Fifteen Years of Probiotic Therapy in the Dental Context
What Has Been Achieved?
Twetman, Svante; Jørgensen, Mette Rose; Keller, Mette Kirstine

Published in:
Journal of the California Dental Association

Publication date:
2017

Document version
Publisher’s PDF, also known as Version of record

Citation for published version (APA):
You are a dentist. One who deserves superior protection and to be rewarded for your loyalty — something this company does quite well. Case in point, the substantial multipolicy discounts with the TDIC Optimum Bundle.

**TDIC Optimum Bundle**

Professional Liability  
Commercial Property  
Workers’ Compensation

Discounts apply to individual policies and are not cumulative. To obtain the Professional Liability premium five (5) percent, two-year discount, California dentists must complete the current TDIC Risk Management seminar. Call 800.733.0633 for current deadlines and seminar details.

**Good**

- 10% discount on Professional Liability when combined with Workers’ Compensation

**Better**

- 10% discount on both Professional Liability + Commercial Property when combined

**Optimum**

- 20% discount on Professional Liability
- 10% discount on Commercial Property
- 5% discount on Workers’ Compensation

**Bonus**

Additional 5% discount on Professional Liability when you take the current TDIC Risk Management seminar.
DEPARTMENTS

513 The Editor/Kiosk Dentistry
515 Letter
517 Impressions
571 RM Matters/Safe and Sober: Managing Employees Who Are Under the Influence
575 Regulatory Compliance/Security Risk Management Plan
578 Ethics/Shedding Light on the Ethics/Well-Being Connection
582 Tech Trends

FEATURES

523 Ecological Approaches to Oral Biofilm Management: Future Perspectives for Dentistry
An introduction to the issue.
Renate Lux, PhD

525 Ecological Events in Oral Health and Disease: New Opportunities for Prevention and Disease Control?
Dental professionals need to be aware of the beneficial functions of the resident oral microbiota, so that treatment strategies are focused on the control rather than the elimination of these natural biofilms.
Philip D. Marsh, PhD

539 Fifteen Years of Probiotic Therapy in the Dental Context: What Has Been Achieved?
The aim of this narrative and subjective review is to briefly provide the general dental practitioner with an update on recent advances concerning the clinical use of probiotics for oral health.
Svante Twetman, DDS, Odont Dr; Mette Rose Jørgensen, DDS, PhD; and Mette Kirstine Keller, DDS, PhD

547 Biofilm Battles: Beneficial Commensals vs. Streptococcus Mutans
The authors describe mechanisms utilized by beneficial bacteria to compete with the dental caries pathogen Streptococcus mutans and how these interactions moderate the pathogenic potential of oral biofilms.
Brinta Chakraborty, PhD; Kyulim Lee, BS; and Robert A. Burne, PhD

557 Targeted Antimicrobial Peptides: A Novel Technology to Eradicate Harmful Streptococcus Mutans
This manuscript reviews current anticaries approaches and a newly developed methodology called specifically targeted antimicrobial peptides (STAMP).
Lihong Guo, PhD, DDS, and Anna Edlund, PhD

565 Oral Microbiota Transplant: A Potential New Therapy for Oral Diseases
This article critically reviews the potential of oral microbiome transplant for managing oral diseases.
Marcelle Nascimento, DDS, MS, PhD
CDA Classifieds work harder to bring you results. Selling a practice or a piece of equipment? Now you can include photos to help buyers see the potential.

And if you’re hiring, candidates anywhere can apply right from the site. Looking for a job? You can post that, too. And the best part—it’s free to all CDA members.

All of these features are designed to help you get the results you need, faster than ever. Check it out for yourself at cda.org/classifieds.

On my first visit to New York City, my parents took us to an Automat. It was a large open room with small glass doors in the surrounding walls. There were labels under each door that described the food item behind the door and stated the cost. You got a tray and went from wall to wall seeking out what you wanted to eat and feeding the requisite number of coins to make the little door open. You put the item on your tray, closed the little door and someone on the other side of the wall immediately replaced the item. A handful of coins got you lunch and dessert and you never had to speak to a waitress or server. It was supposed to be the restaurant of the future.

Automation may be defined as a method of controlling a process by mechanical means thereby reducing human intervention to a minimum. The New York Automat was a primitive adaptation of production-line automation for the food service arena. Now automation is working its way into the medical/dental arena.

We have all seen the drugstore blood pressure centers. A chair with a blood pressure cuff fixed to an armrest is positioned near the pharmacy dispensing window. (I assume that the chair is near the dispensary in case of a malfunction. It could not be a brand enhancer to have a drugstore patron marooned in the soap and deodorant section, squirming and squealing to get free of a cuff that refused to deflate.)

That blood pressure station was the forerunner of the medical kiosk that has made inroads into patient care today. Kiosks run the gamut in design, function and size. Their first tasks were centered on registration or “check-in.” It is easy to see the advantages of automated check-in. The staff is relieved of the patient interview and the information can be immediately integrated into the patient record. But now the kiosk is being used as a more ambitious means of automating the health care experience.

A dental kiosk system has been proposed to reduce cost and inefficiencies associated with dental related emergency room visits. A recent ADA study found that “in 2012, [emergency department] dental visits cost the U.S. health care system $1.6 billion, with an average cost of $749 per visit.” In addition, the study found that 70 percent of the emergency department (ED) visits were “outside of normal business hours.” Some speculate that a significant number of these ED dental visits are repeat patients because when a dentist is not present to administer care, treatment may be limited to pain and antibiotic prescriptions to temporarily alleviate the symptoms. If the patient does not receive follow-up therapeutic care, symptom recurrence could lead to a revolving door of repeated visits to the ED.

The situation presents the potential for technological innovation and a new practice model. Why not reduce the need for dental staffing of the ED with a kiosk interface? At least one company is trying to occupy that niche with a 24 hours a day, seven days a week, 365 days a year triage and treatment referral center. A dental kiosk is placed in the ED (the design displayed on the website is a bit disconcerting: a two-dimensional figure in a white coat, headless, with a touch screen and phone handset attached at the waist).

The kiosk interface connects to a team of “online dental professionals” and the firm’s website information states “a licensed … dental representative will schedule an appointment for the patient at a convenient dental office or clinic within 48 hours.” This could be very effective … or not.

The positive side of the kiosk interface model is clear: triage and appointment. This could be helpful in situations where:
1. The person seeking care may have no dental home.
2. The patient may be unable to seek dental care during normal business hours.
3. The patient may have a dental home in an unsecured area that may be unsafe after hours. (Neither the patient nor the doctor may be eager to meet at night in a deserted or high-crime area.)

However, the drawbacks to the kiosk are also pretty clear. Visual and tactile data may not be easily evaluated due to transmission inadequacies. There may be language barriers. Also, if therapeutic interventions are not immediate and the individual is directed to another location for care, the revolving-door scenario could result from palliative prescriptions.

In addition to these therapeutic problems, kiosks bring with them their own set of technological or security problems.
The word “kiosk” derives from an ancient Persian word meaning outdoor pavilion. A kiosk is open, so privacy and data security are inherently an issue. According to a recent study on medical kiosks, “No one really knows how secure the video streams, credit card information and personal health information are within the kiosks, how easy or not it would be for a cyber thief to hack into the system and steal multiple identities and prescriptions, how secure the cloud is to which the data are being sent and how the data would be destroyed if/when the relationship between the employer and the kiosk manufacturer/supplier ended.”

There is also the question of liability and brand tarnish. A recent article suggested, “When patients see the [respected institution’s] name, for example, they might apply the trust they have for [that institution’s] brand to the kiosk. And if anything were to happen to the data — regardless of whether it’s the fault of [that institution] — the patient is likely to blame the trusted brand that made them comfortable using the kiosk initially.”

A 2012 experiment in using medical kiosks to improve the ED experience provided disappointing results. The failure of the kiosks to be uniformly integrated into the intake process suggested the need to “approach technology implementation as an institutional, social and behavioral change process, not just a technical project.” They observed that the ED workers found ways to work around the technology rather incorporating it into their process.

Automation is the future but the Automat proved that in the food service arena successful integration of automated systems is not a simple linear progression. In medicine and dentistry, social, institutional, technological and cyber security advances will be required before it can be considered an unqualified success.

REFERENCES
Don’t Stop Children’s Inhalers

Congratulations on presenting “The Oral Effects of Inhalation Corticosteroid Therapy: An Update” in the May 2017 issue. It is a well-done and comprehensive article on the potential negative oral consequences of corticosteroid inhalers. While the authors are quite correct about the frequency of oral candidiasis in adults, about 10 percent of patients, clinical thrush is an extremely rare occurrence in children taking low or medium doses of inhaled corticosteroids. In one pediatric study comparing two dose levels of ciclesonide with low-dose fluticasone propionate, for example, there was only one case of thrush among 212 patients in the ciclesonide group and only one case in the fluticasone group, about 0.4 percent for each. In the University of Florida pediatric pulmonary clinic, where thousands of patients have been treated for extended periods with inhaled corticosteroids for persistent asthma, attending physicians could only remember seeing two patients with thrush. It would be a shame if the warnings presented in this article stopped parents from giving this essential medication that prevents exacerbations and need for systemic steroids in children with persistent asthma.

LESLIE HENDELES, PHARMD
Professor emeritus, University of Florida

PHILIP TRASK, DDS, MS
University of California, Los Angeles, pediatric dentistry

REFERENCES
While you’re in charge of running your dental practice, several state and federal agencies are charged with regulating it. That’s why CDA Practice Support has experts and tools to help you keep in compliance. Get guidance on the latest regulations affecting your practice with downloadable checklists, forms, required documents and more.

Call an analyst or explore our library of online resources, including a helpful regulatory compliance manual.

800.232.7645
cda.org/regulatorycompliance
Got Principles?

David W. Chambers, EdM, MBA, PhD

It is usually said that professions such as lawyers, ministers and doctors are more deserving of respect and trust than are beauticians, dog walkers and city planners because they have codes of ethics based on principles. Principles are general guides to behavior. There are dozens and dozens of these, from integrity to helping others, to “first do no harm,” to confidentiality, to loyalty. Anyone who cannot find a principle to support his or her action is not trying very hard. And a few minutes on the web will show that virtually every group, including pro wrestlers and real estate agents now have codes of ethics based on principles.

Trade groups have always been more interested in ethics as principles than have philosophers. Philosophers avoid principles for a couple of reasons.

First, principles suggest what is right to do, but there is a lot of wobble in the system. They are “prima facie” rules, which is just a fancy way of saying “this is a controlling factor unless there are other more controlling factors.” If there is no reason why a dentist should not treat mostly patients on welfare (on some principle such as putting patients’ interests first), the dentist is compelled to do so.

Second, it is absolutely impossible to know when one has found the master moral principle. There is no true north on the moral compass. It is well known that multiple principles conflict. When that happens, professionals are supposed to make a “balanced” decision. There is no “balance” principle and the lead one could vary from person to person causing moral relativism.

Fifty years ago, my college roommate told a stale joke over dinner. He had just been tapped for Phi Beta Kappa and I wanted to know what somebody majoring in topology did. Here is what he said: A slice of bread can always (regardless of shape) be cut so that the two halves are identical in surface area. (You can see right away that this is about fairness.) Now a piece of ham can be added and a cut can still be made that guarantees parity. A third ingredient, perhaps mayo, makes it very difficult but justice can still be served. The fourth plane, always mustard in the story, is the deal breaker. There is no way a plane can be guaranteed to bisect four superimposed planes. Yeah, the punch line is, “Topologists have proven that you can’t always cut the mustard.”

It turns out that this impossibility theorem has been proven mathematically. There is no way we can be sure we have achieved a stable ethical when using all five of the traditional principles of patient respect, beneficence, nonmaleficence, justice and veracity. Even four will be impossible.

David W. Chambers, EdM, MBA, PhD, is a professor of dental education at the University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, and editor of the American College of Dentists.
FOX13 Gene Is Involved in Dental Cusp Formation

Scientists studying the skulls and teeth of pedigreed hairless dogs from the collection of the Phyletisches Museum of the University of Jena in Germany found that hairless dog breeds differ from other dogs not only by lacking a coat but also in the number and nature of their teeth. The study was published in the journal Scientific Reports in July.

Hairless dogs such as the Chinese crested dog and the Mexican hairless dog are among the oldest dog breeds worldwide. The missing hair of these dog breeds is the result of a mutation of the forkhead box I3 gene (FOX13), which belongs to a transcription factor gene family and is also, among others, involved in tooth development, according to the study. The research furthered the understanding of the involvement of the FOX13 gene in the development of teeth, not only in hairless dogs but potentially in other mammals as well, including humans.

By studying the museum’s historical skull collection of hairless and coated dogs, a team led by Kornelius Kupczik, PhD, of the Max Planck Institute for Evolutionary Anthropology, and Martin S. Fischer, PhD, of the Friedrich Schiller Universität Jena, found that hairless dogs are almost completely missing their replacement teeth (i.e., incisors, canines and premolars). The molars, however, were present, according to the study. The deciduous premolars and permanent molars of the hairless dogs repeatedly lacked specific lingual cusps. On the basis of DNA sampled from the more than 100-year-old dog skulls, the researchers demonstrated that these morphological findings are also associated with FOX13 gene variation.

The study also noted that the molar phenotype of the hairless dogs can be found in wild forms of living and extinct carnivores, and the molars of humans and great apes also show varying expressions of the lingual cusps. The researchers from Leipzig and Jena therefore suggest that FOX13 may be of general importance in mammalian tooth development. “It is possible that this gene may have played a role in evolutionary changes of human tooth morphology as well,” Dr. Kupczik said.

Learn more about this study in the Journal of Prosthetic Dentistry (2017); doi: dx.doi.org/10.1016/j.prosdent.2017.02.025.

Minimal Displacement of Zirconia Crowns Occurs in Implants

A study published in the Journal of Prosthetic Dentistry measured the displacement of zirconia crowns in two different implants and found that, while mean vertical displacements were higher than mean displacements in both the mean mesiodistal and buccolingual directions, minimal overall displacement occurred when using these crowns and only the vertical displacement in both implants was statistically significant.

The researchers, led by Hanadi Rebeeaah, BDS, a specialist from the division of prosthetic dentistry at Hamad Medical in Doha, Qatar, and colleagues from The Ohio State University College of Dentistry and College of Engineering in Columbus, Ohio, sought to determine if displacement of screw-retained zirconia-milled single crowns occurred in recently introduced internal conical seal implants (Astra EV, Dentsply Sirona) in three dimensions during screw tightening by hand or torque driver. They compared the amount of crown displacement with the previous internal connection implant (OsseoSpeed, Dentsply Sirona).

According to the study, the crowns on the Astra EV implant tended to undergo more vertical displacement than the crowns on the OsseoSpeed implant. They attributed this increase in displacement to the difference in torque value applied during the torque process — as the tightening torque increases, the abutment displacement increases. However, measured displacements were small and did not exceed 14μm.

The vertical direction had the highest displacement compared with other directions, the researchers found. However, they determined that the differences in displacement directions between the two implants were not statistically significant.

Learn more about this study in the Journal of Prosthetic Dentistry (2017); doi: dx.doi.org/10.1016/j.prosdent.2017.02.025.
Estrogen Therapy May Prevent Gum Disease in Women Over 50

Treatment for osteoporosis may also help prevent gum disease, according to a University at Buffalo study that examined the prevalence of periodontitis in postmenopausal women.

The study, published in the July issue of Menopause: The Journal of the North American Menopause Society, revealed that women over the age of 50 treated with estrogen for osteoporosis are 44 percent less likely to have severe periodontitis than women who did not receive the treatment.

The lack of estrogen, a natural consequence of menopause, places women at risk of osteoporosis as they age. To counter these effects, some women are prescribed estrogen therapy along with supplements of calcium and vitamin D. Although previous studies have investigated the relationship between osteoporosis and tooth loss, few have examined the link between estrogen therapy and periodontitis.

The study research, led by Johelle de S. Passos-Soares, PhD, at the Federal University of Bahia in Brazil, examined nearly 500 postmenopausal women who received service at an osteoporosis diagnosis center in Brazil. Of the 356 women who were diagnosed with osteoporosis, 113 chose to receive estrogen therapy.

The researchers found that women receiving osteoporosis treatment had less periodontal probing depth and clinical attachment loss and less gum bleeding than those who did not receive therapy. The study also found that a higher family income and more frequent consultations with a dentist were associated with a lower prevalence of periodontitis.

Learn more about this study at Menopause: The Journal of the North American Menopause Society (2017); doi: 10.1097/GME.0000000000000830.
New Materials Developed to Stop Degradation of Dentin

A new study led by Salvatore Sauro, PhD, a professor at the CEU Cardenal Herrera University in Spain and a specialist in developing innovative dental biomaterials, researched the development of biomaterials with protective and self-healing remineralizing properties and generated two new dental restorative biomaterials containing bioactive glasses. Dr. Sauro with researchers from Finland, Brazil, Belgium, Germany and the United Kingdom also assessed the biomaterials’ effectiveness in reducing the enzymatic autodegradation of collagen fibers and evoking their remineralization. The study was published recently in the Journal of Dental Research.

The study’s aim was to evaluate the inhibition of endogenous proteolytic enzymes of dentin and the remineralization induced by two different innovative experimental resins containing bioactive glasses: one containing microparticles of Bioglass 45S5 and the other containing microparticles of an experimental bioactive glass enriched with fluoride and a high amount of phosphates. Their antidegradation effects were evaluated on completely demineralized human dentin specimens after immersion in artificial saliva for a period of 30 days by means of an immunohistochemical process. The remineralization evoked by such experimental bioactive resins was also evaluated by means of infrared spectroscopy and scanning electron microscopy.

The resin-based material containing the experimental bioactive glass enriched with fluoride and phosphates resulted to be more efficient in inhibiting the enzymatic-mediated degradation of demineralized dentine collagen and more bioactive than the one containing Bioglass 45S5, according to the study.

“The resin was more effective in reducing the degradation of demineralized dentine collagen via inhibition of matrix metalloproteinases and cysteine cathepsins,” Dr. Sauro said. “This type of experimental bioactive material would therefore be more suitable for the development of new restorative dental materials for the clinical treatment of the dental caries.”

Dr. Sauro spends most of his time in the development of minimally invasive treatments and therapeutic bioactive dental composites for the treatment and restoration of caries-affected dentin, such as those tested in this study.

To learn more about this research, read the study in the Journal of Dental Research (2017); doi: doi.org/10.1177/0022034517709464.

Saliva Molecule Could Heal Wounds

A study published online in The Official Journal of the Federation for American Societies of Experimental Biology (ASEB) delves into the mystifying fact that wounds in the mouth heal faster and more efficiently than wounds elsewhere on the body. Until now, it was understood that saliva played a part in the wound healing process but the extent of its role was unknown. The study examined the effects of salivary peptide histatin-1 on angiogenesis (blood vessel formation), which is critical to the efficiency of wound healing. Researchers found that histatin-1 promotes angiogenesis, as well as cell adhesion and migration.

“These findings open new alternatives to better understand the biology underlying the differences between oral and skin wound healing,” said Vicente A. Torres, PhD, associate professor at the Institute for Research in Dental Sciences within the Faculty of Dentistry at the University of Chile in Santiago, Chile. “We believe that the study could help the design of better approaches to improve wound healing in tissues other than the mouth.”

The study involved experiments at three levels: endothelial, or blood vessel-forming, cells in culture, chicken embryos as animal models and saliva samples obtained from healthy donors. Using these three models, histatin-1 and saliva were found to increase blood vessel formation. Researchers are now taking the next step in this line of study and using these molecules to generate materials and implants to aid in wound healing.

Read more about this study’s research in the FASEB journal (2017); doi:10.1096/fj.201700085R.
Small Molecule Inhibitor Prevents Cavities

Researchers at the University of Alabama at Birmingham have created a small molecule that prevents or impedes tooth cavities in a preclinical model. The inhibitor blocks the function of a key virulence enzyme in an oral bacterium, a molecular sabotage that is akin to throwing a monkey wrench into machinery to jam the gears, according to a study published in July in *Scientific Reports*.

In the presence of the molecule, *Streptococcus mutans* — the prime bacterial cause of tooth decay — is unable to make the protective and sticky biofilm that allows it to glue to the tooth surface, where it eats away tooth enamel by producing lactic acid.

This selective inhibition of the sticky biofilm appears to act specifically against *S. mutans*, and the inhibitor drastically reduced dental caries in rats fed a caries-promoting diet.

New Material Could Fight Cold Sensitivity, Prevent Caries

Researchers have developed a new material with green tea extract that could fix the problem of cold sensitivity and also help prevent cavities in susceptible patients, according to a study published in the journal *ACS Applied Materials & Interfaces*.

Tooth sensitivity commonly occurs when the protective layers of teeth are worn away, revealing a bony tissue called dentin. This tissue contains microscopic hollow tubes that when exposed allow hot and cold liquids and food to contact the underlying nerve endings in the teeth, causing pain. Unprotected dentin is also vulnerable to cavity formation. Plugging these tubes with a mineral called nanohydroxyapatite is a long-standing approach to treating sensitivity. But the material doesn’t stand up well to regular brushing, grinding, erosion or acid produced by cavity-causing bacteria. Researchers wanted to tackle sensitivity and beat the bacteria at the same time.

They encapsulated nanohydroxyapatite and a green tea polyphenol — epigallocatechin-3-gallate (EGCG) — in silica nanoparticles, which can stand up to acid and wear and tear. EGCG has been shown in previous studies to fight *Streptococcus mutans*, which forms biofilms that cause cavities. Testing on extracted wisdom teeth showed that the material plugged the dentin tubules, released EGCG for at least 96 hours, stood up to tooth erosion and brushing and prevented biofilm formation. It also showed low toxicity. Based on these findings, the researchers say the material could be a good candidate for combating tooth sensitivity and cavities.

Learn more about this study at *ACS Applied Materials & Interfaces* (2017); doi: 10.1021/acsami.7b06597.

The researchers explained that their compound is drug-like, non-bactericidal and easy to synthesize and exhibits very potent efficacy in vivo, making it an excellent candidate that can be developed into therapeutic drugs that prevent and treat dental caries.

About 2.3 billion people worldwide have dental caries in their permanent teeth, according to a 2015 Global Burden of Disease study. “If we have something that can selectively take away the bacteria’s ability to form biofilms, that would be a tremendous advance,” said Sadanandan Velu, PhD, associate professor of chemistry in the UAB College of Arts and Sciences and a lead researcher in the study.

