company that produced the treatment and placebo tablets. Two of Shimauchi's researchers were employed by this pharmaceutical company. Participants were also selected from the workers at the factory who produced the tablets.

Xylitol is used as a primary ingredient in many placebo and test products across this selection of studies. Xylitol's inhibition of oral Streptococci, with significance for dental caries, has been well documented.[31] There does not appear to be any published research that describes effects of xylitol on periodontal health, and the anticipation of such an effect is not discussed within these studies. Shimauchi et al used a xylitol base for their probiotic–active and placebo tablets.[22] They reported having observed in their own experimentation that xylitol has no modulating effect on periodontal pathogens by itself; however, xylitol boosts L. salivarius WB21's inhibitory effects on the periodontal pathogen P. gingivalis. They have not included this unpublished data.[22]

Zhu et al also found evidence for the preventive capacity of periodontal probiotics.[25] At least in–vitro, certain probiotic strains can inhibit the growth of P. gingivalis and P. intermedia when allowed to colonize first. In theory, guided pocket recolonization after scaling and root planing could be a strategic clinical use of probiotics in dental hygiene practice, when Chlorhexidine or other antimicrobials are contraindicated. Further research to develop a probiotic mixture for post–operative in–office application may someday be able to assist the longevity of pathogen removal in scaling and root planing procedures. An implication of this study is that further clinical research could be rewardingly directed at probiotic treatment of periodontal patients immediately following professional plaque removal.

The results of Vivekananda et al are surprising for their uniformity – Pathogens A. actinomycet-

emcomitans, P. gingivalis and P. intermedia were each reduced by the L. reuteri test product, ProDentis®, by the same amount (to 10^5 CFU/ml).27 The ProDentis®–only treatment showed statistically significant reductions in pathogen levels by the end of the 6 week trial ($p<0.05$ to $p<0.001$), and a comparison of the combined scaling and root planing plus ProDentis® treatment to ProDentis® alone showed no statistically significant advantage to adding scaling and root planing. The combined treatment of scaling and root planing plus ProDentis® still showed the greatest numerical reduction of each species, even though the added advantage was not statistically significant compared to the ProDentis®–only treatment. Vivekananda et al discuss that this finding corroborated others' observations regarding the limited effect of a single scaling and root planing treatment on the long–term reduction of periodontal pathogens such as A. actinomycetemcomitans.[27]

Teughels et al sampled dogs rather than humans, and is presently the only study examining the use of oral Streptococci as a probiotic treatment in subjects with periodontal disease.[29] Their successful black–pigmented pathogen control within 4 mm pocketing suggests that the search for periodontal probiotics need not be limited to Lactobacilli. Teughels et al were the only investigators to use an intra–pocket treatment application.[7] This approach bears technical similarity to site–specific antimicrobial therapies, such as Arestin® (minocycline HCL 1 mg) and PerioChip® (chlorhexidine gluconate), which are widely regarded as successful.[32]

Discussion of Host Immune Response: Twetman et al reported significant benefits for periodontitis patients chewing a double–dose (2 pieces) of active gum a day.[23] These benefits consisted of significant reductions in the inflammatory mediators TNF–alpha and IL–8, which are known to be associated with inflammatory tissue damage ($p<0.05$). Since the group who chewed 1 active and 1 placebo piece (effectively receiving single–dose) did not experience the significant drop in mediators, it appears that the response may be dose–related. However, the authors state that it was too early to establish a treatment dosage based on these findings and considered the study a pilot. Though these subjects only chewed the test product for 2 weeks, Twetman et al performed a follow–up evaluation of the inflammatory marker levels again at 4 weeks (2 weeks after test product use was stopped) and found that the levels had returned to their values at the first measurement, before any treatment.[23] The benefits were not long–lasting after treatment ceased. The authors acknowledged that because gingival crevicular fluid could only be collected in very small amounts, it's hard to know whether the measurement of inflammatory cytokines, such as TNF–alpha and IL–8, was accurate.[23] Previous in–vitro research published in 2004 by Ma et al supports L. reuteri's ability to modulate TNF–alpha, IL–8 and other human inflammatory cytokines.[33] Krasse et al state that the 2 strains of L. reuteri they examined, LR–1 and LR–2, may have complimentary host benefits, though the data supporting this assertion, based on their own prior research, are not presented or cited.[24]

Though only the current smokers in the Shimauchi et al study test–group showed significant clinical improvements in probing depth and plaque indices ($p<0.05$ and $p<0.01$), the test group as a whole (smokers and non–smokers) showed significantly decreased levels of Lf ($p<0.01$). Lf data for the currently–smoking subset of test subjects reflect these significant Lf reductions, but it was unclear whether the non–smoking subset experienced such a benefit when considered separately.[22] L. salivarius WB21 may therefore be a periodontal probiotic for smokers, but not necessarily for the non–smokers.