Hui Wu, PhD, professor of pediatric dentistry at the UAB School of Dentistry, director of UAB Microbiome Center and also a lead investigator in the study, said their compound is particularly exciting in the broad sense of targeting microbiota using chemical probes tailored to the specific pathogen within a complex microbial community.

“Successful development of this selective lead inhibitor in the dental setting offers a proof of concept that selective targeting of keystone bacteria is promising for the design of new treatments,” Dr. Wu said. “This is relevant for many elusive human diseases as the microbiome is being linked to overall health and disease.”

Read more about this study at *Scientific Reports* (2017); doi:10.1038/s41598-017-06168-1.
Ecological Approaches to Oral Biofilm Management: Future Perspectives for Dentistry

Renate Lux, PhD

Dental plaque, also known as oral biofilm, colonizes all accessible surfaces in the oral cavity. Depending on the composition and behavior of their microbial residents, these biofilms can be beneficial to the host via their ability to keep pathogens in check or foster disease by facilitating overgrowth of disease-associated species. The transition from oral health to disease is the result of ecological shifts triggered by changes in the oral host environment, which lead to microbial imbalance or dysbiosis within these biofilms or dental plaque. Current approaches in dentistry for the treatment of biofilm-associated oral diseases are still predominantly based on biofilm elimination. Recognizing and understanding the underlying ecological factors of oral health-associated symbiosis and disease-causing dysbiosis has led to the possibility of biofilm treatments that aim to restore beneficial plaque ecology with probiotic and prebiotic applications, targeted pathogen elimination as well as microbiota exchange via transplantation. In this issue of the Journal, we present five articles that reflect on the possibilities and challenges of changing oral biofilm ecology from disease to health-promoting microbial biofilm communities.

Oral biofilm composition, behavior and health-/disease-association are governed by ecological concepts. The relationship between host and biofilm is dynamic with a mutually beneficial (symbiotic) relationship during health. Disruption of this balance by environmental changes can trigger drastic shifts in oral biofilm composition and behavior, which lead to microbial dysbiosis and disease. In his article, Philip D. Marsh, PhD, provides an overview of how the oral cavity functions as a microbial habitat and how oral biofilms benefit the host in health. Disruption of this ecological balance leads to disease development. The respective environmental triggers and consequences for oral biofilms are very different for the common oral biofilm diseases caries (nutritionally altered environment) and periodontitis (driven by interplay of host response and inflammophilic biofilm species). Understanding of plaque ecology and knowledge of the underlying processes opens new approaches to dental professionals for the treatment of ecological oral diseases with a focus on biofilm management rather than elimination.

Probiotic treatment to restore and maintain normal function of human-associated biofilm microbiota has been employed as a therapeutic approach for intestinal ailments for more than a century.
Its debut in the oral cavity was only 15 years ago, when a first clinical study showed that the probiotic *Lactobacillus rhamnosus* GG exhibited promising reduction of selected caries risk factors in preschool age children. Svante T wetman, DDS, with co-authors Mette Rose Jørgensen, PhD, and Mette Kirstine Keller, PhD, point out in their article that these findings led to a rapid expansion of probiotic applications to a number of different biofilm-associated oral diseases. Probiotic treatment aims to shift the microbial biofilm communities from a dysbiotic “diseased” state back to a beneficial biofilm via introduction of “good” bacteria, which are safe to use. However, more detailed longitudinal studies are still needed to elucidate the underlying mechanisms and long-term benefits.

Beneficial commensals within oral biofilms have the ability to antagonize pathogenic species and thus prevent disease development. In their article, Robert Burne, PhD, and co-authors Brinta Chakraborty, PhD, and Kyulim Lee, BS, focus on examples of antagonisms against the major cariogenic species *Streptococcus mutans* by commensal streptococci and other beneficial species. Antagonistic actions including alkali generation or hydrogen peroxide production among others are represented by a number of different mechanisms and pathways. This natural anti-*S. mutans* potential of beneficial biofilm species can be enhanced by prebiotic compounds such as arginine, which promote alkali production for plaque pH neutralization.

Caries prevention remains a major oral health challenge. Currently available therapies such as fluoride treatment can be effective but do not remove the etiological disease agent. In their article, Anna Edlund, PhD, and Lihong Guo, DDS, PhD, review an innovative approach to specifically remove the prominent cariogenic species *Streptococcus mutans*. The application of “specifically targeted antimicrobial peptides” (STAMPs) eliminates *S. mutans* from oral biofilms. *S. mutans* removal reduces acid production and alters overall biofilm composition toward a community that is more resistant to *S. mutans* invasion. This novel technology is safe for host cells and comprises a promising future therapeutic.

Curing biofilm diseases via replacement of dysbiotic microbiota with a healthy one is a compelling concept. This approach has garnered a lot of attention due to the success of fecal microbiota transplants in the treatment of severe gastrointestinal infection but is still at the theoretical stage for use in oral diseases. Marcelle Nascimento, DDS, MS, PhD, explores this idea for oral applications and critically reviews the potential of oral microbiome transplants for managing oral biofilm diseases.
Ecological Events in Oral Health and Disease: New Opportunities for Prevention and Disease Control?

Philip D. Marsh, PhD

ABSTRACT The oral microbiome delivers important benefits to the host (symbiosis). Changes to the oral environment drive deleterious shifts in this microbiome (dysbiosis). Low biofilm pH from dietary sugar catabolism selects for acidogenic/acid-tolerating species and promotes dental caries, while inflammation following biofilm accumulation enriches for the proteolytic and anaerobic microbial communities associated with periodontal disease. Prevention depends not only on biofilm control but also on eliminating drivers of dysbiosis, i.e., an ecological approach to disease prevention.

Author

Philip D. Marsh, PhD, is a professor of oral microbiology at the School of Dentistry at the University of Leeds, U.K. He has published more than 250 research papers and review articles and is co-author of a leading textbook on oral microbiology. His research interests include dental biofilms and oral microbial ecology, which has led him to propose the “ecological plaque hypothesis” to explain whether the relationship between the oral microbiota and the host will result in health (symbiosis) or disease (dysbiosis). Conflict of Interest Disclosure: None reported.

Humans have co-evolved with microorganisms and it has been estimated that we are composed of equal numbers of both eukaryotic and prokaryotic cells, i.e., we are 50 percent microbial. These microorganisms, termed the human microbiome, colonize all environmentally exposed surfaces of the body and deliver essential health benefits. In this symbiotic relationship, the resident microorganisms gain a warm and nutritious habitat and in return play an active role in a wide range of important functions, including the:

- Digestion of food and the generation of vitamins and energy.
- Regulation of our cardiovascular system.
- Development of normal intestinal morphology and function.
- Development and regulation of the immune system.
- Exclusion of exogenous and often pathogenic microorganisms (a process termed “colonization resistance”).

The human microbiome varies in composition at, but is characteristic of, distinct surfaces of the body such as the skin, mouth, gastrointestinal and urogenital tracts. Thus, the microbiome found on the skin of a number of individuals is very similar but is markedly different to that found at other body sites on the same person, despite the continued transfer of microorganisms between these sites. These consistent variations in the
microbiome at these habitats around the body are a direct consequence of the selection pressures that operate at each site due to important differences in key physical and biological properties.

The relationship between the host and the microbiome is dynamic and active, and while the composition of the microbiome in health at any site is relatively stable over time, this can be perturbed by, for example, changes in lifestyle, immune status or following broad-spectrum antibiotic therapy. This can cause a deleterious change in the balance of the microbiome at a site that could potentially lead to pathological consequences (dysbiosis). In the gut, this can result in inflammatory bowel diseases (e.g., ulcerative colitis and Crohn’s disease) and colorectal cancer. Changes to the composition and metabolism of the gut microbiota have also been linked to obesity, rheumatoid arthritis, neurological disorders and insulin resistance.

From the above, it can be seen that the microorganisms that comprise the resident human microbiota are not mere passengers but are an integral and intimate part of our makeup and play essential roles in maintaining our general health. The characteristic site specificity of the human microbiome is direct evidence of an important ecological principle, namely that the prevailing environmental conditions at each habitat determine which organisms can colonize and which will predominate or be only minor components of the microbial community. When the same principles are applied to the oral cavity, it is possible to obtain insights into the factors that determine whether the oral microbiome will have a symbiotic or dysbiotic relationship with the host; this will be discussed later.

The Mouth as a Microbial Habitat

The mouth is similar to other habitats in the body in supporting the growth of a natural and characteristic community of microorganisms (the oral microbiome) that also delivers benefits to the host (Figure 1). The mouth is warm and moist and is supplied with a broad array of proteins and glycoproteins from saliva and gingival crevicular fluid (GCF) that are critical for key physiological functions (lubrication, host defenses, etc.), but which can also be exploited by microorganisms as nutrients. Therefore, a diverse range of viruses, bacteria, Archaea, fungi and even protozoa can be commonly isolated from the mouth, of which bacteria are the most numerous and diverse group — approximately 700 species have been identified. These microorganisms colonize mucosal and dental surfaces and persist in the mouth by forming three-dimensional, structurally organized multispecies interactive communities termed biofilms. In general, desquamation ensures that the microbial load on mucosal surfaces is kept relatively low, but the mouth is a unique site in the body in that it provides nonshedding surfaces (teeth, dentures, implants) for microbial colonization. This can result in the accumulation of large numbers of microorganisms, particularly at stagnant and hard-to-clean sites, unless patients practice effective oral hygiene. The biofilms that form on teeth have previously been referred to as dental plaque.

A number of environmental factors influence the distribution and metabolic activity of the resident oral microbiome (Figure 2). The mouth is maintained at a temperature of approximately 35–37 degrees Celsius, which is suitable for the growth of a...
broad range of microbes. Although the mouth is overtly aerobic, the majority of oral bacteria are either facultatively or obligately anaerobic. The distribution of these anaerobic bacteria in the mouth is generally related to the redox potential (Eh), which measures the degree of oxidation-reduction at a site. The gingival crevice has the lowest Eh in the healthy mouth and harbors the largest numbers of obligately anaerobic bacteria.

As oral bacteria exist as members of microbial communities, many anaerobic species survive in more aerobic habitats by existing in close partnership with oxygen-consuming species. Oral anaerobes also express a range of enzymes whose function is to scavenge low levels of oxygen in the environment to enable them to survive.

In the mouth, pH is a major determinant of bacterial distribution and metabolism. The buffering activity of saliva plays a major role in maintaining the intraoral pH at around neutrality, which again is optimal for the growth of most members of the oral microbiome. Changes in environmental pH occur, however, following dietary sugar consumption. Many beneficial oral bacteria can tolerate brief conditions of low pH, but their growth is inhibited by prolonged or frequent exposures to acidic conditions.

Oral microorganisms gain substantial advantages by growing as a biofilm and by functioning as a microbial community. Microorganisms are in close proximity to one another in biofilms, thereby providing many opportunities for synergistic interactions. For example, bacteria with complementary enzyme profiles can collaborate to metabolize structurally complex host molecules, such as salivary glycoproteins, that would be recalcitrant to the action of single species. The metabolism of these communities is also energetically more efficient, with food chains and food webs developing to catabolize substrates to the simplest end products of metabolism. In this situation, the metabolic product of one organism becomes a primary nutrient source for a second, and in this way, a number of nutritional interdependencies develop. This helps to promote resilience (to change) and stability within the microbial community. Oral microbial communities also display a broader habitat range, for example, with obligate anaerobes being able to persist at sites that are overtly aerobic. Biofilms are inherently tolerant to environmental stresses, the host defenses and antimicrobial agents. The latter can be due to the limited access or penetration of molecules within the biofilm, while cross-protection of sensitive species can occur due to co-location near organisms that produce neutralizing enzymes (e.g. β-lactamase, catalase, etc.). In this way, the properties of microbial communities are more than the sum of the constituent species and such multispecies biofilms display “emergent properties.”

**Benefits of the Oral Microbiota to the Host**

As with other habitats in the body, the relationship between the oral microbiota and the host is generally harmonious. The microorganisms are maintained in a favorable growth environment and supplied with a diverse array of host molecules that serve as nutrients while the resultant microbiome provides benefits to the host. As stated earlier, the resident oral microbiota prevents the establishment of the many exogenous microorganisms that serve as nutrients while the resultant microbiome provides benefits to the host.
microbiota is disrupted, for example, by long-term exposure to broad-spectrum antimicrobial agents, a consequence of which can be an overgrowth by yeasts or by environmental organisms.

The relationship between the oral microbiome and the host is not passive but is active and dynamic and as a result is susceptible to change (FIGURES 3A and 3B). There is active communication ("cross talk") between the resident oral microbiota and host cells to avoid a damaging or excessive inflammatory response to these beneficial organisms. Some resident bacteria, especially streptococci, are involved in this cross talk and downregulate potentially proinflammatory host responses to members of the normal oral microbiota, such as the Gram-negative commensals, which could be damaging to host tissues. Despite this, the host is still able to retain the ability to respond to genuine microbial threats.

Resident oral bacteria make a major contribution to the general health of their host by regulating gastrointestinal and cardiovascular systems via the metabolism of dietary nitrate. Approximately 25 percent of ingested nitrate is secreted in saliva, from where it is reduced to nitrite by commensal oral bacteria. Nitrite regulates blood flow, blood pressure and gastric integrity and is converted to nitric oxide in the acidified stomach. This has antimicrobial properties that contribute to the defense against enteropathogens and in the regulation of gastric mucosal blood flow and mucus formation.

These properties emphasize that it is essential to maintain a natural oral microbiome so as not to lose the beneficial functions of these resident oral microorganisms, and this has implications for treatment strategies. As already stated, the symbiotic relationship between the oral microbiome and the host is dynamic (FIGURE 3A) and will be affected by changes in lifestyle (e.g., diet, smoking, antibiotic treatment, etc.), decreased rates of saliva flow, inadequate oral hygiene and compromised host defenses that can alter microbe — microbe interactions and ultimately microbe — host interactions (FIGURE 3B). Oral health is more than the absence of disease and needs active promotion and management.

Dental Biofilms in Disease

Numerous studies using either traditional culture or contemporary molecular approaches have compared the microbiota in biofilms from healthy sites to those with dental caries and periodontal diseases. The principal findings from these investigations have shown that there are substantial differences in the composition of the microbiota in disease (FIGURE 4). Caries is associated with individuals who regularly consume fermentable carbohydrates and have a high plaque index. Periodontal disease is also associated with a high plaque index and the presence of specific bacterial species, such as Porphyromonas gingivalis and Treponema denticola. These bacteria produce toxins that damage the periodontal ligament and destroy bone and connective tissue.

FIGURE 3A. In health, a natural balance is maintained between host and environmental factors that results in a stable microbiota (i.e., microbe-microbe interactions are in equilibrium) and a beneficial relationship with host tissues is established. This symbiotic relationship is susceptible to change.

FIGURE 3B. A major change in the oral environment or lifestyle of the host can upset the delicate balance that exists among the many species that make up the oral microbiota. Previously minor components can become more competitive and predominate, which disrupts the previously symbiotic relationship with the host and increases the risk of disease.

FIGURES 3. A schematic representation of the dynamic relationship between the oral microbiome and the host environment in health and disease.
Carbohydrates, especially sucrose. These dietary sugars are converted to acids which result in prolonged conditions of low pH in the biofilm, while intracellular and extracellular polysaccharides can be formed from sucrose. There is a similar outcome if the flow of saliva is impaired either due to aging or other physiological reasons, radiotherapy or as a side effect of medication. The diversity of the microbiome decreases in samples taken from carious enamel lesions, probably because of the more extreme environment at these sites. Molecular-based (culture-independent) approaches have not always confirmed the numerical dominance of mutans streptococci in caries and support the concept of caries as having a polymicrobial etiology. Recently recognized species such as *Scardovia wiggsiae* and *Slackia exigua*, as well as some more common cariogenic species, have been detected in early childhood caries. No one species is found exclusively in disease.

Common properties of these cariogenic organisms are the ability to rapidly metabolize dietary sugars to acid (mainly lactic acid) and to preferentially grow under the acidic conditions so generated (Figure 4). In addition, some species synthesize intracellular and extracellular polysaccharides (EPS) from sucrose. The former act as energy reserves and can be converted to acid in the absence of dietary sugars while the latter contribute to the extracellular polymeric matrix of the biofilm. These properties are not exclusive to any one group of bacteria, however, and several oral species are saccharolytic and/or synthesize EPS. There is a spectrum of expression of these traits ranging from bacteria that preferentially grow at neutral pH and display relatively slow rates of glycolysis through to organisms that can drive the pH to below pH 4.5 in a few minutes and grow optimally under acidic conditions, with every combination in between. Furthermore, there are strains of streptococci from species such as *S. mitis* and *S. oralis* that are as acidogenic under certain conditions as some isolates of *S. mutans*. However, mutans streptococci are adapted for growth at low pH and display all of the above cariogenic properties.

---

**Figure 4.** A schematic representation of some of the shifts in the composition and activities of the oral microbiome in disease.
By applying these ecological concepts further, it is perhaps not surprising that the microbiota associated with caries affecting the dentine shows an increase in diversity as a consequence of the altered and nutritionally more varied environment found on the root surface during lesion formation. The exposure of proteins including collagen as a root surface lesion progresses provides nutrients to support the growth of proteolytic bacteria, and the presence of consortia of both anaerobic, Gram-negative proteolytic bacteria in combination with saccharolytic species have been reported.

An inflammatory response to biofilm accumulation is one of the common features of periodontal diseases, though lifestyle factors (e.g., smoking) and a compromised immune system can increase the likelihood of disease. In periodontitis, an inflammatory response is triggered if biofilm accumulates around the gingival margin beyond levels compatible with oral health. The flow of GCF is increased to deliver components of the host defenses (immunoglobulins, complement, neutrophils, cytokines, etc.) in response to the microbial challenge. This response will inhibit susceptible species, but a number of subgingival organisms (including Porphyromonas gingivalis) can subvert these defenses, for example, by degrading complement, interfering with neutrophil function and blocking phagocytosis. The species isolated from periodontal pockets have been termed infl ammophilic in that they are capable not only of surviving the host response but also of exploiting and thriving under the altered environmental conditions. An unintended consequence of the increased flow of GCF is that other host molecules present in this exudate can act as nutrients for some of the proteolytic, but normally minor, components of the subgingival microbiota. A consistent feature of the numerous traditional culture and more contemporary molecular-based (culture-independent) studies is that periodontitis is associated with an enormous diversity and richness of bacterial species. Many of these organisms are nutritionally fastidious and obligately anaerobic and remain difficult or currently impossible to grow in even the most advanced laboratories but can satisfy their growth requirements from the catabolism of host molecules. The bacteria found at sites with periodontitis exist as microbial complexes or consortia. In early studies, the strongest association with advanced periodontitis was linked to the presence of three bacterial species (P. gingivalis, Treponema denticola and Tannerella forsythia) that were designated the “red complex.” Their presence was often preceded by other consortia which included various Prevotella species, Fusobacterium nucleatum, Campylobacter species and Eubacterium nodatum (termed the “orange complex”), while other bacterial groupings were associated with periodontal health. More recently, studies using the sequencing of 16S rRNA genes and metagenomic approaches have further emphasized the complexity of the microbiota associated with periodontal diseases and have discovered the presence of a large proportion of novel bacteria, some of which have no cultivable examples and many are currently unnamed.

A large number of studies on different patient cohorts have now been completed using culture-independent approaches, and 400–500 oral taxa have been detected subgingivally. There is no great consistency in defining the predominant species implicated in disease, and generally inflammation is associated with diverse polymicrobial communities. A recent systematic review of the literature comprising data from 41 studies found moderate evidence to support the association of 17 newly identified species with periodontitis. The variations in microbial composition of subgingival biofilms isolated from diseased sites might be a result of fundamental biological differences in the clinical status of the pocket and/or due to the technical methods used to sample, process (e.g., variations in DNA extraction methods) and analyze the data (e.g., the data might be influenced by the bioinformatic pipelines and the sequencing platforms used), but it might also reflect that consortia with a different composition can cause similar clinical signs. To date, the field has been preoccupied with accurately naming the members of these communities, whereas it could be more instructive if we determined the function or role of each organism within the consortium. It is probable that bacteria with different names could be performing identical functions within a community. Hence, we might see a greater consensus across studies looking at diseased sites if we reported by microbial function rather than by bacterial name.

In periodontitis, an inflammatory response is triggered if biofilm accumulates around the gingival margin beyond levels compatible with oral health.
Where Do the Putative Pathogens Come From?

An intriguing question is the source of these potential dental pathogens. Theories have ranged from the acquisition of these organisms from close personal contacts to translocation from mucosal reservoirs. An answer to this question could help with developing effective prevention and control strategies.

In many of the studies of supragingival plaque in relation to caries, although mutans streptococci and/or lactobacilli are generally found in higher proportions where lesions are found, their presence is not diagnostic at a site- or person-level and caries can develop in the apparent absence of these species. These species have also been detected at low levels from clinically sound surfaces.51 Likewise, the application of sensitive, culture-independent molecular techniques has led to the occasional detection from the healthy gingival crevice of many of the bacteria associated with periodontal diseases but in low numbers.51 A logical interpretation of these findings is that these dental diseases have a polymicrobial etiology that is most likely due to deleterious shifts in the composition of the biofilm (dysbiosis) rather than as a result of exogenous “infection” with a classical pathogen.32,38,54 Disease is associated, therefore, with markedly higher proportions of certain species that when present in health are normally noncompetitive with the beneficial bacteria and hence are only minor components in the biofilm. An even more fundamental question that follows, therefore, is what are the drivers of dysbiosis in the dental biofilms that lead to these undesirable changes in microbiota associated with disease? New possibilities of preventing disease open up if these drivers can be identified.

Theories To Explain the Etiology of Plaque-Mediated Diseases

The interpretation of the results from the early cultural cross-sectional studies of plaque in relation to caries or periodontitis was that although the microbiota was complex and diverse, disease was the outcome of the metabolism of a small subset of organisms and the term “specific plaque hypothesis” was coined.53 This was very helpful as it meant that future studies of the etiology of disease and ways to prevent disease could be focused on microbiota of ever increasing richness and complexity. A number of species with relevant traits for provoking inflammation and/or causing tissue destruction were isolated, while disease sometimes occurred without the purported pathogens being detected.48,53 Over time, therefore, as more and more studies were conducted, the “specificity” argument became less clear-cut especially when disease could also occur in the apparent absence of the proposed pathogens, while these organisms could also be detected at low levels at healthy sites. During this period, an alternative viewpoint was being put forward, which proposed that disease is the outcome of the overall activity of the entire microbial community (the “nonspecific plaque hypothesis”).59 It should be noted, however, that if the bacteria found at diseased sites were not identical, they did share common properties. So if the etiology was not entirely specific in terms of bacterial name, they demonstrated evidence of specificity in terms of metabolic function and activity and certain species were found consistently from sites exhibiting disease.

A Contemporary Approach To Explain the Relationship Between the Resident Oral Microbiota and Dental Disease – an Ecological Perspective

An alternative hypothesis was subsequently proposed in order to reconcile the strengths and inconsistencies in the above hypotheses while recognizing and emphasizing the important role of lifestyle and the host environment in causing dysbiosis. It is not disputed that there is a substantial change in the microbial composition of the biofilm in disease compared to oral health (Figure 4). In other ecosystems, such dramatic
shifts in microbiota are associated with a major alteration to the habitat, such as changes to the nutrient status (e.g., the overgrowth of algae in rivers following the wash-off of nitrogenous fertilizers from neighboring farmland), pH (e.g., the disruption of aquatic life in lakes by acid rain) and atmosphere or immune status (e.g., reactivation of latent Mycobacterium tuberculosis in the lungs of HIV-infected patients).

Therefore, against this background the ecological plaque hypothesis was put forward to explain the role of oral bacteria in dental diseases (FIGURES 5A and 5B). This hypothesis proposed that major changes to key determinants in the local environment will alter the competitiveness of individual bacteria within the biofilm leading to the enrichment of organisms most suited to the new environment. In certain situations, these changes will increase the risk of dental caries or periodontal diseases.

In caries, an increased frequency of sugar intake, or a reduction in saliva flow, results in supragingival biofilms spending more time at low pH. This selects for acid-producing and acid-tolerating species at the expense of health-associated bacteria that grow preferentially at pH values around neutrality (FIGURE 5A). Mutans streptococci and lactobacilli, among other species, have evolved to have the metabolic machinery to rapidly consume dietary sugars and grow optimally at pH values around 5.0–5.5, unlike health-associated bacteria that utilize more complex substrates, such as host glycoproteins, and whose growth is inhibited at low pH. Laboratory studies of communities of oral bacteria have shown that cariogenic species, such as S. mutans and Lactobacillus casei growing on mucin at neutral pH (conditions reflecting a healthy mouth), are noncompetitive with other oral species, and the consortium was dominated by streptococci associated with health, such as S. gordonii and S. oralis. Even when a fermentable sugar is introduced but the pH is artificially controlled at pH 7.0, the cariogenic bacteria remain less than 1 percent of the community. However, when the pH is allowed to fall naturally after each carbohydrate pulse, as would occur in real life, the numbers and proportions of the cariogenic species rise incrementally causing the pH to fall further and faster. This occurs at the expense of the beneficial bacteria with the consequence that after 10 days of pulsing, the cariogenic species made up more than 50 percent of the microbial community.

These changes have two major impacts:
- Conditions of low pH rather than sugar availability per se drives dysbiosis in terms of the selection of cariogenic bacteria.

![FIGURE 5A. The ecological plaque hypothesis. In caries, if the biofilm spends more time at a low pH due to increases in sugar consumption (amount and/or frequency) then beneficial bacteria are inhibited and outcompeted by organisms that are adapted to growing under acidic conditions. These organisms include mutans streptococci, bifidobacteria and lactobacilli, but not exclusively. These acidogenic and acid-tolerating species can make even more acid and at faster rates, thereby increasing the risk of lesion development. The selection of these cariogenic bacteria also occurs if saliva flow is reduced, but the process can be reduced by dietary control, effective oral hygiene and the appropriate use of fluoride-containing oral care products.](image-url)
The increased levels and activity of these cariogenic bacteria results in even lower pH values being reached in the biofilm, which provides an even stronger selection pressure for acidogenic and aciduric bacteria. In this way, positive feedback loops develop, which accelerate dysbiosis and create an environment that promotes demineralization. Some supragingival bacteria are also able to adapt to regular conditions of low pH. This has been reflected in the extended ecological plaque hypothesis. In both versions of the ecological plaque hypothesis, however, the major driver of dysbiosis is the acid produced (and resultant low environmental pH) from the bacterial catabolism of dietary sugars and especially sucrose because of its additional role in intracellular and extracellular polysaccharide production. In these laboratory studies, it was established that there was an inverse relationship between the terminal pH reached and the proportions of cariogenic species that suggested that dysbiosis could be reduced or prevented if the environmental pH could be controlled. Subsequent studies confirmed that inhibitors of acid production could indeed prevent the enrichment of acid-tolerant and potentially cariogenic streptococci by eliminating the requisite conditions of low pH that give these organisms a competitive advantage over beneficial species.

In periodontal diseases, the subgingival environment undergoes significant change because of the host mounting an inflammatory response when plaque accumulates beyond levels that are compatible with health (FIGURE 5B). The host increases the flow of GCF in order to deliver the host defenses into the crevice. However, if the biofilm is not reduced or removed, an unintended consequence of this host response is that a number of host molecules present in GCF (including transferrin, haptoglobin, hemopexin, hemoglobin, etc.) can be exploited as primary nutrient sources by proteolytic Gram-negative anaerobes that have the potential to act as periodontal pathogens. For example, black-pigmented anaerobes such as Prevotella intermedia and P. gingivalis have an absolute requirement for hemin for growth and derive this cofactor from the catabolism of host molecules such as hemoglobin. Laboratory and animal studies have shown that an increase in hemin availability dramatically increases the protease activity and virulence of P. gingivalis. A further consequence of this proteolytic metabolism is an increase in local pH and a fall in the redox potential. These environmental changes also promote the upregulation of some of the virulence factors associated with...
these putative pathogens and favor their growth at the expense of the species associated with gingival health (i.e., increases the competitiveness of the potential pathogens). If sustained, the combined selective pressures of changed nutrient supply, elevated pH and lower redox potential leads to a rearrangement of community structure and an enrichment of the proportions of the anaerobic and proteolytic components of the microbiota (FIGURE 5B).

Microbial proteolytic activity in the developing pocket results in cleavage of host defense molecules and a subverted and exaggerated inflammatory response causing bystander damage to the subgingival tissues. This provides an even broader range of host molecules for the increasingly metabolically versatile microbial community. Most of the tissue damage in the developing periodontal pocket is a consequence of this excessive and inappropriate host response. Thus, in the ecological plaque hypothesis for periodontal disease, a positive feedback loop can develop in which, if the host fails to control the initial microbial insult, the disproportionate response to the subgingival biofilm inadvertently provides conditions that will further select for the pathogens that will subsequently continue to drive inflammation. It is recognized that disease is an outcome of the activity of multiple species acting in concert (pathogenic synergism or polymicrobial synergy). Some of the putative pathogens can be present in low numbers but exert a disproportionate influence on other members of these communities to drive inflammation and have been referred to as keystone pathogens.

**Ecological Plaque Hypotheses: Implications for Treatment**

The ecological plaque hypothesis in its original and extended form, and the more recent polymicrobial synergy and dysbiosis model for periodontal disease, recognizes the direct and dynamic link between local environmental conditions in the mouth and the activity and composition of the biofilm community, so that any change to the host environment will induce a response in the microbiota and vice versa. A key principle of the original ecological plaque hypothesis, however, is that long-term prevention of dental diseases will only be achieved by interfering with the underlying changes in host environment that drive the deleterious shifts in the microbiota. This could be by improving oral hygiene practices to more effectively disrupt or remove biofilm by lifestyle changes, such as altering the diet or by using oral care products or other approaches that interfere with the drivers of dysbiosis and/or promote symbiosis (TABLE).

The repeated production of acid from the microbial fermentation of dietary sugars and the regular lowering of the pH in dental biofilms over time selects for acidogenic and acid-tolerating bacteria while inhibiting the growth of beneficial species (FIGURE 5A). Approaches that could reduce microbial acid production include the use of oral care products containing inhibitors (TABLE). Fluoride, in addition to its role in preventing demineralization and promoting remineralization, can interfere with several aspects of sugar metabolism by oral saccharolytic bacteria, including the inhibition of sugar transport and glycolysis. Antimicrobial agents that are commonly formulated into oral care products persist in the mouth for long periods at sublethal concentrations. At these levels, these agents can also inhibit sugar metabolism and acid production and also inhibit enzymes (glucosyltransferases) that synthesize polymers that form the biofilm matrix. Patients can be encouraged to reduce the intake of sugar between main meals either by dietary restriction or by consuming snack foods and beverages that contain sugar substitutes (e.g., sugar alcohols such as xylitol or erythritol or intense sweeteners like aspartame or saccharin). These sugar substitutes cannot be fermented at all (or only slowly) by oral bacteria, thereby reducing damage from bacterial
acids to dental hard tissues and removing the environmental conditions needed for acid-tolerating bacteria to outcompete beneficial species, while they also stimulate saliva flow, which delivers numerous important benefits to the oral ecosystem. Attempts have also been made to raise the pH in the biofilm, for example, by delivering supplements such as arginine or urea that can be metabolized by plaque bacteria to alkali\(^\text{19,80,81}\) (TABLE). In silico, modeling has provided evidence to support the favorable accumulative impact that small but regular inhibitory effects can have over prolonged periods on maintaining a healthy, beneficial biofilm by suppressing the growth and activity of potentially deleterious bacteria.\(^\text{82–84}\)

The majority of the bacteria associated with periodontal diseases are both obligately anaerobic and highly proteolytic. The growth of these bacteria, therefore, depends on the plentiful supply of essential nutrients (proteins, peptides) and cofactors, such as hemin, and a low redox potential. Strategies have been investigated to alter the subgingival environment to make it unfavorable for the growth of putative periodontal pathogens (TABLE). These include delivering redox agents that raise the local Eh in the periodontal pocket,\(^\text{87}\) novel anti-inflammatory agents that promote tissue healing while also reducing the flow of GCF,\(^\text{89,90}\) which in turn denies the microbiota access to factors essential for their growth and antimicrobial agents in oral care products that at sublethal concentrations inhibit bacterial proteases.\(^\text{90}\)

Disease can also be treated by targeting the putative pathogens directly (e.g., with conventional antimicrobial agents or by novel compounds such as the specifically targeted antimicrobial peptides).\(^\text{91}\) Other approaches that are being investigated include identifying oral probiotic bacteria\(^\text{92}\) and/or creating nonpathogenic strains that can prevent colonization by wild-type organisms (replacement therapy, e.g., using molecular biology to produce strains of \(S.\) mutans that are unable to produce lactic acid but which also secrete a bacteriocin to inhibit and exclude natural strains of mutans streptococci).\(^\text{93}\) Oral streptococci have recently been isolated from caries-free individuals that could form the basis of novel oral probiotic bacteria. These streptococci were arginolytic (i.e., could raise the \(\text{pH}\) in biofilms following acid production) and also produced natural antimicrobial agents that could inhibit the growth of \(S.\) mutans.\(^\text{95,86}\)

The deliberate re-implantation of resident bacteria into surgically treated periodontal pockets has also been evaluated as a means of promoting colonization resistance and tissue healing.\(^\text{94}\) Research is also underway to identify molecules (prebiotics) that might actively promote the growth of the resident microbiota.\(^\text{95}\) Recent studies have identified a number of compounds that are able to selectively stimulate the growth of commensal beneficial bacteria in mixed culture laboratory models.\(^\text{96}\)

**Conclusions**

Diverse communities of microorganisms naturally inhabit the mouth and play an active and important role in the normal development of host tissues and functions and in the maintenance of health. The symbiotic relationship between the oral microbiome and the host can be disrupted by changes in environmental conditions in the mouth leading to the outgrowth of previously minor components of the biofilm, thereby increasing the risk of disease (dysbiosis). The drivers for dysbiosis differ between caries and periodontal disease. The metabolism of dietary carbohydrates (and especially sucrose) selects for acid-producing and acid-loving species while the inflammatory response to excessive plaque accumulation around the gingiva enriches for proteolytic, obligately anaerobic and inflammophilic microbial consortia.

The dental team deals with the consequence of the deleterious shifts in the microbial composition and metabolism of the biofilm. These changes can be explained by ecological principles, and disease has been likened to minor ecological catastrophes.\(^\text{54}\) An ecological plaque hypothesis has been proposed to explain the relationship between the environment, the oral microbiome and the integrity of host tissues. Implicit in the ecological plaque hypothesis is the fact that disease will inevitably reoccur and the patient will continue to present with caries or periodontal diseases unless the underlying predisposing factors that are driving these deleterious shifts in the microbiota are addressed. An ecological approach to thinking about dental diseases creates opportunities for novel therapeutic strategies and supports a more holistic and personalized approach to treatment planning for patients. Dental professionals need to be aware of the beneficial functions of the resident oral microbiota, so that treatment strategies are focussed on the control rather than the elimination of these natural biofilms.
REFERENCES

peptostreptococci and fusobacteria. A Van Leeuw J Microb
65. ter Steeg PF, van der Hoeven JS, de Jong MH, van Munster
Pj, Jansen MJH. Modelling the gingival pocket by enrichment of
subgingival microflora in human serum in chemostats. Microbial
66. ter Steeg PF, van der Hoeven JS. Development of
periodontal microflora on human serum. Microbial Ecology in
hemin on the physiology and virulence of Bacteroides gingivalis
68. Join S, Darveau RP. Contribution of Porphyromonas
gingivalis polysaccharide to periodontitis. Periodontol 2000
2010;54(1):53–70.
69. Marsh PD, McKee AS, McDermid AS. Continuous culture
studies. In: Shah HN, Mayrand D, Genco RJ, eds. Biology of
the species Porphyromonas gingivalis. Boca Raton, Fla.: CRC
70. O’Brien-Simpson N, Veth PD, Dashper SG, Reynolds EC.
Porphyromonas gingivalis gingipains: The molecular teeth of a
71. Ryder MI. Comparison of neutrophil functions in aggressive
72. Meyle J, Chapelle I. Molecular aspects of the pathogenesis
73. Lamont RJ, Hajishengallis G. Polymicrobial synergy
and dysbiosis in inflammatory disease. Trends Mol Med
74. van Steenbergen TJM, van Winkelhoff AJ, de Groot J.
Pathogenic synergy: Mixed infections in the oral cavity. Antimicrob
75. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen
clinical study to determine the effect of a toothpaste containing
enzymes and proteins on plaque oral microbiome ecology. Sci Rep
2017;7:43344.
the oral ecosystem induced by the use of 8% arginine
78. Marsh PD. Controlling the oral biofilm with antimicrobials. J
79. Marsh PD. Contemporary perspective on plaque control. Br
80. Gordan VV, Garvan CW, Ottenegger ME, et al. Could algal production be considered an approach for caries control?
Caries Res 2010;44:547–54.
81. Burne RA, Marquis RE. Algal production by oral bacteria
and protection against dental caries. FEMS Microbiol Lett
82. Head DA, Marsh PD, Devine DA. Norlethral control of the
cariogenic potential of an agent-based model for dental plaque.
pone.0105012.
83. Marsh PD, Head DA, Devine DA. Ecological approaches
to oral biofilms: Control without killing. Caries Res 2015;49
Suppl 1:46–54.
84. Marsh PD, Head DA, Devine DA. Dental plaque as a
biofilm and a microbial community—Implications for treatment.
85. Lopez-Lopez A, Camelo-Castillo A, Ferrer MD, Simon-
Soro A, Mira A. Health-Associated Niche Inhabitants as
Oral Probiotics. The Case of Streptococcus dentisani. Front
Microbiol 2017;8:379.
Streptococcus Species That Potently Antagonizes Streptococcus
87. Wilson M, Gibson M, Strahan D, Harvey W. A preliminary
evaluation of the use of a redox agent in the treatment of
88. van Dyke TE. The management of inflammation in
89. Hasturk H, Kantarci A, van Dyke TE. Paradigm shift in the
pharmacological management of periodontal diseases. Front Oral
90. Marsh PD, Head DA, Devine DA. Ecological approaches
to oral biofilms: Control without killing. Caries Res 2015;49
Suppl 1:46–54.
91. He J, Yarbrough DK, Kreth J, et al. Systematic approach
to optimizing specifically targeted antimicrobial peptides
against Streptococcus mutans. Antimicrob Agents Chemother
92. Gruner D, Paris S, Schwindewicke F. Probiotics for managing
caries and periodontitis. Systematic review and meta-analysis. J
93. Hillman JD. Replacement therapy for dental caries. In:
biofilms in health and disease. Cardiff: Bioline; 1999. pp
587–99.
periodontal pocket re-colonization: A proof of concept. J Dent
95. Devine DA, Marsh PD. Prospects for the development of
probiotics and prebiotics for oral applications. J Oral Microbiol
2009;1 DOI: 10.3402/jom.v1i0.1949.
Nutritional stimulation of commensal oral bacteria suppresses

THE AUTHOR, Philip D. Marsh, PhD, can be reached at
p.d.marsh@leeds.ac.uk.

Bring in a new member, get $200.

Refer a new member to organized dentistry and receive
double the rewards!

Get our thanks, plus a $100 check from CDA and a
$100 American Express gift card from the ADA for every
referral.* Share with your peers why you love being part
of a community of 27,000 CDA members—all who are
making the profession stronger than ever.

Visit cda.org/mgm to learn more.

* Total rewards possible per calendar year are
limited to $500 from CDA and $500 in gift
cards from the ADA.
More than 27,000 members working together means that CDA is growing stronger than ever. With an easier online application, valuable special offers and rewards for referring new members, there’s no better time to share our support, advocacy, education and protection. See how membership helps build professional connections and creates pathways for success.

Connect to the best and brightest in dentistry.

cda.org/member
Fifteen Years of Probiotic Therapy in the Dental Context: What Has Been Achieved?

Svante Twetman, DDS, Odont Dr; Mette Rose Jørgensen, DDS, PhD; and Mette Kirstine Keller, DDS, PhD

ABSTRACT Many oral diseases are driven by an ecological shift from a balanced microbial consortium to dysbiotic communities with reduced diversity. Probiotic bacteria offer an opportunity to prevent and manage conditions such as dental caries, periodontal conditions and candidiasis. Regular intakes may support a healthy microbiome via direct interference with the biofilm and systemically through modulation of the host’s immune response. Placebo-controlled trials have shown substantial beneficial effects but further research is needed for general treatment recommendations.

AUTHORS

Svante Twetman, DDS, Odont Dr, is a professor of cariology in the department of odontology at the Faculty of Health and Medical Sciences at the University of Copenhagen, Denmark. His interest is in the prevention and treatment of biofilm mediated oral diseases.

Mette Rose Jørgensen, DDS, PhD, is assistant professor in the section for oral medicine, department of odontology at the Faculty of Health and Medical Sciences at the University of Copenhagen, Denmark.

Conflict of Interest Disclosure: None reported.

Mette Kirstine Keller, DDS, PhD, is an assistant professor in the section for pediatric dentistry in the department of odontology of the Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

Conflict of Interest Disclosure: Dr. Keller received a full doctoral scholarship from BioGaia AB in Stockholm.

More than 15 years have passed since the first clinical study on the effect of probiotic bacteria on caries risk and caries development in preschool children was published. Milk supplemented with L. rhamnosus GG was served in day care settings over a period of seven months, and the results indicated clear beneficial effects on selected caries risk factors. The interest generated around these findings was the virtual starting point for a novel avenue of research in clinical dentistry, widening the outcome measures to gingivitis, periodontal disease, implantitis, peri-implantitis, mucositis, candidiasis and halitosis. In fact, the probiotic concept became a hot topic and soon there were far more review publications available than original studies. So, one may ask if another review really is needed? The answer could be yes in light of the rapid advances in the human microbiome and microbial ecology. Molecular and functional studies have provided insights that bacterial biofilms have co-evolved with humans and play an important role in health and well-being. Consequently, the composition and function of the oral microbiota plays an active role in the oral cavity. A diverse and balanced microbiota is associated with oral health while dysbiosis, commonly driven by ecological stress, is linked to a variety of oral conditions and opportunistic infections. In this context, probiotic therapy may offer an additional measure to established and evidence-based interventions. The background principle behind the use of probiotic bacteria (or bacteriotherapy) is quite simple: to modulate or replace unwanted microbes with the aid of harmless or friendly bacteria instead of using antibiotics or antimicrobial agents. Probiotic bacteria
A general clinical recommendation are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” The aim of this narrative and subjective review is to briefly provide the general dental practitioner with an update on recent advances concerning the clinical use of probiotics for oral health. The paper is based mainly on systematic reviews and human trials with clinical endpoints of importance for the individual patient.

Genera, Strains and Dose

The main probiotic strains used for oral bacteriotherapy belong to the Lactobacillus (Figure 1) and Bifidobacterium genera but also some Streptococcus species may express probiotic properties. Generally, the effects of probiotic bacteria are strain specific and properties of one strain cannot necessarily be applied to others. The very same strain may also display different effects in different individuals. A general clinical recommendation is that the probiotic bacteria must be ingested on a regular basis, which in clinical practice means at least four days per week. There is currently no evidence to support a permanent colonization of probiotic bacteria in the oral cavity although exceptions following early-in-life exposures seem to exist. It should, however, be stressed that a permanent colonization is not a prerequisite for probiotic action in the oral biofilm. The common vehicles for administration are dairy products (milk, yogurt, sour cream) or tablets, capsules, lozenges and drops. Any “optimal” dose for oral care is unfortunately not established. The common recommendations of 1–2 deciliter of yogurt/milk per day with each milliliter containing 1 x 10^8 live cells or 1–2 tablets per day (1 x 10^9 live cells in each tablet) are derived from gastrointestinal health. For infants, the recommended regime is five probiotics drops per day, sometimes in combination with vitamin D. It is possible, and perhaps even likely, that there is dose-response relationship for oral effects but this is yet to be explored.