The results of Staab et al suggested modulation of the host's immune response by L. casei Shirota in the absence of mechanical plaque removal during "experimental gingivitis."[26] The re–elevation of inflammatory marker (PMN elastase and MPO) and MMP–3 levels after the "experimental gingivitis," while no probiotic was consumed, suggested that the effects on immune response are not lasting. L. casei Shirota did not seem to reduce plaque build–up. The key finding was immunomodulation, as demonstrated by altered levels of MPO, MMPs and PMN elastase. Further research on this strain, such as a comparison of delivery systems and a controlled trial contrasting the probiotic to other types of treatment, could expand the profile of L. casei Shirota as a probiotic. This study was considered a pilot study due to its limited scope and uncontrolled variables, and more research is needed.[26]

Conclusion

At this point in time, a dental professional's response to patient inquiries about probiotic treatments for periodontal health should be cautious. While supportive research exists, our understanding of the complex and interconnected factors that must be part of any treatment recommendation is too undeveloped for us to offer such recommendations to our patients yet. Much of the relevant data is very recent, published in 2005 or later. Overall, the results of these clinical and in–vitro studies are encouraging to the development of effective probiotic treatments to help maintain and

possibly help restore patients' periodontal health. However, further experimentation is needed. Periodontal disease severity and other health factors, such as systemic disease and lifestyle choices, are variables that have not been fully explored, and trials to establish the optimal delivery methods and treatment schedule are still needed. Currently, L. reuteri and several Streptococci species are available in formulations intended to support periodontal health. Other species that possess promising characteristics for probiotic periodontal use have yet to be examined in clinical treatment. Finally, possible conflicts of interest exist within some of the available studies, particularly the most conclusive clinical trials. A thorough collection of clinical trials from truly independent sources is needed before clinical application can be considered grounded in science.

Allegra Raff, RDH, BS, is a practicing Dental Hygienist in Washington, DC. Lynne Carol Hunt, RDH, MS, is a Visiting Clinical Assistant Professor with the Department of Dental Ecology at the UNC–CH School of Dentistry.

References

1. Eke PI, Genco RJ. CDC Periodontal Disease Surveillance Project: Background, Objectives, and Progress Report. *J Periodontol*. 2007;78(7 Suppl):1366–1371.

2. National Institute of Dental and Craniofacial Research. Periodontal Disease in Adults (Age 20 to 64). NIDCR [Internet]. 2011 January [cited 2011 February 2]. Available from: http://www.nidcr.nih.gov/datastatistics/finddatabytopic/gumdisease/periodontaldiseaseadults20to64.htm

3. American Association of Periodontology. Gum Disease Found to Be Significant Public Health Concern. American Academy of Periodontology [Internet]. 2010 October [cited 2011 February 3]. Available from: http://www.perio.org/consumer/cdc–research.htm

4. Li Y, Lee S, Hujoel P, et al. Prevalence and severity of gingivitis in American adults. *Am J Dent*. 2010;23(1):9–13.

5. Taubman MA, Valverde P, Han X, Kawai T. Immune response: the key to bone resorption in periodontal disease. *J Periodontol*. 2005;76(11 Suppl):2033–2041.

6. Teughels W, Newman MG, Coucke W, et al. Guiding periodontal pocket recolonization: a proof of concept. *J Dent Res*. 2007;86(11):1078–1082.

7. Loesche WJ, Giordano JR, Soehren S, Kaciroti N. The nonsurgical treatment of patients with periodontal disease: results after five years. *J Am Dent Assoc*. 2002;133(3):311–320.

8. Slots J. Selection of antimicrobial agents in periodontal therapy. *J Periodontal Res*. 2002;37(5):389–398.

9. Antibiotic Resistance Questions & Answers. Centers for Disease Control and Prevention [Internet]. 2009 June [cited 2011 September 11]. Available from: http://www.cdc.gov/getsmart/antibiotic–use/antibiotic–resistance–faqs.html

10. Macy E, Poon K–Y T. Self–reported antibiotic allergy incidence and prevalence: age and sex effects. Am J Med. 2009;122(8):778.