Mechanisms of Action

It is generally thought that the intake of probiotic bacteria can trigger a chain of direct (local) and systemic (indirect) effects. The direct events in the oral biofilm include co-aggregation, competitive exclusion, bacteriocin (hydrogen peroxide) production and competition for nutrients. The ability to produce toxins, particularly H2O2-like agents, is perhaps the most powerful local property and probiotic bacteria can thereby modify the composition of the oral biofilm and/or its metabolic activity. The systemic effects rely on immunomodulation of the host’s innate and adaptive inflammatory response through activation of T-cells. Consequently, significant effects on IgA and cytokine expression in the guts and the gingival crevicular fluid (GCF) have been displayed. It is however important to emphasize that the detailed mechanisms of action are not fully known and that there are conflicting reports on the probiotic-induced effects on the host response in the oral environment. For example, one recent study failed to show the effects on salivary immunoglobulins and inflammatory mediators while others found increased levels of s-IgA and human neutrophil peptides 1-3 in saliva immediately after probiotic exposure. It has also been demonstrated that the presence of Lactobacillus reuteri in saliva coincides with higher salivary IgA in young adults after the intake of probiotic lozenges. Contradictory data are also present for periodontal conditions. Studies have shown that Lactobacillus brevis CD2 can delay gingivitis development and inhibit periodontitis by downregulating the inflammatory cascade in GCF. These anti-inflammatory effects have been attributed to the presence of arginine deaminase which prevented nitric oxide generation. Similar findings reported by Ince et al. show that the GCF levels of the matrix metalloproteinase inhibitor TIMP-1 increased and the MMP-8 levels decreased in patients with chronic periodontitis when probiotic L. reuteri was added to traditional nonsurgical therapy. On the other hand, Hallström et al. found no effects on the cytokine levels using the same strains in subjects with healthy periodontal conditions, indicating a therapeutic role of the probiotic supplements rather than a preventive. Another interesting but open question is whether the intake of probiotics can influence the composition of the oral bacterial community. Two studies were unable to demonstrate a shift after two to three weeks of probiotic exposure. However, a prolonged study over 12 weeks with L. reuteri displayed an
altered biofilm composition on teeth although the richness of species seemed to be unaffected. The shift was, however, of a transient nature and was “normalized” within one month after termination of the exposure. This may indicate that there is a “colonization memory” in the oral biofilm similar to that of the guts. Clearly, more studies are needed to elucidate both the local and systemic avenues of action.

Safety
The safety of probiotic administration must of course be considered. Probiotic supplements are from a regulatory point of view classified as food additives and labeled “generally recognized as safe” (GRAS). There have been no reports of adverse effects in healthy humans although interventions for critically ill patients or the very fragile elderly should be considered with some caution. On the other hand, probiotic therapy may be used for cancer patients. Sharma and co-workers have shown that lozenges with \textit{L. brevis} CD2 can reduce the incidence and alleviate the symptoms of radiation- and chemotherapy-induced mucositis in patients with head and neck cancer. Concerns have also been raised for the cariogenic abilities of lactobacilli. Indeed, probiotic lactobacilli are highly acidogenic but there is at this time no data to support that a regular intake of these bacteria would increase the caries risk.

Probiotics and Caries
The potential of probiotic bacteria to influence the caries process is commonly addressed with intermediate endpoints rather than caries lesion development. Two systematic reviews, based on 19 and 23 papers respectively, have concluded that probiotic interventions clearly can reduce the mutans streptococci counts in supragingival plaque and saliva, thereby suggesting a positive effect in the prevention of caries. With respect to lesion development, seven placebo-controlled studies are currently available in preschool children/primary dentition, two in adolescents and one on root caries arrest in the elderly. The studies are summarized in Table 1. For the infants and preschool children, the probiotic supplements were typically administrated via drops or gruel from the parents or with milk served in day care settings. The duration of the intervention ranged from six to 21 months. The effectiveness in preventing childhood caries is illustrated in Figure 2. The probiotic supplements were better than placebo in all seven studies although the difference was statistically nonsignificant in two of them. The prevented fraction ranged from 11 to 61 percent with a median of 48 percent. It is important to underline that virtually all families reported regular use of fluoride toothpaste in parallel with the probiotic supplements.

### Table 1

<table>
<thead>
<tr>
<th>First author, year</th>
<th>n/age</th>
<th>Vehicle</th>
<th>Strain</th>
<th>Follow-up</th>
<th>Outcome, a comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention during infancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taipale, 2013 &amp;superscript;26</td>
<td>106/newborn</td>
<td>pacifier/spoon</td>
<td>\textit{B. animalis}</td>
<td>4 yr.</td>
<td>NS, low-risk population</td>
</tr>
<tr>
<td>Hasslöf, 2013 &amp;superscript;27</td>
<td>180/4–13 mo.</td>
<td>gruel</td>
<td>\textit{L. paracasei}</td>
<td>9 yr.</td>
<td>NS</td>
</tr>
<tr>
<td>Stensson, 2013 &amp;superscript;28</td>
<td>188/newborn</td>
<td>drops</td>
<td>\textit{L. reuteri}</td>
<td>9 yr.</td>
<td>S, primary teeth only</td>
</tr>
<tr>
<td>Intervention to preschool children</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Näse, 2001 &amp;superscript;1</td>
<td>594/1–6 yr.</td>
<td>milk</td>
<td>\textit{L. rhamnosus}</td>
<td>after 7 mo.</td>
<td>NS/S = 3–4-year-old subgroup</td>
</tr>
<tr>
<td>Stecksén-Blicks, 2009 &amp;superscript;29</td>
<td>248/1–5 yr.</td>
<td>milk</td>
<td>\textit{L. rhamnosus}</td>
<td>after 21 mo.</td>
<td>S, milk contained 2.5 ppm F</td>
</tr>
<tr>
<td>Hedayati-Hajikand, 2015 &amp;superscript;30</td>
<td>138/2–3 yr.</td>
<td>lozenges</td>
<td>\textit{Streptococcus}</td>
<td>after 12 mo.</td>
<td>S, high-risk population</td>
</tr>
<tr>
<td>Rodriguez, 2016 &amp;superscript;31</td>
<td>261/2–3 yr.</td>
<td>milk</td>
<td>\textit{L. reuteri}</td>
<td>after 12 mo.</td>
<td>S, high-risk population</td>
</tr>
<tr>
<td>Intervention to schoolchildren</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keller, 2014 &amp;superscript;32</td>
<td>36/12–17 yr.</td>
<td>lozenges</td>
<td>\textit{L. reuteri}</td>
<td>after 3 mo.</td>
<td>S, assessed with QLF &amp;superscript;b</td>
</tr>
<tr>
<td>Teanpaisan, 2015 &amp;superscript;33</td>
<td>122/12–14 yr.</td>
<td>milk-powder</td>
<td>\textit{L. paracasei}</td>
<td>after 6 mo.</td>
<td>S, high-risk group</td>
</tr>
<tr>
<td>Intervention to adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* S = significant difference in caries prevalence/increment compared with placebo; NS = no significant difference
* QLF = quantitative light fluorescence
Interestingly, two of the studies reported significant improvements in the general health of the children on top of the dental outcome and a reduced need for antibiotic prescriptions.\(^{29,35}\) The study by Stensson et al.\(^{28}\) was of particular interest. Probiotic drops were given to newborn babies during their first year of life and a reduction of caries in the primary dentition was scored eight years later. The results may be interpreted as a “proof of concept” that an early start of probiotic exposure is important in order to support a diverse colonization of the oral biofilm on a “first-come, first-served” basis.\(^9\) The studies carried out in the young permanent dentition point also toward a caries-preventive effect with the best results in schoolchildren with increased caries risk.\(^{32,33}\) The only adult study focused on root caries over 15 months and both fluoride and probiotic supplements could reverse soft, leathery lesions in a significant way compared to placebo.\(^{34}\) None of the abovementioned caries studies were, however, free from risk of bias, so further independent studies are required to ascertain efficacy, both from the patient perspective as well as from a public dental health point of view with health-economic analyses.

Probiotics and Periodontal Disease

A large number of studies have addressed the use of probiotics for gingivitis and periodontitis in recent years. Typical clinical endpoints are plaque index, gingival bleeding index, bleeding on probing, periodontal probing depth and clinical attachment loss. In addition, a number of periodontal pathogens and the levels of cytokines and chemokines in gingival crevicular fluid are often assessed as biomarkers of the inflammatory activity. A recent systematic review and meta-analysis has summarized that probiotic therapy compared with placebo reduced bleeding on probing and gingival bleeding in a significant way but did not affect the amount of plaque.\(^{36}\) Likewise, a meta-analysis supported the adjunctive use of \(L.\) reuteri to scaling and root planing in the treatment of chronic periodontitis at short-term, especially in deep pockets.\(^{37}\) Similar conclusions were drawn from the systematic review of Matsubara and co-workers.\(^{38}\) Based on 12 included studies, it was summarized that oral administration of probiotics improved the recognized clinical signs of chronic and aggressive periodontitis such as probing pocket depth, bleeding on probing and attachment loss, with a concomitant reduction in the levels of major periodontal pathogens. The authors highlighted that a continuous probiotic administration was necessary to maintain these benefits and that the adjunctive use to conventional mechanical treatment was likely to reduce the need for antibiotics. The included studies were however disparate and of limited size which may reduce the strength of these conclusions. Probiotic bacteria have also recently been applied for the prevention and treatment of peri-implant mucositis. Flichy-Fernández and co-workers\(^{39}\) found that a one-month exposure to \(L.\) reuteri significantly improved clinical parameters around the implants in edentulous patients compared with placebo.

Probiotics and Candida

Oral candidiasis is a common problem among the fragile elderly but it may also appear in young individuals. Over the last years, a number of randomized controlled clinical trials investigating the antifungal effects in the oral cavity from probiotic therapy have been published\(^{40-43}\) (Table 2). It is known that probiotic bacteria have the ability to co-aggregate with various \(Candida\) species, interfere with hyphae formation and inhibit growth via production of bacteriocins.\(^{44}\) Therefore, it was not surprising to find that all clinical studies resulted in reduced salivary counts of \(Candida\) albicans, the most common fungi in the oral cavity. Interestingly, the significant reductions seemed to be obtained irrespective of probiotic strains, administration mode and frequency. It should however be underlined that also for opportunistic \(Candida\) infections probiotic therapy should be regarded as a bioecological adjunct rather than an alternative to the conventional pharmaceutical treatment.
Probiotics and Halitosis

A number of placebo-controlled studies have focused on the treatment of oral malodor as summarized in TABLE 3. Although all studies but one reported a short-term reduction of halitosis, the studies were heterogeneous with respect to the intervention. The probiotic strains were administrated alone or as adjunct to mechanical cleaning, scaling and root planing and/or antibacterial rinses. It is also important to stress that the main outcome measure was organoleptic scores, which calls for some subjectivity. Due to the short duration of the studies, another issue is whether the improvements in malodor are stable over time.

Probiotics and the Future

What will happen in the next 15 years? In today’s -omics era, it is obvious that the general awareness concerning the co-evolvement and symbiosis between the human host and bacteria is increasing along with a demand for “health-by-nature” instead of an overuse of chemicals and antibiotics. Hopefully, probiotic administration to combat biofilm-mediated diseases will help to reduce the need for antibiotics in the future. Furthermore, the common risk factor approach with close links between oral and general diseases (diabetes, metabolic syndrome, obesity), which calls for a patient-centered holistic view, will unite the efforts of dental and medical professionals in health promotion. In fact, due to evidence supporting the role of probiotics for the prevention of eczema in infants, management of side effects related to antibiotics and alleviation of functional bowel symptoms, five states within the European Union have recognized probiotics in their national dietary guidelines. The interest for prebiotic substances that induce the growth or activity of beneficial microorganisms is also emerging. Recently, arginine was described as a genuine oral prebiotic because of its ability to promote a healthy oral ecology from a caries point of view. It is therefore likely that an
increasing number of consumer oral care products with prebiotics and/or orally optimized probiotic strains will be developed and available over the counter in the coming years.

The next probiotic area to investigate in clinical dentistry could very well be oral wound healing and control of postoperative pain and discomfort, for example after third molar surgery. Research has indicated that lactobacilli-derived probiotics may enhance chronic wound healing, which could also be applicable in the oral cavity. In this context, our research group has recently shown that bacterial products secreted from *L. reuteri* are noncytotoxic for human gingival fibroblasts and may stimulate the production of prostaglandin E2. Thus, probiotic bacteria may play a role in the resolution of inflammation in human gingival fibroblasts, which is an important first step in accelerated oral wound healing.

**Clinical Considerations**

Will general dental practitioners include probiotic therapy in their toolboxes for the prevention and maintenance of oral health? The current literature displays a wide range of probiotic products have been marketed directly to the consumer with none or very limited background research. It is the responsibility of each clinician to advocate for safe products with documented effectiveness from clinical trials.

**Conclusions**

The current literature displays without a doubt a growing body of evidence that probiotics might help to improve oral conditions such as dental caries, periodontitis, halitosis, mucositis and oral Candida load. Probiotic supplements are safe to use and may very well be added to the general practitioner’s preventive and therapeutic toolbox. However, further research is needed to verify and expand the current knowledge base and particularly, long-term randomized clinical trials with a health-economic approach would be welcome.

**References**

coincidence with higher salivary IgA in young adults after intake of probiotic lozenges. Benef Microbes 2016;Nov 22.
Say hello to the TDSC Marketplace, a shopping site exclusive to CDA members, which offers the supplies you use every day at discounts that really add up.

- Negotiated discounts for exceptional savings
- 25,000+ items from your favorite vendors
- Free shipping on every order and fast delivery
- No fees, no minimum order size, no hassle

Ready to make your practice competitive and efficient? Start shopping today and explore other powerful solutions from The Dentists Service Company.

Get started at tdsc.com or call 888.253.1223.

*Price comparisons are made to the manufacturer’s list price. Actual savings on the TDSC Marketplace will vary on a product by product basis.
Biofilm Battles: Beneficial Commensals vs. Streptococcus Mutans

Brinta Chakraborty, PhD; Kyulim Lee, BS; and Robert A. Burne, PhD

ABSTRACT A healthy human oral cavity is colonized by biofilms composed of a very diverse group of eubacteria with minor representation of fungi and archaea. Beneficial commensal bacteria, particularly oral streptococci, play essential roles in the establishment and persistence of biofilms that are compatible with oral health. We describe mechanisms utilized by beneficial bacteria to compete with the dental caries pathogen Streptococcus mutans and how these interactions moderate the pathogenic potential of oral biofilms.

Humans harbor and co-evolved with a large and complex population of microbes, forming mutually beneficial relationships for the host and microorganisms. By way of example, the lower gastrointestinal tract of mammals contains an astounding number of resident commensal bacteria that exist in homeostasis with the host and its immune system. Colonization of commensal bacteria within a complex ecosystem like the gut is a prerequisite for the proper maturation of the immune system, shaping responses to pathogens, influencing autoimmune diseases and impacting other critical processes, including neurological development. The mechanisms by which the microbiota influences host immune responses and development remains an active area of research with important implications for human health.

Polymicrobial communities at other body sites can protect against pathogens and provide additional benefits to the host. Of relevance here, the biofilms formed on the many different surfaces of the oral cavity protect the host from the caries causing pathogen S. mutans. The human mouth is heavily colonized by bacteria, but archaea, fungi, protozoa and viruses are intermittently detected. This review focuses exclusively on bacteria because of their relative abundance and their established dominant role in the most common oral infectious diseases, dental caries and periodontal diseases.

Oral Bacterial Colonization

The human mouth is home to approximately 700 physiologically and genetically diverse microorganisms, many of which are not normally found elsewhere. In the past, 16S rDNA studies...
have revealed that a healthy individual is colonized with about 100–200 different taxa, with others comprising < 0.1% of the microflora at a given site. However, with the remarkable sensitivity of next-generation sequencing and metagenomics, more than 1,000 different taxa have been identified as resident or transient members of oral biofilms. Dental plaque, which is a complex bacterial milieu on the teeth, is similarly diverse and contains bacteria that may be overtly beneficial and some that are clearly opportunistic pathogens. Dental plaque formation on a recently cleaned tooth surface occurs in an ordered manner, beginning with the adhesion of “early colonizers” to the acquired enamel pellicle, followed by co-adhesion events between the adhered bacteria and so-called “late colonizers.” Streptococcus and Actinomyces spp. are the most abundant early colonizers of the soft and hard tissues of the human oral cavity. Fusobacterium spp. can co-adhere to early colonizers and serve as bridging organisms between early and late colonizers. Collectively, these biofilm formation and maturation processes give rise to temporally and spatially organized degradative communities that can cooperatively catabolize complex host- and diet-derived nutrients.

The biofilms that colonize tooth surfaces are generally compatible with maintenance of the integrity of these tissues. Saliva also helps in maintaining the integrity of mineral composition of tooth surfaces by providing buffering, cleansing and remineralizing capacity to the hard tissues of the oral cavity. However, when the human diet becomes enriched with certain carbohydrates, such as sucrose and starches, the organisms can ferment these sugars to produce relatively strong organic acids (e.g., lactic, formic) that lead to lowering of pH, as observed in the Stephan Curve and demineralization of the tooth. If the acid challenge is sufficient to overcome the natural protective forces of saliva, dental caries can develop. Importantly, dental health is associated with greater proportions of comparatively acid-sensitive bacteria that can utilize particular substrates to neutralize plaque acids. These organisms include Streptococcus sanguinis, Streptococcus gordonii and certain other oral streptococci. Conversely, the initiation and progression of caries lesions is strongly associated with increased proportions of highly acidogenic and aciduric (acid-tolerant) bacteria, particularly Streptococcus mutans, but also certain Lactobacillus and Scardovia spp.

Health-associated dental biofilms can protect the host against infections by opportunistic and overt pathogens. One of the main reasons caries pathogens emerge as dominant members of tooth biofilms is because they can grow and metabolize at acidic pH values, whereas commensal and beneficial organisms often cannot. Thus, a primary strategy used by oral commensal bacteria to foster a biofilm that discourages the emergence of caries pathogens is to metabolize certain substrates to produce basic compounds, especially ammonia, that alkalinize the cytoplasm of the
commensals and the local environment. It is noteworthy, then, that plaque pH and ammonia levels are elevated in biofilms of fasting subjects who are caries free, compared to caries-active individuals. However, similar to other polymicrobial ecosystems, homeostasis in dental biofilms can be perturbed by substantial changes in the environment, including high levels of fermentable carbohydrates in the diet, diminished saliva flow due to medications and other conditions and extended periods of decreased biofilm pH following ingestion of carbohydrates19 (FIGURE 1). Not only does the sustained exposure to low pH provide a selective advantage to caries pathogens over commensals, but exposure to a sub-lethal pH can induce an adaptive acid tolerance response in S. mutans that alters gene expression and cellular physiology in a way that increases aciduricity and acid production, thereby enhancing the virulence of the organism.22–24 These changes in the physiology of S. mutans play crucial roles in the competitiveness of S. mutans in conditions that are favorable for the development or worsening of carious lesions. Importantly, healthy plaque communities are disrupted by these environmental changes in a way that fosters the outgrowth of a cariogenic microflora.19,25,26

Microbiome Studies With a Focus on Oral Health and Disease

Several in vitro and in vivo studies highlight the importance of beneficial commensals and their role in the “ecological plaque hypothesis”19 that postulates that initiation and progression of dental caries is fostered by increases in the proportions of acidogenic and acid-tolerant microbes in dental biofilms. Periodontal diseases are also characterized by induced dysbiosis leading to significant compositional differences in the microbiomes in health and disease.22 Sequencing of 16S rDNA from a longitudinal study spanning over 10 years looking at different sites in the oral cavity of 200 individuals have identified bacterial species that constitute about 95 percent of the human salivary microbiota, namely operational taxonomic units (OTUs) from the genera Streptococcus, Veillonella, Granulicatella, Rothia and Fusobacterium.28 OTUs are often used to identify taxonomically related species29 based on 16S rDNA sequence and/or the conservation of certain genes. Interestingly, despite substantial intersubject microbial diversity, Streptococcus mitis was present in all subjects associated with health (subjects with healthy periodontia and no active white spot lesions or caries), consistent with a separate study on the oral microbiome of healthy individuals that found S. mitis to be a dominant organism colonizing the oral cavities of healthy subjects.30 Therefore, increased proportions of S. mitis could be described as a potential bacterial “fingerprint” for a health-associated oral microbiota.31 In diseased conditions, such as periodontitis, a shift in the periodontal microbiota occurs away from mostly Gram-positive organisms to a flora enriched for Gram-negative, asaccharolytic organisms, particularly members of the so-called “red complex” Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola, which show a strong association with active periodontal disease.32,33 Recent advancement in high throughput technologies have allowed the screening of microbial communities as a whole, identifying the presence of new species involved in periodontitis, such as species belonging to genus Filifactor and Scardovia.34 In the case of dental caries, two members of the mutans streptococci, Streptococcus mutans and Streptococcus sobrinus, were considered the primary pathogens of human dental caries for decades. While evidence still confirms S. mutans as a primary contributor to dental caries, more in-depth microbiome analyses have shown that caries can occur in the absence of increased proportions of S. mutans.35 Mounting evidence supports the polymicrobial nature of dental caries, with studies showing acidogenic Bifidobacterium,36 Lactobacillus and Scardovia spp. being present in increased proportions in advanced caries lesions37 and PCR amplification and high throughput metagenomics revealing the presence of diverse bacterial genera, such as Veillonella, Atopobium and Corynebacterium31,38–42 in diseased dental plaques. In vitro studies also show cocultivation of S. mutans and Veillonella alcalescens produce more acid than when growing separately, highlighting the potential for multispecies interactions enhancing the overall capacity of the biofilms to cause demineralization.43-45 Other microbiome studies focusing on the influence of different variables, including individual dietary habits, oral hygiene practice and availability of dental care, also influence the oral microbiome composition.46
How Do Beneficial Commensal Bacteria Promote Health?

Over the years, plate-based competition assays have been considered the classical method to observe antagonistic behaviors between commensals and pathogens (FIGURE 2). Multiple factors contribute to manifestation of antagonism where commensals employ several strategies to inhibit the growth of the pathogens and create an environment in which they can thrive. Similarly, in the oral cavity the commensals and beneficial species in the oral microbiome can play an important role in promoting oral health by interfering with the colonization, persistence and/or virulence of pathogens in biofilm communities. As described earlier, these biofilms are exposed to widely fluctuating environmental conditions that affect the composition, structure and biochemistry of the biofilms with pH and carbohydrate availability having the most profound effect on the development of dental caries (FIGURE 1). Organic acids produced by the fermentation of dietary carbohydrates by cariogenic bacteria elicit demineralization of tooth enamel. These periods of acid challenge to the tooth are followed by periods of alkalization, which neutralizes plaque pH and promotes remineralization and enamel surface integrity. While many factors contribute to the alkalization of oral biofilms (e.g., buffers in saliva, diffusion of acids out of biofilms), alkali generation by oral bacteria plays a major role in pH homeostasis in oral biofilms and inhibits the initiation and progression of dental caries. A subgroup of bacteria in dental biofilms can protect themselves against acidic conditions by breaking down substrates that yield alkaline end products such as ammonia. The two major substrates utilized by these bacteria are urea and arginine. Urea, which is secreted in major and minor salivary glands as well as in gingival crevicular fluids, is present in human saliva at relatively high concentrations (3–10 mM). Urea can be rapidly hydrolyzed to two molecules of ammonia and one molecule of carbon dioxide by bacterial ureases produced by oral bacteria, mainly Streptococcus salivarius, Actinomyces naeslundii and certain oral haemophili. Urea metabolism contributes to maintenance of health-associated biofilms by counteracting the acidification of biofilms. For example, subjects with chronic renal failure (CRF) have high levels of salivary urea but are refractile to caries development despite ingesting high carbohydrate diets and having reduced stimulated saliva flow, both of which can greatly increase the risk of caries development. The observed low caries incidence in CRF subjects has been attributed to increased salivary urea levels found in these patients, resulting in higher baseline plaque pH values and less acidification of biofilms after a carbohydrate challenge compared to healthy subjects. Several studies now support that the decreased alkaligenerating capacity of oral biofilms from urea and also from arginine is associated with caries incidence and severity.

The other primary mechanism by which oral biofilms generate ammonia is via the arginine deiminase system (ADS), a three-enzyme pathway that converts arginine to one molecule of ornithine and CO₂ plus two molecules of ammonia with the concomitant generation of one molecule of ATP (FIGURE 3). Arginine can be found in micromolar concentrations in ducal saliva, but is much more abundant in salivary peptides and proteins. When arginine is catabolized through the ADS pathway, it provides key bioenergetic benefits to health-associated oral commensals, neutralizing their cytoplasm and biofilm pH and generating ATP that can be utilized for growth and maintenance. A variety of oral streptococci express ADS activity, including Streptococcus gordonii, Streptococcus parasanguinis, Streptococcus sanguinis and certain lactobacilli. The most extensively studied ADS of oral bacteria is that of S. gordonii, where the ADS genes are clustered: arginine deiminase (AD) (arcA), ornithine carbamoyl transferase (arcB), carbamate kinase (arcC), which are the genes for the enzymes of the pathway and an arginine-ornithine antiporter (arcD) and arginine aminopeptidase (arcT) are co-transcribed in a single operon. Also linked to the operon are arcR, which encodes a transcriptional activator responsive to arginine, and an Fnr-like protein (encoded by flf) that serves as an activator of the arcA promoter in anaerobic conditions. Among ADS-positive oral species, S. gordonii is unusual in that queA, which catalyzes the final step in queuosine modification of tRNAs in
other organisms, is encoded with arcR, and loss of queA affects ADS expression under certain growth conditions. Preferred carbohydrates, including glucose, are able to repress ADS expression via carbon catabolite repression (CCR) mediated by catabolite control protein A (CcpA) binding to two cis-acting catabolite response elements (CREs) in the arcA promoter region. Gene arrangements of the ADS operons and the primary sequence of the enzymes in the pathway have been fairly well-conserved through evolution, although the regulation of ADS production can vary considerably between species. In most oral streptococci studied to date, arginine and low pH usually result in increased ADS expression, whereas growth with preferred carbohydrates and elevated oxygen levels lead to lower ADS expression.

More recently, the role of arginine metabolism in oral ecology and its beneficial properties in maintaining oral health and preventing caries has been documented. In children, caries status was shown to be significantly associated with ADS activity, as dental plaque from caries-free (CF) children showed higher ADS activity compared to plaques from caries active (CA) subjects. Higher ADS activity levels were also observed in plaque samples of CF adults when compared to those of CA adults. Increasing the availability of arginine to these patients, in the form of a fluoride-free, arginine-containing toothpaste, significantly increased ADS activity in plaque in a matter of weeks. When evaluating the microbial profile of CA patients treated with the arginine dentifrice, a significant shift in the microbiome composition toward one that more closely resembled that of the communities in CF subjects was also noted. While the underlying mechanisms of the change in microbial profile in that study was not described, it is now established that exogenous arginine may disrupt S. mutans biofilm matrix assembly, as demonstrated in a mixed-species model with S. mutans (cariogenic) and S. gordonii (arginolytic) under cariogenic conditions. Arginine was shown to impact biofilm architecture, significantly reducing insoluble glucan exopolysaccharides (EPS) formed by S. mutans glucosyltransferase (Gtf) enzymes. Arginine negatively affected expression of the gtfB gene of S. mutans gene required for insoluble EPS synthesis from sucrose. Additionally, arginine is thought to have a multidimensional effect on dental biofilm stability; including an ability to destabilize multispecies oral biofilms and disrupt biofilm architecture, to modulate cell-to-cell signaling and cause biofilm dispersion; and to reduce antimicrobial tolerance, enhancing the susceptibility of pathogens to killing.

The basis for why health-associated oral biofilms express higher ADS activity has not been established, in part because it is now known that there is considerable genomic and phenotypic heterogeneity within and across species of oral streptococci that harbor the ADS. In an effort to better understand the basis of oral arginolytic bacterial communities and their relationship to dental health, Huang et al. isolated a panel of ADS-positive bacteria from supragingival plaque samples, including strains of S. sanguinis, S. gordonii, S. parasanguinis, Streptococcus intermedius, Streptococcus australis and Streptococcus cristatus and evaluated ADS expression patterns of individual isolates in response to a variety of environmental stimuli. Considerable variation in ADS expression was exhibited in response to pH, to the availability of oxygen or arginine and to carbohydrate source. For most strains, optimal ADS expression was dependent on the presence of supplemental (25 mM) arginine; however, a number of strains expressed high levels of ADS activity levels without arginine supplementation. While low pH induced higher ADS activity in S. gordonii DL1, some isolates expressed high ADS activity even when cultured at neutral pH and did not show induction of ADS expression at lower environmental pH.

FIGURE 3. Overview of the bacterial arginine deiminase system. One primary route by which oral bacteria generate an alkaline environment is via the arginine deiminase system. See text for more details.
Similarly, while glucose repressed ADS activity in all clinical strains, certain strains did not show nearly as much repression of the ADS by glucose as the reference strain S. gordonii DL1. Of note, during the characterization of these clinical isolates, a Streptococcus strain designated as A12 was found to be highly arginolytic in nearly all growth conditions tested, but it also has a particularly potent ability to antagonize the growth of S. mutans.76 Phylogenomic comparison of the entire genome sequence of A12 showed that it is most closely related to S. australis and S. parasanguinis, although it remains to be determined if A12-like bacteria constitute a distinct species.

Multiple clinical studies have now demonstrated the beneficial properties of introducing arginine to oral biofilms, as well as the combined effects of arginine in a calcium-containing dentifrice in preventing caries and promoting remineralization. Effective inhibition of initiation and progression of caries was noted in a clinical study with patients receiving a dentifrice containing arginine bicarbonate/calcium carbonate (CaviStat) (n = 304) compared with arginine bicarbonate/calcium carbonate receiving a dentifrice containing fluoride.63,64 Larger clinical studies comparing fluoride- and 1.5% arginine-containing dentifrices with fluoride dentifrice alone also reported similar results. A two-year, double-blind randomized clinical trial that included ~6,000 children in Bangkok with low to moderate caries risk showed statistically lower decayed, missing and filled teeth (DMFT) and decayed, missing and filled surface (DMFS) scores after two years of treatment with an arginine-containing toothpaste.64 More recently, significant reductions in caries incidence were observed in a clinical trial performed in Southern Thailand after using toothpaste containing 1,450 ppm F and 1.5% arginine versus fluoride alone for two years.44 A separate two-year clinical trial compared the efficacy of toothpastes containing 1.5% arginine and fluoride versus fluoride alone on ~5,500 children in China and found that the use of dentifrice containing arginine demonstrated significant greater reductions in DMFS.45 While the anticaries benefits of exogenous arginine treatment appear promising from some of these studies, which were primarily funded by corporations that are marketing arginine-containing oral health products, additional studies are needed to confirm these results, to evaluate the benefits of arginine in higher-risk populations and to more directly probe the basis for how arginine influences the composition and behavior of the microbiome in different populations and in individuals with varying behaviors (oral health maintenance) and diets that may impact their risk for caries. For example, recent in vitro work has shed new light on how arginine adversely affects the expression of multiple virulence-related properties of S. mutans, including growth and stress tolerance. Clearly, much remains to be learned about arginine metabolism in the context of the oral microbiome.86

Among the different antagonistic strategies employed by commensal bacteria against S. mutans, hydrogen peroxide (H2O2) generation by these species is thought to have a profound impact on oral bacterial ecology.87,88 S. gordonii, S. oralis, S. mitis, S. sanguinis and many other commensal oral streptococci produce substantial amounts of H2O2 in the presence of oxygen. H2O2 serves as a protective mechanism against competing species and a potent growth inhibitor of S. mutans and other oral pathogens.89,90 Multiple enzymatic pathways can contribute to the production of H2O2 in oral biofilms, but pyruvate oxidase (Pox) encoded by spxB appears to be the dominant source of H2O2 for many arginolytic species, including S. sanguinis, S. gordonii and Streptococcus A12.76,90-92 In the presence of oxygen, pyruvate oxidase catalyzes the conversion of pyruvate and inorganic phosphate to H2O2, carbon dioxide (CO2) and acetyl phosphate (AcP), and AcP can be used to produce ATP by the enzyme acetate.
kinase.\textsuperscript{97} The Pox enzyme therefore not only provides bioenergetic benefits (ATP) but also provides some buffering against biofilm acidification as the released CO\textsubscript{2} is converted to bicarbonate. Pox also diverts pyruvate away from lactate and toward higher pK\textsubscript{a} organic acids. Although the H\textsubscript{2}O\textsubscript{2}-producing activity of other enzyme systems in oral streptococci is significantly lower than Pox, some have been shown to yield sufficient H\textsubscript{2}O\textsubscript{2} to inhibit the growth of S. mutans, including the lactate oxidase (Lox) system utilized by \textit{Streptococcus oligofermentans} to convert lactic acid to pyruvate and H\textsubscript{2}O\textsubscript{2} under aerobic conditions, which has the added benefit of removing lactate from the environment.\textsuperscript{93,94} L-amino acid oxidases and NADH oxidases also contribute to H\textsubscript{2}O\textsubscript{2} in oral biofilms, albeit not as robustly as Pox under conditions tested thus far.\textsuperscript{95,96}

The production of Pox is sensitive to CCR in commensal streptococci, including \textit{S. gordonii}, \textit{S. sanguinis} and \textit{Streptococcus A12}\.\textsuperscript{76,97} High availability of glucose or sucrose can inhibit \textit{spxB} expression and reduce H\textsubscript{2}O\textsubscript{2} production in \textit{S. gordonii}.\textsuperscript{97,98} On the other hand, \textit{spxB} expression in \textit{S. sanguinis} is not dependent on carbohydrate availability and appears to be repressed until other environmental input is received.\textsuperscript{99} It has been proposed that this mode of regulation exists because of the relative sensitivity of \textit{S. sanguinis} to H\textsubscript{2}O\textsubscript{2}, so as to ensure that cells do not generate levels of H\textsubscript{2}O\textsubscript{2} that would be detrimental to the organism itself.\textsuperscript{99,100} While H\textsubscript{2}O\textsubscript{2} is clearly able to inhibit \textit{S. mutans}, there are likely conditions in vivo where commensals are limited in their capacity to produce this compound, e.g., in relatively anaerobic biofilms or when excess carbohydrate is consumed by the host. Both factors may be favorable to caries formation since \textit{S. mutans} growth and biofilm formation is inhibited by oxygen.\textsuperscript{101} Thus, there was likely evolutionary pressure on the commensals to acquire and retain additional systems to interfere with the caries pathogen \textit{S. mutans}, especially since \textit{S. mutans} dominates cariogenic biofilms at the expense of health-associated commensals.

In addition to pH neutralization of the oral environment and H\textsubscript{2}O\textsubscript{2} generation, amino sugars such as N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) appear to play a role in modulating the competitiveness of commensals against \textit{S. mutans}.\textsuperscript{102} Amino sugars are important constituents of bacterial cell envelopes, fungal cell walls and salivary glycoproteins.\textsuperscript{103} Bacteria have well-conserved pathways for amino sugar metabolism.\textsuperscript{104} A recent study by Zeng et al. has shown that commensal streptococci grew faster and to a higher optical density than \textit{S. mutans} in planktonic culture in a chemically defined medium (FMC)\textsuperscript{105} containing GlcNAc as sole carbohydrate source. Likewise, in a dual-species biofilm model with \textit{S. gordonii} DL1 and \textit{S. mutans} UA159 in synthetic medium (biofilm medium)\textsuperscript{106} supplemented with amino sugars (GlcNAc or GlcN), the commensal gained an advantage over \textit{S. mutans} compared to when glucose was used as the primary carbohydrate source. Amino sugar catabolism in oral streptococci requires the expression of the \textit{nagA} and \textit{nagB} genes, which are regulated by NagR.\textsuperscript{107} Interestingly, production of H\textsubscript{2}O\textsubscript{2} by \textit{S. gordonii} reduces the production of \textit{NagA} and \textit{NagB} by \textit{S. mutans}.\textsuperscript{102} Furthermore, metabolism of amino sugars results in an elevated pH in the mixed species biofilm model, compared to when glucose is the carbohydrate source, due to the release of ammonia from glucosamine or N-acetylglucosamine.\textsuperscript{102}

The isolation and molecular characterization of commensal organisms like A12 is beginning to facilitate a more in-depth understanding of additional mechanisms utilized by beneficial bacteria to suppress caries pathogens. For example, \textit{S. gordonii} produces a protease that can inhibit a key intercellular communication pathway used by \textit{S. mutans}. In particular, \textit{S. mutans} has multiple two-component systems for stress tolerance\textsuperscript{108} and bacteriocin production by \textit{S. mutans} is primarily regulated by a peptide CSP (competence stimulating peptide) interacting with the ComDE two-component signal transduction system.\textsuperscript{76} A secreted protease, designated as challisin and encoded by the \textit{sdc} gene of \textit{S. gordonii}, is able to degrade CSP of \textit{S. mutans} and thereby block the activation of bacteriocin production and genetic competence by \textit{S. mutans}\.\textsuperscript{109} \textit{Streptococcus A12} produces a challisin-like protease (60.4% amino acid sequence identity)\textsuperscript{76} and an sgc mutant of \textit{Streptococcus A12} lacks the ability to block CSP-dependent signaling and bacteriocin production. Importantly, it was also shown that the \textit{sdc} protease of A12 is able to protect a sensitive commensal (\textit{S. sanguinis}) from killing by \textit{S. mutans} mutacins. Of note, \textit{S. gordonii} is unable to block the \textit{comX}-inducing peptide (XIP) signaling pathway that is required for genetic competence, but A12 is highly effective at doing so, apparently through a mechanism distinct from expression of the challisin-like protease.\textsuperscript{76} More recently,
supernates from A12 were shown to alter EPS production and gtf gene expression by S. mutans in a way that destabilized S. mutans biofilms.310 Clearly, we are just beginning to understand the many ways that beneficial streptococci and other oral commensals may interfere with the colonization, persistence or induction of disease by oral pathogens.

**Application**

The majority of efforts directed at eradicating caries have taken a disease-focused approach, eradicating biofilms entirely or targeting pathogens in diseased biofilms. As basic and clinical research into caries etiology over the last decade has been greatly accelerated by various technological advances, including next-generation sequencing and metabolomics, the importance of beneficial commensal bacteria in shaping the ecology — and therefore the pathogenic potential — of dental biofilms has become evident. Clearly, beneficial bacteria can moderate acidification of oral biofilms and directly antagonize the growth and expression of virulence-related attributes of cariogenic bacteria, which must have a key role in inhibition of the initiation and progression of dental caries. Thus, significant interest has now been generated as to whether individual or combinations of beneficial bacteria (probiotics) can be utilized alone or in combination with prebiotic compounds (e.g., arginine) to prevent the initiation of dental caries and to repair incipient lesions. While probiotic formulations already exist that are targeted at improving oral health through the use of live or killed microorganisms, none of these products has been tested in rigorous clinical trials. None are FDA-approved and there remain major gaps in our knowledge of the mechanisms of action of beneficial bacteria, how arginine may inhibit caries and affect the microbiome and whether probiotic or symbiotic approaches can be truly effective against a strong cariogenic challenge.

The recent characterization of clinical strains isolated from CF human plaque samples and the demonstration of substantial intra- and interspecies variability in beneficial properties greatly complicates the analysis of the microbiome and our ability to correlate certain taxa with disease or health.111,112 Thus, it is of importance to know the spatiotemporal distribution of a certain dental plaque bacteria in health and disease before rational protective measures can be designed.113 Additionally, recent advances in high-throughput sequencing and metaproteomics will surely provide more comprehensive information regarding the phenotypic potential of commensals, which in turn will disclose additional mechanisms by which these organisms may influence the stability of healthy biofilms and combat pathogenic bacteria in dental caries and periodontitis. Therefore, while much knowledge has been gathered over recent years about commensals and their beneficial effects on their hosts, it is likely that in the coming years oral health researchers can begin to make use of the spectrum of benefits of commensal organisms for the more effective prevention and treatment of dental caries and promotion of overall oral health.

**ACKNOWLEDGMENTS**

The authors thank Jenna Shuman for her assistance with antagonism studies. This work was supported by the NIH-NIDCR R01 DE25832 and T90 DE21990. Funding was also received by RAB and a collaborator at the University of Florida from the Colgate-Palmolive Company, which markets arginine-containing dentifrices.

**REFERENCES**

21. Margolis HC, Duckworth JH, Moreno EC. Composition of pooled resting plaque fluid from caries-free and caries-


Targeted Antimicrobial Peptides: A Novel Technology to Eradicate Harmful Streptococcus mutans

Lihong Guo, PhD, DDS, and Anna Edlund, PhD

ABSTRACT  Dental caries remains the most common chronic oral disease in major developed countries. We review current anticaries approaches and a newly developed methodology called specifically targeted antimicrobial peptides (STAMP). STAMP specifically eradicates the cariogenic Streptococcus mutans bacterium in the human oral cavity but does not disturb the benign and beneficial bacteria. It promises impacts far beyond dentistry and could possibly be used to treat and prevent other microbiome-related diseases.

Dental caries is one of the most prevalent bacteria-related infectious diseases worldwide.\(^1\),\(^2\) It is a “silent epidemic” and results in a financial burden that leaves many cases untreated in underprivileged socioeconomic regions and countries, eventually resulting in tooth extraction as the last remedy.\(^3\),\(^4\)

Therefore, several measures have been developed for preventing caries and one of the most effective is the use of sodium fluoride toothpaste and/or rinse. This approach has a well-documented clinical efficacy as it inhibits the activity of cariogenic bacteria besides the remineralizing capacity and its recovery of demineralized enamel.\(^5\),\(^6\) Previous studies also show that silver diamine fluoride (SDF) treatment is highly efficient in both preventing and arresting dentine caries.\(^7\) The treatment procedure is simple, inexpensive (i.e., it requires no expensive equipment), noninvasive and the risk of spreading infection is low. However, SDF is not a complete solution to caries risk, as single application has been reported to be insufficient for sustained benefit.\(^8\) Its downsides include an unpleasant metallic taste, potential to irritate gingival and mucosal surfaces and the characteristic black staining of the tooth surfaces to which it is applied.\(^8\),\(^9\)

Cariogenic bacteria have the capacity to consume carbohydrates at a rapid rate, resulting in the accumulation of organic acid in a short period of time.\(^10\) This process leads to a dramatic drop in plaque pH causing the inactivation of health-associated community members and enamel remineralization processes.\(^11\),\(^12\) As the pH drops below the demineralization
threshold (5.5–5.7), cariogenic bacteria thrive and the solubilization process of the tooth mineral becomes rapid and irreversible. Despite fluoride being able to prevent plaque formation, it has limited impact on directly killing and extinguishing cariogenic bacteria residing in dental plaque. This is a major explanation for why caries persists in many communities and remains a global health problem. The improved understanding of oral microbial ecology, especially the importance of the balance between cariogenic and commensal residents, has highlighted the fundamental need to develop novel measures to selectively inhibit cariogenic species and modulate the microbial composition of dental plaque for caries control.