11. Periodontal disease: Medications. University of Maryland Medical System [Internet]. 2009 January [cited 2011 September 12]. Available from: http://www.umm.edu/patiented/articles/how_antibiotics_being_used_long–term_prevention_of_periodontal_disease_000024_9.htm

12. McCoy LC, Wehler CJ, Rich SE, Garcia RI, Miller DR, Jones JA. Adverse events associated with chlorhexidine use: results from the Department of Veterans Affairs Dental Diabetes Study. *J Am Dent Assoc*. 2008;139(2):178–183.

13. An Introduction to Probiotics. National Center for Complementary and Alternative Medicine [Internet]. 2008 August [cited 2011 January 25]. Available from: http://nccam.nih.gov/health/probiotics/introduction.htm

14. Food and Agriculture Organization of the United Nations; World Health Organization. Guidelines for the Evaluation of Probiotics in Food. FAO [Internet]. 2002 April. Available from: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf

15. Meurman JH, Stamatova I. Probiotics: contributions to oral health. *Oral Dis*. 2007;13(5):443–451.

16. Broekaert IJ, Walker WA. Probiotics and chronic disease. *J Clin Gastroenterol*. 2006;40(3):270–274.

17. How it Works. Oragenics [Internet]. [cited 2011 March 4]. Available from: http://www.oragenics.com/probiotics/evorapro–dental–professionals/how–it–works

18. About Biogaia. BioGaia [Internet]. 2009 [cited 2011 March 1]. Available from: http://www.biogaia.com/about–biogaia

19. For Dental Professionals. Sunstar Americas [Internet]. 2009 [cited 2011 March 4]. Available from: http://commerce.jbutler.com/dentalprofessionals.aspx

20. Kõll–Klais P, Mändar R, Leibur E, Marcotte H, Hammarström L, Mikelsaar M. Oral Lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. Oral Microbiol Immunol. 2005;20(6):354–361.

21. Mayanagi G, Kimura M, Nakaya S, et al. Probiotic effects of orally administered Lactobacillus salivarius WB21–containing tablets on periodontopathic bacteria: a double–blinded, placebo–controlled, randomized clinical trial. *J Clin Periodontol*. 2009;36(6):506–513.

22. Shimauchi H, Mayanagi G, Nakaya S, et al. Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: a randomized, double–blind, placebo–controlled study. *J Clin Periodontol*. 2008;35(10):897–905.

23. Twetman S, Derawi B, Keller M, Ekstrand K, Yucel–Lindberg T, Stecksen–Blicks C. Short–term effect of chewing gums containing probiotic Lactobacillus reuteri on the levels of inflammatory mediators in gingival crevicular fluid. *Acta Odontol Scand*. 2009;67(1):19–24.

24. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic Lactobacillus reuteri. *Swed Dent J*. 2006;30(2):55–60.

25. Zhu Y, Xiao L, Shen D, Hao Y. Competition between yogurt probiotics and periodontal pathogens in–vitro. *Acta Odontol Scand*. 2010;68(5):261–268.

26. Staab B, Eick S, Knöfler G, Jentsch H. The influence of a probiotic milk drink on the development of gingivitis: a pilot study. *J Clin Periodontol*. 2009;36(10):850–856.

27. Vivekananda MR, Vandana KL, Bhat KG. Effect of the probiotic Lactobacilli reuteri (Prodentis) in the management of periodontal disease: a preliminary randomized clinical trial. *J Oral Microbiol*. 2010;2:5344.

28. Komine K, Kuroishi T, Ozawa A, et al. Cleaved inflammatory lactoferrin peptides in parotid saliva of periodontitis patients. *Mol Immunol*. 2007;44(7):1498–1508.

29. Teughels W, Loozen G, Quirynen M. Do probiotics offer opportunities to manipulate the periodontal oral microbiota? *J Clin Periodontol*. 2011;38(Suppl 11):159–177.

30. Lactobacillus reuteri Prodentis effective in the treatment of periodontitis. BioGaia [Internet]. 2010 November [cited 2011 March 27]. Available from: http://www.biogaia.com/news/pressreleases/archive/2010/new–study–published

31. Burt BA. The use of sorbitol– and xylitol–sweetened chewing gum in caries control. *J Am Dent Assoc*. 2006;137(2):190–196.

32. Paquette DW, Ryan ME, Wilder RS. Locally delivered antimicrobials: clinical evidence and relevance. *J Dent Hyg*. 2008;82(Suppl 3):10–15.

33. Ma D, Forsythe P, Bienenstock J. Live Lactobacillis reuteri is essential for the inhibitory effect on tumor necrosis factor alpha–induced interleukin–8 expression. *Infect Immun*. 2004;72(9):5308–5314.