Other approaches to reduce caries include the neutralization of plaque pH with sodium bicarbonate,13 abstinence from dietary sugars or substitution with sugar analogues14 and self-performed mechanical removal of the dental plaque using a toothbrush and interdental floss. The effect of these approaches, however, is unsustainable and requires repeated application or change of dietary habits for sustained effects. Aseptic mouth rinses and indiscriminant topical antibiotics6,7,15 are also adopted to reduce the total bacterial load in the oral cavity. Though temporarily effective to various degrees in reducing caries incidence, the nonselective interventions often lead to severe antibiotic-associated infections due to the vacated niche available for cariogenic species re-infection.16

Cariogenic Traits of Streptococcus mutans and Its Prevention

According to the ecological plaque hypothesis stated by Marsh,17 the microbial homeostasis within dental plaque is suggested to shift when the oral environment changes, such as the uptake of fermentable sugars. Continued acid production from dietary sugars by the acidogenic species eventually reduces the pH below the critical threshold of 5.5, triggering a shift in the enamel demineralization/mineralization equilibrium toward demineralization.18 As the principal causative organism of dental caries,5,19,20 S. mutans possesses many physiological traits relevant to cariogenesis.21 By rapid fermentation of carbohydrates, it can generate acidic end products (acidogenicity), which is not only the direct cariogenic factor for demineralization of tooth surfaces but also an environmental determinant that may affect the caries-related microbial flora during cariogenesis.21 Meanwhile, S. mutans has also developed an adaptive acid tolerance response (ATR) to combat the destructive nature of the acidic environment it produces (aciduricity).22 The ability to produce the insoluble extracellular polysaccharide glucan is another critical virulence trait contributing to S. mutans’ cariogenicity.23 This not only promotes attachment and biofilm formation but also provides binding sites that fuel accumulation of a variety of microorganisms on the tooth surface. In addition, the produced glucans can also retain protons from the acidic environment to precondition the bacterium for acid stress.24

Numerous efforts have been attempted to prevent S. mutans from acid production via replacement therapy, which includes applying a genetically engineered S. mutans strain to outcompete indigenous acidogenic bacteria.25 Other methods include colonization control via anticaries vaccines, e.g., immunization against either cell surface adhesins16 or a glucosyltransferase enzyme that is responsible for glucan production.27 However, no favorable results have yet been reported from these treatment approaches.28,29 The current treatments frequently rely on general biocide mouthwashes and broad-spectrum antibiotics administered in the oral cavity. Treatment with broad-spectrum antibacterial agents is known to cause destruction of the entire oral bacterial flora, thus allowing for equal competition between S. mutans and commensal organisms to recolonize the tooth surface. If an individual has poor oral hygiene and a high uptake of dietary sugars, S. mutans will re-infect the oral cavity without difficulty.30,31 and the re-established oral biofilm will retain a persistent cariogenic condition. Conversely, individuals with low levels of S. mutans are resistant to exogenous colonization from cariogenic species and have shown long-term protection from dental caries.6,15,12 Therefore, there is a need to develop an antimicrobial agent with the specific ability to kill S. mutans that can eradicate the primary pathogen of dental caries from the oral microbial community while leaving the remaining commensal organisms intact. If this can be achieved, the major initiator of caries, S. mutans, can be eliminated and a healthy oral biofilm established, which might provide long-term caries protection.6
Application of Oral Antimicrobial Peptides

As part of the innate immunity, antimicrobial peptides (AMPs) have been shown to play important roles in controlling viability of a vast range of pathogens.33,34 Many AMPs have been identified in the oral cavity and represent promising candidates for the development of new oral antimicrobial therapeutics.35,36 The known AMPs belong to six functional families, including cationic peptides, bacterial agglutination and adhesion, metal ion chelators, peroxidases, protease inhibitors and AMPs with activity against bacterial cell walls.37–39 The physical traits of these AMPs are highly dependent on their interaction with the phospholipid bilayer.41 Because of their attraction to negatively charged structural molecules on the bacterial membrane, development of resistance to these peptides is rare,42 making them potentially useful as antibiotics. However, the broad-spectrum antimicrobial characteristics of AMPs alter the ecological balance of the oral microbial community and eliminate the entire oral flora along with any protective benefits provided,43 which has prompted interest in the design of target-specific AMPs.

Specifically Targeted Antimicrobial Peptides

Our research group has initiated a targeted approach to controlling oral microbial pathogenesis via a new class of antimicrobials called specifically targeted AMPs (STAMPs).44 The STAMP requires two functionally independent peptide domains, a killing moiety comprised of a nonspecific AMP that can rapidly kill bacterial cells and a targeting moiety consisting of a species-specific, high-affinity binding peptide.44,45 The two moieties are then integrated through a small linker, generating a fusion AMP without detrimental changes in the independent functions of the two domains. The major strength of such an AMP is that the targeting moiety can guide the conjoined peptide to selectively recognize the target organism, allowing peptide-guided killing. Furthermore, the fusion peptide, which is constructed from two short moieties, can be chemically synthesized with high yields.

By using the structure of STAMP as a template, a number of novel STAMPs with S. mutans-selective activity were generated.46 These potential STAMPs were investigated for their killing potency and selectivity against S. mutans. Among them, C16G2 was selected because of its improved minimum inhibitory concentration (MIC), greatly enhanced killing kinetics and selectivity against S. mutans (FIGURE 1). The STAMP C16G2 was designed by utilizing an S. mutans-produced pheromone, i.e., a competence stimulating peptide (CSP) as the STAMP targeting domain for effective delivery of the AMP without detrimental changes in the independent functions of the two domains. The major strength of such an AMP is that the targeting moiety can guide the conjoined peptide to selectively recognize the target organism, allowing peptide-guided killing. Furthermore, the fusion peptide, which is constructed from two short moieties, can be chemically synthesized with high yields.

By using the structure of STAMP as a template, a number of novel STAMPs with S. mutans-selective activity were generated.46 These potential STAMPs were investigated for their killing potency and selectivity against S. mutans. Among them, C16G2 was selected because of its improved minimum inhibitory concentration (MIC), greatly enhanced killing kinetics and selectivity against S. mutans (FIGURE 1). The STAMP C16G2 was designed by utilizing an S. mutans-produced pheromone, i.e., a competence stimulating peptide (CSP) as the STAMP targeting domain for effective delivery of the AMP without detrimental changes in the independent functions of the two domains. The major strength of such an AMP is that the targeting moiety can guide the conjoined peptide to selectively recognize the target organism, allowing peptide-guided killing. Furthermore, the fusion peptide, which is constructed from two short moieties, can be chemically synthesized with high yields.

STAMPs — Selectivity and Killing Ability

C16G2 has been shown to specifically eliminate S. mutans without affecting closely related noncariogenic oral streptococci in planktonic and saliva-derived biofilm systems.44,45 Our group further investigated the antimicrobial specificity of C16G2 by expanding the panel of streptococci species closely related to S. mutans. This study showed that C16G2 treatment did not significantly affect the diversity of total Streptococcus spp. A panel of 20...
different bacterial species, including oral and non-oral Gram-positive and Gram-negative bacteria in monoculture was also tested. The results revealed an overall low capacity of C16G2 against Gram-negative species. Among the oral Gram-positive bacteria tested, C16G2 was most potent in killing \textit{S. mutans}.\textsuperscript{51}

C16G2 has a rapid mechanism of action, affecting bacteria in less than one minute of exposure, a duration short enough for the application of most oral care products. It is also soluble in aqueous solutions, indicating that the STAMP is readily amendable for delivery to the oral cavity in a mouth rinse vehicle.\textsuperscript{44,46,52,53} In another study,\textsuperscript{50} a 40 s rinse with a mouth rinse formulation containing 0.04\% C16G2 was administered only once at the start of a four-day test phase (no fluoride toothpaste was used during this time period). We observed that C16G2 was highly effective in decreasing levels of plaque and salivary \textit{S. mutans}. The fact that the placebo group showed a significant increase in the relative amounts of \textit{S. mutans} confirms that growth conditions were favorable. The study also supported that at day four the concentration of \textit{S. mutans} was significantly lower in the C16G2-treated group, which suggests that the antimicrobial activity of C16G2 is \textit{S. mutans} selective. In addition, further evidence for \textit{S. mutans} selectivity was shown, as the overall bacterial community composition at day four was highly similar for the C16G2 treated and placebo groups. This study also strongly suggested C16G2 had high efficacy for preventing \textit{S. mutans} from regrowing despite frequent exposure to sugar during the four-day period.\textsuperscript{50} Although C16G2 show strong inhibitory effects, reinfection is highly likely due to shared lifestyles and environments among family members who may be \textit{S. mutans} carriers. Therefore, it is likely that the C16G2 treatment will have to be repeated.

\textbf{Modes of Action}

The STAMP-targeting region drives the enhancement of antimicrobial activity due to increased binding to the surface of a targeted pathogen utilizing specific determinants such as overall membrane hydrophobicity, charge and/or pheromone receptors, which in turn leads to increased selective accumulation of the killing moiety.\textsuperscript{44,48} The exact mechanism through which AMPs kill targeted bacteria is not well understood and likely varies peptide by peptide, but membrane disruption and subsequent interference with intracellular targets are thought to be the main processes responsible.\textsuperscript{54–57} Sequence analysis of C16G2 suggests that it is an amphipathic and cationic \(\alpha\)-helical peptide, similar to traditional AMPs.\textsuperscript{54} The hydrophobic moment of C16G2 is considerably greater than that of its individual moieties due to the stacking of hydrophobic residues in the STAMP. Our group’s data suggest that CSPC16-\textit{S. mutans} binding is species-specific but is independent of the ComD surface receptor,\textsuperscript{44} which can sense pheromone CSP and triggers the signaling cascade for bacteriocin production and other cell density-dependent activities.\textsuperscript{58} A natural \textit{S. mutans}-specific targeting sequence in this pheromone might bind to an alternative receptor (e.g., lipids, exopolysaccharides or teichoic acids) on the bacterial surface prior to interaction with ComD. An explanation of the selective killing activity against \textit{S. mutans} by CSPC16 might be the absent avidity or hydrophobic interactions of CSP16 with the membrane of untargeted oral organisms, resulting in poor binding and/or retention as well as a lack of \(\alpha\)-helical adoption, resulting in decreased hydrophobic moment and membrane activity. The proper folding of CSPC16 on the surface of \textit{S. mutans} may retain a role in sequestering and retaining STAMP. Although the exact mechanism of selective membrane disruption by C16G2 remains unclear, it may involve early membrane binding or partition steps governed by the targeting moiety of C16G2.\textsuperscript{53} Recent studies have indicated that C16G2 kills \textit{S. mutans} through membrane disruption with small molecules subsequently leaking out of the cell followed by a loss of membrane potential and cell death.\textsuperscript{53} It seems likely that the amphipathic characteristic shared between C16G2 and AMPs results in the STAMP functioning as a membrane disrupting peptide but with greater specificity for its target.\textsuperscript{53}

The authors’ study and the study by Eckert et al.\textsuperscript{44} showed significantly enhanced killing of \textit{S. mutans} cells but no activation of the signal transduction pathway or its regulated genes.\textsuperscript{59,60} This may be because all fusion peptides lack a C-terminal structural motif of CSP, which is known to activate the signal transduction pathway.\textsuperscript{59}

\textbf{Impacts on Microbial Community Ecology}

Microbial communities usually result from complex intraspecies, interspecies and microbe-host interactions. Any change in the abundance of a particular species within the community could have drastic effects on its interacting partners, eventually resulting in a
change of community profile as well as community level functions. To explore if the application of C16G2 affects the composition shift of the oral microbial community, a saliva-derived in vitro model system containing more than 100 species approaching the diversity and overall metabolic functionality of the human oral microbiome was applied. We treated S. mutans-containing in vitro planktonic oral microbial communities with C16G2 for 30 minutes followed by extensive washing to remove the residual C16G2. The treated communities were then allowed to recover by being cultured in fresh nonselective medium. The microbial composition of the recovered community was determined by 454 pyrosequencing analyses to examine how the removal of S. mutans may affect other species within the same community.

The most intriguing finding was that the targeted removal of S. mutans had a community-level impact on the species composition and abundance within the same community. Data showed that 21 bacterial genera could be detected from regrowth of untreated samples with Streptococcus, Veillonella, Parvimonas, Prevotella and Peptostreptococcus spp., being the most dominant genera (FIGURE 2). In contrast, only 16 bacterial genera were detected from the regrowth of the C16G2-treated samples, with Streptococcus, Granulicatella and Prevotella being the most dominant ones (FIGURE 2). Interestingly, although the relative abundance of S. mutans reduced drastically, the overall sequence counts of all Streptococcus spp. increased from 30 percent to 81 percent in the culture recovered after C16G2 treatment. Meanwhile, many bacterial genera, most of which were Gram-negative bacteria, including Fusobacteria, Campylobacter, Neisseria and Parvimonas spp., which were present at less than 5 percent, could no longer be detected at

![Cluster analyses of oral taxa-weighted abundance profiles obtained from regrowth after treatment with Carrier (negative control) and C16G2. Relative proportions of the total taxa abundance are indicated in the heat map, which shows how the dominant taxa varied. The figure is modified from Guo and colleagues.](image-url)
the depth of sequencing obtained from the regrowth of the C16G2-treated samples, whereas genera such as Veillonella suffered drastic reductions in relative abundance within the community (from 20 percent to less than 1 percent).\(^\text{31}\)

Our study indicated that the reduction in the S. mutans population by C16G2 was accompanied by an increase in the abundance of several streptococci from the mitis group, including S. mitis, S. cristatus, S. oralis and S. sanguinis, signature bacterial species identified from the oral microbial community of healthy subjects.\(^\text{62–64}\) The antagonism between S. mutans and streptococci of the mitis group, particularly S. sanguinis and S. gordonii, at the ecological level has been well-documented.\(^\text{64}\) Epidemiological studies revealed that high levels of S. mutans are always concurrent with low levels of S. sanguinis\(^\text{65}\) whereas high levels of S. sanguinis in the oral cavity correlate with delayed S. mutans colonization.\(^\text{50}\) Recent work by Kreth et al.\(^\text{66}\) showed sophisticated interspecies interactions between these two species that might play an essential role in balancing competition and coexistence within the oral community. The targeted removal of S. mutans could shift the balance and provide a competitive growth advantage to the mitis group.

After overnight regrowth, the C16G2-treated community showed decreased microbial diversity compared with the negative control. Many Gram-negative species, such as Veillonella, experienced drastic reductions in abundance whereas F. periodonticum, Campylobacter, Gemella and Neisseria, which are implicated in the pathogenesis of periodontal disease,\(^\text{67}\) could not be detected by pyrosequencing from communities recovered from the C16G2 treatment, although they were only present at abundances of less than 5 percent. The results might be caused by the nonspecific killing of the peptide. However, the data showed that some of these species, including F. periodonticum, displayed high levels of resistance against the C16G2 treatment, suggesting that the reduction or elimination of certain species could be directly or indirectly associated to the removal of S. mutans. For example, it has been shown that lactic acid, a metabolic product of S. mutans, is required for the growth of Veillonella spp.\(^\text{68}\)

The reduction in the S. mutans population as a result of the C16G2 treatment may, therefore, have had a negative effect on the growth of Veillonella spp., such as was seen in our metagenomic data.

The use of STAMP C16G2 to modulate the microbiome structure allows insight into the therapeutic potential of C16G2 to achieve a healthy oral microbiome, because several bacterial species with metabolic dependency or physical interactions with S. mutans suffered drastic reduction in their abundance, whereas S. mutans’ natural competitors, including health-associated oral streptococci, became dominant.\(^\text{31}\)

**STAMP Stability and Safety**

The half-life of C16G2 was estimated to be 18.8 minutes in pooled human saliva, suggesting the STAMP is unlikely to be retained at meaningful quantities in the oral cavity after long durations, indicating its favorable safety.\(^\text{50}\) C16G2 could be formulated in phosphate buffered saline with overnight stability at least 4 degrees Celsius without excipients or stabilizers; it remained active and capable of penetrating dental plaque to inhibit S. mutans and could be freshly prepared up to four hours before treatment if stored at room temperature.\(^\text{50}\) Also, the therapeutic concentrations of 25–100 μM had no hemolytic activity against human red blood cells, isolated human cells or defined tissue,\(^\text{83}\) which suggests that C16G2 does not interfere with human host cell integrity and is therefore relatively safe.

**STAMP C16G2 Protective Effects**

Compared to AMPs with wide spectra of activity, the STAMP C16G2 has demonstrated specificity for S. mutans in multispecies communities, resulting in the complete killing of S. mutans while leaving noncariogenic oral streptococci in the environment unaffected.\(^\text{51}\) Moreover, 0.04% (w/v) C16G2 rinse usage can effectively lessen lactic acid production and protect enamel against demineralization in an in vitro model under the conditions of accelerated demineralization induced by frequent exposure to sucrose, which suggests that C16G2 is effective against S. mutans and its cariogenesis in vivo.\(^\text{50}\)

C16G2 was also shown to significantly elevate the resting pH of dental plaque compared to the placebo rinse.\(^\text{50}\) The higher resting pH creates conditions that are favorable for growth of healthy bacteria and unfavorable for cariogenic (acidoduric) bacteria. This may be in part responsible for helping keep the S. mutans population from recovering in spite of the frequent exposure to sugar. According to the report by Sullivan,\(^\text{50}\) a single STAMP treatment was able to
selectively eliminate *S. mutans* from plaque and salivary bacterial populations while leaving the remaining flora relatively undisturbed. The effect resulted in an *S. mutans*-free “healthy plaque” that resisted *S. mutans* overgrowth despite sucrose challenges of up to four times daily for the entire course of treatment. It is well known that *S. mutans* is the critical and central facilitator of caries development, at least for caries linked to intake of dietary sugars and not resulting from pre-existing pathologies. Therefore, it may be possible to generate a “healthy” noncariogenic microbial ecosystem in the oral cavity through STAMP intervention at the clinical level, as has been demonstrated.50 An intact dental biofilm without *S. mutans* could resist future exogenous *S. mutans* colonization or overgrowth due to sucrose consumption and could delay or postpone cariogenesis. The oral community that recovered from C16G2 treatment exhibited a health condition with an increase in the population of the noncariogenic species, *S. mitis* and *S. sanguinis*, and a reduction in many periodontitis-associated Gram-negative species, such as *Fusobacteria.*51

In contrast to current aseptic interventions, the selective hallmark of STAMP C16G2 drives its development into “probiotic” antibiotics, which could selectively eliminate caries-causative species while preserving the protective colonization effects associated with noncariogenic oral flora that overtake *S. mutans* colonization sites or antagonize the growth of the bacterium directly. The established *S. mutans*-free biofilms through STAMP treatment can reduce the competitive advantage of *S. mutans* even in the presence of high sugar content,50 thus preventing the shift in the biofilm composition toward cariogenesis. Furthermore, the prior establishment of an *S. mutans*-free biofilm provides considerable protection against subsequent reestablishment of this oral pathogen in oral biofilm. In this regard, the STAMP C16G2 may represent a remarkably effective weapon against dental caries that is easy to formulate, easy to administer, complements existing oral hygiene regimens and can be dosed infrequently compared to other oral care ingredients.

Conclusions and Future Directions

As an alternative to conventional antibiotics, antibacterial peptides such as C16G2 have been explored for therapeutic uses. C16G2 is a highly attractive solution to caries disease as it has robust and selective activity against cariogenic *S. mutans* planktonic and biofilm cells in vitro. When available as a mouth wash or gel trays, the treatment will likely have multifold benefits, such as an intact oral ecosystem (i.e., no vacated niches open up for pathogens colonization) and no threats of drug resistance development. In the future, if C16G2 passes clinical trials, it could be prescribed as a mouth rinse or as gel trays for treating clinically diagnosed caries disease. Monitoring of treatment efficiency would have to be conducted by the treating dentist who also would make decisions on treatment time. Posttreatment with fluoride and follow-up visits at the dentist would serve as reinfection prevention. STAMP C16G2 is developed under an investigational new drug authorization with the Food and Drug Administration and is currently in Phase 2 clinical trials. This new technology could have an impact far beyond dentistry and could possibly be used to treat and prevent other microbiome-related diseases.

**ACKNOWLEDGMENT**

The authors thank Dr. Xiaoyu Tang for help with the editing of this manuscript. They also thank Dr. Pierre Kyme and Dr. Brian C. Varnum at C3J Therapeutics Inc. and Dr. Wenyan Shi at the University of California, Los Angeles, School of Dentistry for providing images and knowledge on STAMP C16G2 development.

**REFERENCES**


THE CORRESPONDING AUTHOR, Anna Edlund, PhD, can be reached at aedlund@jcv.org.
Oral Microbiota Transplant: A Potential New Therapy for Oral Diseases

Marcelle Nascimento, DDS, MS, PhD

ABSTRACT Dental caries and periodontitis are among the most common diseases affecting humans worldwide. There is an evolving trend for dental and medical research to share knowledge on the etiology and promising therapies for human diseases. Inspired by the success of fecal microbiota transplant to manage gastrointestinal disorders, oral microbiome transplant has been proposed but not yet tested in humans. This article critically reviews the potential of oral microbiome transplant for managing oral diseases.

Recent systematic review has called attention to the fact that untreated dental caries is the most common disease and severe periodontitis is the sixth most common disease affecting humans globally.1 Of further concern are the serious implications that these oral diseases can have on general health.2 The oral microbiome is comprised of hundreds of microbial species that co-inhabit and functionally interact in oral biofilms to cause disease or to maintain homeostasis.3,4 Caries and periodontitis are closely related to a dysbiosis of the microbial consortia driven by environmental changes, such as a sugar-frequent/acidic-pH environment in caries and a protein-rich/neutral-to-weakly alkaline-pH environment in periodontal disease.5–7 In caries, continuous acid production from the metabolism of dietary carbohydrates results in the emergence of acid-producing and acid-tolerant organisms in supragingival biofilms, a selective process that alters the pH homeostasis of biofilms and shifts the demineralization-mineralization equilibrium toward loss of tooth minerals. Accumulation of subgingival plaque leads to inflammation of the gum tissues, or gingivitis, which may progress to periodontitis. In periodontitis, certain members of the microbial community can destabilize the host immune response, which may result in destruction of periodontal tissues in susceptible individuals. Conventional therapies for caries and periodontitis aim at controlling the formation and metabolic activities of supra- and subgingival biofilms. But caries and periodontitis still remain as major public health problems worldwide. Clearly there is an urgent need to identify novel and more efficient strategies for intervention of these oral diseases that can be widely and safely utilized in a cost-effective manner.
There has been an increasing interest in therapeutic interventions that modulate microbial ecology to restore homeostasis of human biofilms and thus health. Such interest follows insights provided from the Human Microbiome Project revealing that ecological balance in biofilms plays a significant role in health. Fecal microbiota transplant (FMT) is an example of therapy based on altering the dysbiotic microbiota to restore microbial ecological balance. The remarkable success of FMT to treat persistent Clostridium difficile infections suggests that the gut microbiota has sufficient plasticity to undergo ecological interventions that improve health. Specifically, Clostridium difficile can be replaced by commensal and beneficial gut bacteria that has been killed or suppressed, usually from the continuing use of antibiotics. Inspired by the fecal transplantation in medicine, oral microbiota transplant (OMT) has been hypothetically proposed by some dental researchers. This article critically reviews the potential of OMT as a new therapy for managing oral diseases such as caries and periodontitis.

**Fecal Microbiota Transplant**

The human gut microbiota is highly complex and functions to support health in a similar way as the microbiota of other organ systems. Treatment options for gastrointestinal disorders include changes, probiotics, prebiotics and FMT. In particular, the FMT procedure involves administration of fecal material (stool) from a healthy donor to a patient with a disease or condition related to dysbiosis or alteration of their normal gut microbiota. The donor may be an intimate, long-time partner, friend or an unrelated volunteer. The stool suspension taken from the donor is mixed with saline or other solution, strained and introduced into the gastrointestinal tract of the recipient via colonoscopy, enema or a nasogastric tube. FMT usually involves a single administration dose but the use of several doses has been proposed. Different from probiotic therapies in which few bacterial species are dispensed, fecal transplant introduces thousands of naturally occurring gut microorganisms into the colon. Theoretically, the native microbiota used in FMT is more likely to thrive in the acidic environment and during intestinal transit to adhere to the intestinal mucosa and to produce antimicrobial substances that contribute to their beneficial health effects.

FMT has been used to manage chronic inflammatory bowel diseases, insulin sensitivity, ulcerative colitis and autism spectrum disorders (ASD). However, better outcomes were shown when FMT was used to treat persistent Clostridium difficile infection. The figure illustrates the use of FMT to increase the diversity of the gut microbiota and eradicate bacteria containing antibiotic resistant genes. Current clinical and best practice guidelines with indications for fecal transplants and protocols for donor selection and screening, stool preparation and methods of administration were reviewed elsewhere. Although FMT is a promising approach to alter the gut ecosystem and improve gastrointestinal health, evidence of its true effectiveness remains questionable and concerns have been raised regarding short- and long-term safety and tolerability. FMT remains classified as an experimental treatment and complications with regulatory agencies have limited the general use of this therapy.

**Oral Microbiota Transplant**

Involuntary transmission of oral microorganisms from one individual to another via saliva is a common life occurrence. Whereas OMT is not part of this natural event, this therapy aims at transferring oral biofilms from a healthy donor to a patient with caries or periodontitis. Human OMT has been hypothetically suggested by Floyd Dewhirst, DDS, PhD, and Diane Hoffmann, JD, MS, (unpublished data, online PowerPoint presentation) and others, but thus far no actual oral transplantation has been reported.
The procedure that was hypothetically proposed by Dewhirst and Hoffmann involves collection of supragingival plaque from a caries-free donor (potentially a relative of the recipient patient), storage of plaque in saline and the use of a nylon swab to transfer the collected plaque to the teeth of a caries-active patient. According to their proposed protocol, the donor should have a healthy oral microbiota that excludes cariogenic bacteria such as S. mutans and presents minimal pH drop in response to sugar challenge.

Pozhitkov et al. proposed to introduce health-associated oral microbiota into the oral cavity of periodontitis patients. First, they confirmed that the microbiomes of subjects with periodontitis were distinct from those of healthy or edentulous patients. Next, they tested in vitro antimicrobial protocol to be used on the oral cavity of the recipient patient prior to OMT. It was shown that application of sodium hypochlorite (NaOCl) followed by its neutralization with sodium ascorbate buffer may be a valid option for suppressing the disease-associated microbiota to allow for a more pronounced microbial shift to a healthier microbiota. In that same study, the authors suggested an OMT procedure consisting of collecting sub- and supragingival plaque from a healthy donor (spouse or partner), performing deep cleaning, root planing and applying a broad-spectrum antimicrobial agent to the periodontitis patient and, finally, neutralizing the antimicrobial agent immediately following by rinsing with a microbial suspension harvested from the healthy donor in the periodontitis patient.

**Considerations**

The oral cavity is a complex ecosystem in which a rich and diverse microbiota has evolved since birth. The most abundant taxa in oral biofilms display remarkable phenotypic plasticity, e.g., health-associated and disease-associated bacteria can morph rapidly in response to oral environmental changes. In other words, the composition and metabolic activities of microbial communities fluctuate according to the constant environmental changes in pH, nutrient availability, oxygen tension and redox environment, shedding effects of oral surfaces and composition of salivary and crevicular fluids. These changes in the environment, whether imposed by diet, behavior, systemic conditions or medications, may disturb the homeostasis and lead to endogenous infections or susceptibility to exogenous infections. Evidently, intermicrobial species interactions and immunostimulatory effects are expected to play a key role in OMT therapy. Transplanted oral biofilms must exhibit the capacity to:

- Endure the selective pressure of the environment effectively.
- Colonize the oral sites.
- Compete with the disease-microbiota for adhesion sites and nutrient sources.
- Produce substances like bacteriocin and hydrogen peroxide to inhibit the growth of pathogens.
- Modulate local and systemic immune functions.

Safety concerns related to the potential application of OMT are similar to those for oral probiotics. As with probiotics, transplanted biofilms must not cause disease and should possess a high degree of genetic stability. At this point, the mechanisms of action and ideal vehicles for OMT have not been extensively discussed. For example, it is critical to determine whether oral biofilms should be transplanted directly from a healthy donor to a diseased patient or pretreated with methods that eliminate (or attempt to reduce the proportions of) pathogenic organisms prior to transplantations, or even if biofilms created in vitro but composed by naturally occurring commensals organisms would be the best option for OMT. Biofilms composed of clinical strains with beneficial and health-associated properties may be proven effective at interacting and replacing disease-associated biofilms. Other topics for discussion include the need for disinfecting the oral cavity of the recipient patient prior to OMT and whether one dose or multiple doses of oral transplants would be necessary for an effective and permanent colonization of the oral cavity in order to restore and maintain health.

Evidence from oral microbiome studies points to a progressive increase in complexity and diversity from birth to adulthood. In health, the oral microbiome appears to be more stable than those of other body niches like the gut but still with a substantial degree of within-individual variability. In disease, microbial diversity appears to be lower in caries than health, which may reflect the ecological pressure of low environmental pH. Contrasting with caries, periodontal diseases are associated with an increase in microbial diversity, which could be the result of impaired local immune function, increased availability of nutrients or a reflection of the diverse environmental niches at the periodontal pocket. Hence, it is important to keep in mind that while the goal of OMT for caries therapy may be to increase bacterial diversity, the goal of OMT for
periodontitis may not be the same because the bacterial diversity is already high. —

Ongoing and future metagenomics and metabolomics studies ought to increase our understanding of the oral microbiota dynamics and provide new insights on how a dybiotic microbiota can be successfully replaced by a health-beneficial flora. Moreover, studies involving other kingdoms, such as viruses, fungi, archaea and protozoa, should provide a more realistic picture of the complex interactions contributing to the compositional and functional stability of the oral ecosystem. Undoubtedly, well-conducted in vitro and animal studies as well as clinical trials with a proper study design are needed to clarify the questions raised by this review. Future clinical trials must be conducted using clinical (carious lesions and loss of periodontal attachments) outcomes as endpoints measurements rather than microbial measurements alone and extensive follow-up times should be included. Of great importance, if OMT is to be implemented in the future, the success will be dependent on the association of this therapy with other therapeutic strategies aimed at reducing the risk of caries and periodontitis.

Conclusion

Despite limited scientific and clinical evidence, oral microbiota transplant holds promise as a new therapy for managing caries and periodontitis. OMT may represent a cost-effective approach and have the ability to better reach difficult-to-access, high-risk populations. However, clinical recommendations for the use of OMT cannot be provided at this point based on the current state of knowledge. It is crucial to have a better understanding of the retentiveness of transplanted oral biofilms while maintaining the natural balance of the resident oral microbiota with the host immune responses.

Understood issues include best practices for optimal donor selection, sample preparation, vehicles, follow-up timing and number of administrations. ■

REFERENCES


to practicing on your terms.

Every practice is unique. That’s why CDA created The Dentists Service Company as a new subsidiary focused on helping you achieve your vision of success. Grow your patient base, streamline operations and boost productivity with TDSC’s practice management advising services.

Ready to see results? Let’s go.

888.253.1223  
tdsc.com
SOLD

LOS ANGELES COUNTY

BEVERLY HILLS — Modern designed Periodontal practice in multi story medical professional bldg. Has 3 eq ops and 2,100 sq ft. Property ID #5157


LOS ANGELES — GP established in 1968 in a 6 story bldg. NO HMO. Has 4.5 eq ops in a 1,217 sq ft suite. GFC $531K in 2016. Property ID #5163.

MOTEBELLO — GFC approx. $1.1M in 2016, located in a free standing bldg w/ 5 eq ops. Established in 2002. Property ID #5168

PASADENA — Located in the heart of Pasadena w/ 60 years of goodwill. GP w/ 5 eq ops. GFC $616K in 2016. Has 3 eq ops in a ~385 sq ft suite. Property ID #5147.


VALENCIA — GP + Equipped with 9 eq ops in a busy single shopping center. GFC $1.6M in 2016. Property ID #5171.


KERN, VENTURA, & SAN LUIS OBISPO COUNTIES


SB COUNTY

ANAHIM — Established in 1960’s this practice is on a single story bldg w/ 4 eq ops. GFC $735K in 2016. Net $308K. Property ID #5187.

BREA — Beautiful well established practice located on a corner location. Has 8 equipped ops and 3 chairs in open bay. GFC $1.5M. On a busy major street of the city. Property ID #5190.

COSTA MESA — GP w/ 5 eq ops and 3 chairs in open bay in a busy retail shopping center. Established in 2005. 4 day/wk practice. GFC approx. $656K in 2016. Property ID #5121.


LAGUNA HILLS — With over 30 yrs of goodwill this GP is located in a 2 story med bldg. Has 5 eq ops in a 1,600 sq ft suite. Grossed $304K for 2016. Property ID #5127.

MISSION VIEJO (TURN-KEY) — Modern designed GP located in a 2 story med/dent bldg. Has 3 eq / 3 plmbd for expansion. Property ID #5138.

MISSION VIEJO — Group solo practice in a 2 story medical building. Has 6 eq ops with digital x-ray. PPO/Cash/50% NET OF $515K. Property ID #5142.


SAN DIEGO COUNTY

EL CENTRO (GP) — Located in a single story bldg. Building is for sale. 5 equipped operatories. GFC $347K for 2016. Property ID #5023.


LA JOLLA — Beautiful practice — LH & Equip Only!

OCEANSIDE — Well established practice near the ocean in a 2 story bldg. Has 3 eq ops w/ 2 hygiene ops. Lots of foot traffic. GFC $422K in 2016. Property ID #5191.

RIVERSIDE & SAN BERNARDINO COUNTIES

BANNING — LH & Equipment only! Consists of 3 eq ops in a 925 sq ft suite. Property ID #5184.

BEAUMONT — GP + Real Estate! Modern GP w/ 6 eq ops in 2,400 sq ft bldg to be suites. GFC $960K in 2016. Property ID #5182.

CHINO — Real Estate Only! This is a rare opportunity to purchase a condo located in a single story strip mall. Has been a dental practice for 40 years. Property ID 5076.

FONTANA — GP + Real Estate!! Premier office with 50 years of goodwill in a 3,000 sq ft bldg with 8 eq ops. Has the latest technology. GFC approx. $2.3M in 2016. Net of $968K. Property ID #5140.

CORONA — GP w/ over 30 years of goodwill in single story medical building. Has 3eq ops. Buyer’s net $1.4 mil. Property ID #5133.


BUYER’S NET OF $153K. Property ID #4847.


TEMECULA — Modern designed practice w/ 3 eq ops. Projecting approx. $1.2M . Net of $444K. Property ID #5155.


Visit our Website and Social Media pages for Practice Photos and Videos

www.calpracticesales.com

Phone: (800) 697-5656

Contact Us for a Free Consultation
Your practice needs the entire team to be sharp and focused for the schedule to run smoothly. So if you’ve ever had an employee show up in the morning unable to perform duties, you can expect to have both a hard conversation and a long day. The impact of an employee’s substance abuse on a practice extends beyond impaired performance and productivity. Substance abuse leads to higher rates of absenteeism, workplace accidents and patient injuries — all of which impede safety and increase practice liability.

Because every situation is unique, it’s best to exercise an abundance of caution when dealing with employees who appear to be intoxicated or under the influence. The Dentists Insurance Company’s Risk Management team advises dentists to contact their human resources specialists or an employment attorney for counsel specific to their situations. However, there are some essential steps you can take and a few actions to avoid if faced with this challenge.

It starts before you even experience an incident. TDIC recommends that all practices have a detailed drug-free workplace policy, either as part of the employee manual or as a stand-alone document signed at the time of hire. The policy should cover rehabilitation/counseling options and disciplinary actions, including grounds for dismissal. If you intend to conduct reasonable suspicion fitness for duty testing, this should be detailed as well. Contact your attorney for advice specific to your practice.

In addition to having a specific policy in place, the practice owner and the individual who performs human resources duties should be trained on how to handle these types of sensitive situations. The U.S. Office of Personnel Management publishes an online guide called Alcoholism in the Workplace: A Handbook for Supervisors, which can be a helpful training tool.

If an employee does come in to work displaying unusual behavior, observe carefully for the following signs:

- Breath smell.
- Bloodshot eyes.
- Slurred speech.
- Lack of balance.

In a case reported to TDIC, a dental assistant came into work on more than one occasion smelling of alcohol. Her performance and interactions with colleagues and patients were declining and she was using language and a tone inappropriate for the workplace. During
one incident when she was acting highly emotional and erratic, the dentist talked to her and addressed the behavior and then gave her the day off to take care of personal issues. The situation escalated as the employee continued to demonstrate unprofessional behavior, absenteeism and declining performance. The office did not have a drug and alcohol policy in place. Without knowing how to pursue the matter, the dentist contacted TDIC for advice and was referred to an employment attorney.

If an employee in your practice exhibits unusual behavior, document your observation in an objective manner and note only the observable facts in the employee’s file. Making a diagnosis or accusation can heighten an already stressful situation and open the practice up to liability. Rather, express concern for patient and coworker safety and state the facts in a manner such as, “I am concerned. I have observed you slurring your speech.” As there are situations in which an employee behaves erratically due to a prescription medication or a health issue, addressing the underlying behavior and workplace safety is prudent.

Chris Onstott, an employment attorney at Kronick Moskovitz Tiedemann & Girard in Sacramento, California, emphasizes the importance of having an additional person at the practice observe and address the uncharacteristic behavior.

“If the managers observe behaviors that support a reasonable suspicion of intoxication or impairment, then the next steps can be taken as appropriate to the practice’s drug-free workplace policy.”

If your drug policy includes fitness-for-duty testing and the employee refuses to comply, the employee’s refusal may lead to a finding that he or she is being insubordinate. But regardless of an employee’s willingness to comply with the testing, you should help provide him or her a safe ride home. Document the interaction and its outcome in the employee’s file along with the employee’s behaviors that led to the reasonable suspicion and all of the actions and outcomes that followed.

Every member of the dental team should have a clear understanding of the practice’s expectations and the gravity of the drug policy. The role of a practice leader is not to diagnose an alcohol problem but to exercise responsibility in dealing with performance or conduct problems, hold the employee accountable, refer to the practice policies and take appropriate disciplinary actions. This role is crucial to a safe and productive team.

TDIC’s Risk Management Advice Line at 800.733.0633 is staffed with trained analysts who can answer drug policies and other questions related to a dental practice.
**Matching the Right Dentist to the Right Practice**

**4150 SANTA CRUZ GP**
Seller retiring from successful 33 year general practice. Fee-for-service only practice. Fully-equipped 4 op facility in beautiful, remodeled Seller owned building. Building also for sale.

**4162 PETALUMA GP**
Retiring Seller looking to transition a stable and loyal patient base. Averaging 10-15 new patients per month with 2 dedicated days of hygiene and approx. 3 doctor days per week. 2016 Gross Receipts $304K+. Asking $150K for practice. 7 ops (5 fully equipped) in 2,400 sq. ft. Single story, stand alone dental building available to purchase, or Seller will supply long-term lease with a Right of First Refusal to buy the building.

**4145 ROSEVILLE GP**

**4129 PETALUMA GP**
GP located in stunning 1,856 sq. ft. seller owned facility. State-of-the-art office includes 6 ops, staff lounge, reception area, private office, business office, lab area, sterilization area, consult room, separate storage area, bathroom plus private bathroom. Asking $525K.

**4192 REDWOOD CITY GP PATIENT RECORDS**
Patient records of profitable, established general practice for sale. 1,302 active patients & approximately $800k net collections per annum. Doctor willing to assist with patient transition. Asking $370.

**4177 SAN JOSE PROSTHO**
Implant, cosmetic and prosthodontic practice, established 25+ yrs in desirable West San Jose area close to several amenities and referral sources. Ideal for the restorative general dentist inspired by cosmetic and implant dentistry, or a prosthodontist. 3 fully equipped ops in 1,100 square ft. Bright and modern treatment rooms in well established professional medical building. Lots of on-site parking, EZ freeway access. 3 yr. average GR $1.2M+ with adjusted net of $500K+. Asking $813K.

**4133 NAPA GP**
Napa County GP in newly furnished, fully equipped 2 op facility with digital x-ray. 4 doctor day/week with 3 hygiene days. Monthly average revenue of $36K. Seller willing to help for a smooth transition. Asking $331K.

**4185 SILCON VALLEY ORTHO**
Compact, well-run practice available due to relocation. Established 23 years in convenient, high traffic location near major routes. 1,100 sq. ft. leasehold with reception, waiting room, 3 chairs, exam room, lab/sterilization area, storage area, private office + bathroom, patient bathroom. 2.5-day doctor week offers ample opportunity to expand. Asking $186K.

**4190 SAN JOSE GP**
Excellent location on west side of SJ in the Blossom Hill area. Easy access to Hwy 85 & 87 and light rail. Offering 17+ years of goodwill. Beautifully appointed 3 op office in 950 sq. ft. Plentiful on-site parking. 730 active patients with 1.5 days of hygiene. Average two years gross receipts $389K with adjusted net of $154K.

**4127 MENLO PARK GP**
GP offering 35+ yrs of goodwill, this gem on the Peninsula is truly a find. 4 ops in 950 sq. ft. 2016-2014 average GR $567K with average adj. net of $156K. 750+ active patients & 1 hygiene day a week generate 40-45% of the revenue. Asking $417K.

**4108 HUMBOLDT COUNTY GP**
Well-established, high performing general practice boasts 6 fully equipped ops in 2,900 sq. ft. free standing office w/ Digital X-ray, 2 platinum Dexis sensors, & Cerec Omnicam & MCXL units. Perfect for a dentist who wants to escape the grind and live along the coastline. 2016 GR $1.5M+. Asking $995K.

**UPCOMING:**
Endodontic practices in Sonoma County, Peninsula and East Bay.

GP in Campbell, GP on the Peninsula and GP in San Bruno.
SAN FRANCISCO: Financial District office, 5 Ops, room for 6th, 1760 sq. ft. 2015 GR $1.2 mil. Adj. Net $480,000.  #353
SAN FRANCISCO: Periodical Practice & Condo Unit, 1,160 sq. ft. w/4 Op, 2014 GR $714K w/363K Adj. Adj. #324
SAN RAMON FACILITY 4: Facility w/high-end Pelton & Crane Equipment, Digital X-Ray, Digital P. O. Cameras, 1654 sq. ft. corner suite. #3370
SAN RAMON: New Listing! General Dentistry, emphasis on children, 5 treatment chairs, 1700 sq. ft. 2016 GR $444k, min work week due to dentists. Pan. Paperless Charts, Digital Sensor, Digital Pan.  #3439
SONOMA COUNTY: 2,145 sq. ft. office w/5 Ops, Digital X-Rays, Digital Cameras. 2016 GR $131k, low overhead. Building available for sale. #3330
WINEOFT Winery: Perico, 2016 GR $1,997,000 w/978K adj. net in 3,000 sq. ft. office space. Building for sale. #3348
STOCKTON: 7 Ops, 2,052 sq. ft. great lease and options to renew. GR $750k with PPO, HMO and FFS patients. #3341
VALACI0: 1,342 sq. ft. ex. Ops, Intraoral Camera, Digital X-ray, Pan. Laser, Dentistry Software. 2015 GR $911k, 4 day/week. #3389
CENTRAL CALIFORNIA
FRESNO: New Listing! GR. GR past 3 years averaging $1.5M. 4 days hygiene. Owner is retiring. Priced for an incoming sale. Delta PPO but no HM0s. #3345
MODESTO: New Listing! 1,110 sq. ft. 3 Ops, Dentrix software, Digital Digital X-Ray, Fiber Optics, Laser. 2016 GR of $706K 4 day/week. #33451
MODESTO: 2015 GR $918k, adj. net $441k. 2,000 sq. ft., 8 Ops, digital x-rays, Dentrix Pan. Software. Owner retiring. #33451
SANTA CRUZ: New Listing! Approx. 1000 sq. ft office w/4 Ops, established for over 55 years. 2016 GR of $383K w/adj. of $186k for 3 day work week. #33427
SOUTHERN CALIFORNIA
CENTRAL ORANGE COUNTY: New Listing! 4 Ops, Intra-Oral Camera, Pan. 3 days of hygiene/week. 50+ yrs goodwill w/retiring seller plus previous owner. #33424
COASTAL ORANGE COUNTY: Perio. 5 ops. addl. 30 year owner. Great loc. Dig software and Pan. 2016 GR of $80k Adj. work week. #33425
FULLERTON: New Listing! 5 Ops. C Equipped in shopping center, PracticeWorks, Cerec, Digital, 4 days of Hyg/wk. GR $693K w/325K Adj. Net. #33453
HAWTHORNE FACILITY ONLY: New Listing! 8 Ops (6 furnished), Pan, wired for networking, great location on Hawthorne Blvd. W/signage. Busy area with street traffic. Make offer. #33455
LA QUINTA: 8 Ops in busy shopping center, 25 years of Goodwill. Last year's GR $1,64M. State-of-the-art, Dental. Seller Retiring. #33452
LOS ANGELES: 5 Ops, 4 Equipped. LA's only Private practice, Main in local great visibility. Open Dental, Digital X-rays. 2016 GR of $461K w/185k Adj. Net. #33415
ORANGE COUNTY: New Listing! 4 Ops, 40+ years goodwill, OpenDental, Digital X-rays. 2016 GR $533k with $170k Adj. Net. #33440
PALM DESERT: 8 Ops, 1200 sq. ft. 2016 GR $1.1M, Digital x-rays, nice location, big signage. Owner is an Associate in the practice. #33426
Palm Desert: 8 Ops, Equipped. High-end build-out w/Dentrix, Digital X-rays, Panos, and Lasers. Selling dentist works 4 days/week, GR $1.3MM and Adj. Net $388k. #33491
Palm Desert: New Listing! 4 Ops, Equipped. Seller is here only 12 hrs/week. Some patients included in the asking price. PracticeWeb, digital x-rays. #33429
Palm Springs: 4 Ops, 30 years of goodwill. Good location, Doctor wants to transition work part time if possible. 2015 GR $549k. #3351
Palm Springs: 5 Ops in free-standing historic building. 50+ yrs of Goodwill. Building also for sale. Dentist, 2016 GR $253k. #3351
SAN PEDRO: New Listing! 4 Ops, EagleSoft, Digital, 60 year old equipment. Bright and spacious build-out, modern design. 2016 GR $359k. #33449
SANTA BARBARA: Creekside location. 4 Ops in 1500 sq. ft. est. for 40+ years. 7 days Hygiene/week, long-term staff. FFS. GR $955k on 4 day/week. #33291
SANTA CLARITA VALLEY: New Listing! Great Small Specialty Practice. 8 Ops, 5 chair. Ortho Bay. Great visibility. 100 NpNp. PPO/HMO w/large CAP check. #33435
SOUTH PASADENA AREA: New Listing! 5 Ops, 4 Equipped, PracticeWeb, Digital, Pan. 2016 GR $548K w/adj. Net on 3 day wk. #33430
TEMECULA: 5 Ops, 4 Equipped. PracticeWeb, Digital, Pan. 2016 GR $548K w/adj. Net on 3 day wk. #33430
UPLAND: New Listing! 3 Ops (possible 4th Op used as an office) in a shopping center with 30 years in business. 2016 GR of $324K. #33450

UPLAND: New Listing! Facility Only – 4 Ops, 2 Equipped, All Brand New Equipment, OpenDental w/ workstations, hand imaging, in-room treatment, small patients. #33434
VALENCIA: 4 Ops, 1 addl friend, E4D, Digital, DEXIS Pacifies. 20 yrs Goodwill, 4 days hygiene/wk. 2016 GR $721K w/358k Adj. Net. #33413
WESTERN SAN FERNANDO VALLEY: Pedo Practice in upscale location, 4 Ops, EagleSoft, Digital X-rays, Panos, Lasers. 2016 GR of $559k. #33499
YUCCA VALLEY: 5 Ops, retiring seller works a day 4 week w/27 years of Goodwill. Office condo also for sale. 2016 GR of $586k. #33423

SAN DIEGO
NORTH COUNTY, SAN DIEGO: 5 Ops, Dentrix, Digital, Pano, PPO Practice, Excellent and Convenient location. 2016 GR of $535k, $242k Adj. Net. #33428
NORTH COUNTY, SAN DIEGO: 5 Ops, 26 years of Goodwill, with some HMO. 2015 GR of $335, low overhead. #33435
SAN DIEGO: 3 Ops, EagleSoft, Digital, Paperless, Laser, 3 day work week – room to grow! 2016 GR $323K. #33404

OUT OF CALIFORNIA
CENTRAL OAHU, HAWAII: 3 Ops in Central Oahu. Dentrix, Dentix, Panos. 2016 GR $357K. #33102
CENTRAL OREGON COAST: New Listing! 4 Ops, well equipped, Pan, Digital X-rays and Daisy software. Building Available. Owner retiring. #33410
HONOLULU, HAWAII: 5 Ops in the Pearl City/Waipahu area. Dentrix, Digital, Laser, Excellent Location, Highly-profitable, Rare opportunity. #33108
The Health Insurance Portability and Accountability Act (HIPAA) requires a covered entity to conduct an initial and periodic risk analysis, as well as develop and regularly update its risk management plan. The risk analysis process described in last month’s column prioritizes information security issues that a dental practice must attend to. The practice must document in its security risk management plan how it addresses the major risks. The plan must also include information on how the dental practice meets the specifications of the HIPAA Security Rule and describe other security measures.

Addressing Identified Risks

The risk analysis process described in last month’s column uses a six-point scale. Items rated five or six are major risks to the dental practice. For each major risk, consider one of the following approaches to address it:

- Eliminate.
- Reduce probability of occurrence.
- Mitigate potential consequences.

For example, a covered entity may identify the lack of updated policies and procedures to be a major risk. Why is this a risk? Policies and procedures should describe how a dental practice complies with the HIPAA and state privacy and security rules. If staff is not trained on the policy and procedures to respond appropriately to a patient’s request to access his or her records and staff wrongly denies the patient’s request, this can lead to a complaint against the practice with both the Dental Board and the Department of Health and Human Services Office of Civil Rights, the agency that enforces HIPAA. Ideally, a dental practice will eliminate this risk by updating its policies and procedures.

Another example of a major risk is storing patient information unencrypted on a server. Theft of the server would cause the dental practice to comply with mandated breach notification procedures — a potentially costly process — and to experience some loss of reputation. If data encryption is not reasonable or appropriate, a dental practice could reduce the likelihood of theft by reinforcing the physical security of the server, for example, by placing it in a locked cage that is bolted to a cabinet or the floor. The dental practice also should continue to look and plan for reasonable and appropriate solutions that will allow for encryption.

In considering what is reasonable and appropriate, a covered entity should take into account the cost of implementation, physical limitations, staff resources and other operational necessities.

Required and Addressable Safeguards

The HIPAA Security Rule has specifications, also known as safeguards, which are either required or “addressable” (FIGURE). An addressable safeguard is one that a covered entity must implement unless its implementation is unreasonable or inappropriate. In such cases, the covered entity must document the reasons why implementation is unreasonable or inappropriate and describe an equivalent measure that is reasonable and appropriate and that will accomplish the same purpose. Also, the covered entity must document if the standard can still be met if implementing the safeguard or an alternative would not be reasonable or appropriate.
Let’s review one of the specifications — access control and validation procedure — which is an addressable physical safeguard. Many large health care facilities use electronic keycard systems to comply, while moderately sized facilities may use a visitor sign-in/sign-out system while requiring visitors to wear a “visitor” badge at all times. A covered entity should consider how access to the electronic information system is controlled and whether it meets the standard. Is the use of electronic badges reasonable and appropriate for a dental practice? Are visitor badges reasonable and appropriate? Do all visitors enter through the same door or are they allowed to use the employee entrance? A covered entity determines the reasonableness and appropriateness of the policies and procedures implemented to control physical access to the information system.

A series of Security Rule guidance documents are available online at hhs.gov/hipaa/for-professionals/security/guidance/index.html. The documents contain the standards and suggest questions for a covered entity to consider when doing the risk analysis and documenting the risk management plan.

### Assess and Monitor

The work does not stop when the risk management plan is complete. Risk mitigation measures must be monitored and evaluated on an on-going basis. The risk analysis and risk management plan must be periodically reviewed and updated in response to changes in the environment. Although there is no mandated time period for review, a reasonable time to do so is when new risks are identified, when new software or hardware are introduced or when there is a significant change in the physical environment or workforce.

---

**TABLE**

<table>
<thead>
<tr>
<th>Security 101 for Covered Entities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Administrative safeguards</strong></td>
</tr>
<tr>
<td><strong>Standards</strong></td>
</tr>
<tr>
<td>Security management process</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Assigned security responsibility</td>
</tr>
<tr>
<td>Workforce security</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Information access management</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Security awareness and training</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Security incident procedures</td>
</tr>
<tr>
<td>Contingency plan</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Evaluation</td>
</tr>
<tr>
<td>Business associate contracts and other arrangements</td>
</tr>
</tbody>
</table>

| **Physical safeguards**           |
| Facility access controls          | Contingency operations [A]  |
|                                  | Facility security plan [A]  |
|                                  | Access control and validation procedures [A]  |
|                                  | Maintenance records [A]  |
| Workstation use                   | [R]  |
| Workstation security              | [R]  |
| Device and media controls         | Disposal [R]  |
|                                  | Media reuse [R]  |
|                                  | Accountability [A]  |
|                                  | Data backup and storage [A]  |

| **Technical safeguards**          |
| **Standards**                     |
| Access control                    | Unique user identification [R]  |
|                                  | Emergency access procedure [R]  |
|                                  | Automatic logoff [A]  |
|                                  | Encryption and decryption [A]  |
| Audit controls                    | [R]  |
| Integrity                        | Mechanism to authenticate electronic protected health information [A]  |
| Person or entity authentication  | [R]  |
| Transmission security             | Integrity controls [A]  |
|                                  | Encryption [A]  |

[R] = Required, (A) = Addressable
Source: hhs.gov/sites/default/files/ocr/hipaa/administrative/securityrule/security101.pdf

6130 MARIPOSA  Relaxed lifestyle in Sierra Foothill community.  2016 collected $1 Million.  Extremely strong Hygiene Department. Seller can work-back if Buyer desires.

6129 PROSTHODONTIC PRACTICE – SAN MATEO  2016 collected $775,000 on 3.5 day week.  Beautiful 5-op office.  Excellent candidate for acquisition by nearby practice. Seller shall work back to assist in orderly transition. Acquire here or move into nearby practice. Choice is yours.


6127 SAN RAFAEL’S NORTHGATE  Collected $210,000 in 2016 on part-time schedule.  Available Profits of $106,000.

6126 FRESNO Located at busy intersection.  Available Profits of $166,000 with profits of $364,000.  4-Ops.

6125 OAKLAND AREA  Collections average $735,000 per year.  High income zip code with well employed Millennials next door. 10+ new patients per month.  Digital and paperless.  Seller can work-back if Buyer desires.

6124 SAN RAMON  100% Out-of-Network.  5-Ops.  6-days of Hygiene.  $700,000 per year performer.

6123 SANTA CLARA - STARBUCKS "LIKE" LOCATION!  Best exposure in beautiful strip center.  Office just remodeled.  5-Ops.  2017 trending $1+ Million in Collections on 4-days.  Perfect platform to operate 6-days a week.  Wants to do $1.5-$2 Million.

6121 NAPA VALLEY FAMILY PRACTICE  Highly respected community asset.  Collections last 5-years have averaged $1.28 Million per year. Beautiful facility. Condo optional purchase.

6120 OAKLAND'S PIEDMONT AREA  Highly coveted area.  Right off Highway 13.  3-days of Hygiene.  4-Ops with 5th available.  2016 collected $650,000.

6119 NORTH BAY ORTHO  Desirable family community.  Best technology, cone beam and paperless.  Owner works part-time. Revenue streams averaged $600,000/year in past. Strong profits.  Does no marketing to local Dental Community.

6118 SAN FRANCISCO’S EAST BAY  Forty percent partnership in well positioned and branded practice.  2016 collected $2.53 Million.  2017 trending $3.2+ Million in collections.  Full complement of specialties.  6-month Trial Association wherein interested Candidate shall see ability to make $350,000+ per year.

6107 EUREKA  100% Out-of-Network with insurance industry.  2016 collected $930,000+ on Doctor’s 20-hour week.  Doctor’s schedule booked 3-months.  5 1/2 days of Hygiene. Highly respected.  Full Price $250,000.  Condo is optional purchase.

6089 MOUNT SHASTA  Small town living renowned for outdoor lifestyle.  3-day week collected $100,000.  Very strong bottom line. Digital including Pano.

ANAHEIM – NEAR DISNEYLAND  4-ops.  Grosses $40,000+/mth. Includes building.  Full Price $650,000.

ANTELOPE VALLEY  Prior DDS grossed $1.8 Million.  60,000 autos pass intersection daily.  $80 Billion in government contracts will make this highest growth area in SoCal next 10-years.  New DDS overwhelmed.  Will work back for MSO or Specialist.  Renovated 8 op office.  Working Owner will net $500,000 at $1.5 Million, and $800,000 when grossing $2 Million. Full Price $250,000.

ANTELOPE VALLEY  Established 50 years.  Absentee Owner.  Grossing $1 Million.  6 ops. Rent $2,000/month. Full Price $800,000.

BAKERSFIELD  50 year old practice and renovated building.  10,000 sq.ft. lot.  5 ops. Grosses $400,000-to-$600,000.  Seller will let you work before buying. Practice and RE $685,000 includes AR.

BURBANK / GLENDALE  Absentee Seller. Grosses $1-to-$1.2 Million.  6 ops.  Gorgeous high identity corner building.  Refers Endo, Os, Implants.  $300,000 in recent renovations. Full Price 85% of Gross.

CERRITOS – EMERGENCY SALE  Grossing $450,000.  3 Hygiene days.  Digital with Pano. Well equipped for Implants. Full Price $350,000.

DIAMOND BAR  5-Ops.  Grosses $500,000.

INLAND EMPIRE - EMERGENCY SALE  Shopping Center.  Operated by part-time Associate.  Fantastic staff.  Grossing $350,000.  Owner-Operator will do $500,000+.  5-Ops plumbed, 3 equipped.  Gorgeous office. Full Price $350,000.

INLAND EMPIRE - EMERGENCY SALE  High identity Target Center.  Grosses $1 Million.  No marketing.  5-days Hygiene.  200,000 autos pass daily. Recently renovated at cost of $300,000.  Bargain.

IRVINE / SANTA ANA  Vons Shopping Center.  10-years old.  Grosses $50-to-$60,000/month.  Seller will work back 2 days.  Near South Coast Plaza. Lots of new patients. Success assured.

LAKE ELSIMORE - HMO  Established 40 years.  Popular Seller wants to work back 2 days.  Grossing $550,000.  Lots of room to go to $800,000 1st year.  6 Ops. Low rent.


LOS ANGELES - HMO  Grossing $1.5 Million.

NEWPORT BEACH’S FASHION ISLAND  “Coming Up!” Contact Tom Fitterer and register interest.

ORANGE COUNTY BEACH CITY - HMO  Grossing $1.5 Million. Full Price $1.3 Million.  Hands-on Owner will do $2 Million.


SAN FERNANDO VALLEY  Established 40-years.  Recently renovated with best.  Absentee Owner.  Previously did $1 Million, 6 ops. Grossing $550,000.

TorrANCE  Strip center on Hawthorne.  3 ops.  Grosses $300,000.  Refers Endo, Os, Implants, Perio and Ortho. Close to Palos Verdes. Full Price $295,000.

MORE OPPORTUNITIES AVAILABLE  Bellflower, Corona, Dana Point, East LA, Ladera Ranch, Norco.  San Juan Capistrano established 40 years.  Lawndale Galleria, Anaheim, Irvine, Orange/Tustin.
After years of providing quality patient care, Dr. Solo Practice develops chronic musculoskeletal pain. At the end of his busy, stressful workday, he enjoys a drink or two with dinner as a coping strategy. This behavior continues, as it is the path of least involvement for relief. In time, he realizes that his end-of-the-day coping strategy has escalated and is affecting aspects of his personal and professional life. Taking time off to address the problem is not an option, as there are overhead expenses to cover and mouths to feed. Internally, he is in conflict with doing the right thing, denial, self-will and feeling as if there is no way out. The nightcap behavior continues even though his managerial skills are becoming neglected and his patient care has diminished. His office manager notices these changes. Out of concern for the dentist and his patients, she wonders if she should do something and consults with the dentist from across the hall.

What is the appropriate ethical response when you become aware that a dentist colleague is practicing dentistry while potentially impaired? The majority of CDA’s Well-Being program referrals are from concerned individuals who have known about a situation. Having that human ethical dilemma, they seek guidance in wanting to do the right thing.

This article will attempt to answer that sensitive question with the help of CDA and ADA written materials. Quite often it is said that with knowledge and understanding, clarity can be found.

The ADA’s Principles of Ethics and Code of Professional Conduct, Section 2D states: “It is unethical for a dentist to practice while abusing controlled substances, alcohol or other chemical agents that impair the ability to practice. All dentists have an ethical obligation to urge chemically impaired colleagues to seek treatment. Dentists with first-hand knowledge that a colleague is practicing dentistry when so impaired have an ethical responsibility to report such evidence to the professional assistance committee of a dental society.”

In the CDA Well-Being program mission statement, patient safety is mentioned multiple times. As dentists, we are in service to the public and are bound by core ethical principles. Beneficence and professionalism are two of the ethical principles in the CDA Code of Ethics:

- Beneficence, often cited as a fundamental principle of ethics, is the obligation to benefit others or to seek their good.
- “Professionalism: Self-governance is a hallmark of a profession and dentistry will thrive as long as its members are committed to actively support and promote the profession and its service to the public.”

Section 2 of the CDA Code of Ethics states: “Every profession receives from society the right and obligation to regulate itself to determine and judge its own members. Such regulation is achieved largely through the influence of the professional societies, and a dentist has the dual obligation of becoming part of a professional society and observing its rules of ethics.”

The CDA Well-Being program offers solutions to the question posed earlier. By aiding a fellow practitioner to seek help through the Well-Being program, you are helping the public, the profession, your colleague and their loved ones (staff, family, etc.). More times than not, the impaired dentist, when approached, surrenders to the reality that he or she needs help.

According to the program manual, “The Well-Being program is committed to ensuring the health and welfare of those affected. Through use of proven recovery methods and using appropriate treatment facilities, the CDA Well-Being program provides assistance, referral and follow-up support. The program assists not only dental professionals and allied dental health professionals but will also provide support to families who may be affected by those who are impaired.”

Addiction is a disease. Recovery is a solution. We are fortunate to have the Well-Being program that supports member dentists in the rehabilitation process, helping them to get their lives back and getting them back to helping the public. If an impaired dentist does not get needed help, he or she could end up in legal trouble, which can be punitive and costly with irreparable damage to all involved, including staff and family.

Our ethical obligation to our patients, the public, our peers and our profession is supported by the existence of our Well-Being program.

Ronald V. Surdi, DDS, practiced general dentistry in San Pedro, Calif. He volunteers as an adjunct professor at the Ostrow School of Dentistry of USC and serves on the CDA Judicial Council.

For more information on the Well-Being program, go to cda.org/Portals/0/pdfs/cda_wellbeing_brochure.pdf. For more information or further guidance, contact your local ethics committee or Brittney Ryan, CDA judicial council manager, at 800.232.7645.
QUESTIONS MOST OFTEN ASKED BY SELLERS:

1. Can I get all cash for the sale of my practice?
2. If I decide to assist the Buyer with financing, how can I be guaranteed payment of the balance of the sales price?
3. Can I sell my practice and continue to work on a part time basis?
4. How can I most successfully transfer my patients to the new dentist?
5. What if I have some reservation about a prospective Buyer of my practice?
6. How can I be certain my Broker will demonstrate absolute discretion in handling the transaction in all aspects, including dealing with personnel and patients?
7. What are the tax and legal ramifications when a dental practice is sold?

QUESTIONS MOST OFTEN ASKED BY BUYERS:

1. Can I afford to buy a dental practice?
2. Can I afford not to buy a dental practice?
3. What are ALL of the benefits of owning a practice?
4. What kinds of assets will help me qualify for financing the purchase of a practice?
5. Is it possible to purchase a practice without a personal cash investment?
6. What kinds of things should a Buyer consider when evaluating a practice?
7. What are the tax consequences for the Buyer when purchasing a practice?

Lee Skarin & Associates have been successfully assisting Sellers and Buyers of Dental Practices for nearly 30 years in providing the answers to these and other questions that have been of concern to Dentists.

Call at anytime for a no obligation response to any or all of your questions
Visit our website for current listings: www.LeeSkarinandAssociates.com
### BAY AREA

**AC-566 SAN FRANCISCO:** Spectacular views of Washington Square. 3 ops +2 add’l, 1400sf $200k

**AC-624 SAN FRANCISCO:** Wonderful patients, solid income in great stand-alone bldg $475k

**AC-640 SAN FRANCISCO:** On 23rd Floor of prestigious bldg, 2 ops in 700sf. Seasoned Staff. Seller Retiring $175k

**AC-649 SAN FRANCISCO Facility:** Richmond District, 3 ops +1 add’l, Equipment less than 1yr old $120k

**AG-652 SAN FRANCISCO:** Strategically located with huge growth potential. 2 ops + 1 add’l, 600sf $175k

**AG-645 SAN FRANCISCO:** Low Overhead, compact practice ready for expansion or relocation. Retail/Commercial area. 2nd Floor $99k

**AG-669 SAN FRANCISCO:** RARE opportunity in the heart of the city! 2 ops LOW OVERHEAD! $88k

**AN-513 REDWOOD CITY:** Practice of your dreams! 900sf w/ 4 ops + 2 add’l $350k

**AN-686 SAN FRANCISCO:** Office designed w/ patient flow & maximum office efficiency. 1000sf w/ 4 ops $825k

**AN-712 SAN FRANCISCO:** Easy accessibility, exceptional visibility, free parking & extremely low rent! 1000sf w/ 2 ops + 2 add’l $89.5k

**BC-663 DANVILLE:** Remodeled office located in semi-rural community, 1000sf w/ 4 ops $432k

**BC-681 WALNUT CREEK:** Located in desirable, bustling community w/ seasoned, caring staff. 836sf w/ 3 ops $224k

**BC-710 WALNUT CREEK:** Desirable location in stand-alone, single-story bldg. 1313sf w/ 3 ops $150k / RE $850k

**BG-724 RICHMOND:** Spacious office w/ enormous growth potential! 2000sf w/ 4 ops Practice $138k / Real Estate $700k

### BAY AREA CONTINUED

**BG-731 LAFAYETTE:** Well-educated, health conscious patient base. 1,000 sf w/ 3 ops 35+ years goodwill $265k

**BN-504 RICHMOND:** Established Practice & Real Estate! 1450sf w/ 2 ops + 2 add’l $100k / RE $700k

**BN-736 BERKELEY:** Step into this quality practice and you’ll know you belong here! 906sf w/ 3 ops, $495k

**BG-734 ANTIQUE:** The perfect place to work, live and play! Located in desirable professional neighborhood. 1,323 sf w/ 4 ops. $315k

**CG-632 SAN RAFAEL:** Small town life in vibrant, growing city. 3 ops in 800sf office. Beautiful views of Tamalpias from each operatory window, 4 ops, $224k

**CC-669 SAN RAFAEL:** Compact practice ready for expansion or relocation. Retail/Commercial area. 2nd Floor $175k

**CC-720 SONOMA COUNTY:** Well-established practice. Seller retiring from this area. 6 npts/mo, 3 ops in 900sf $375k

**CG-616 NAPA:** State-of-the-Art practice. Seller moving out of state! $425k

**CG-735 ROHNTERT PARK:** Collections over $600k, Net Profit over $230k and expertly located near major anchor tenants! $370k

**DC-480 SILICON VALLEY:** Multi-specialty practice. 14+ ops in 7500sf. Owner financing available. $1,075M

**DC-431 SAN JOSE:** Starter practice. Desirable area. 6 npts/mo, 3 ops in 900sf $150k

**DC-692 DUBLIN Facility:** Modern digital office. 5 ops 1800sf / $210k w/ Cone Beam Unit or $165k without

**DC-738 WATSONVILLE:** 6 ops in beautiful remodeled 2,600sf office, visibly located in attractive shopping complex $480k

**DG-635 CASTRO VALLEY:** Excellent location & stellar reputation! Solo Group Practice $650k

---

**800.641.4179**

**WPS@SUCCEED.NET**
BAY AREA CONTINUED

DG-726 SAN JOSE: Busy, Vibrant Practice. Collections over $1.1M on a relaxed 4 day work week. ~2850sf w/ 7 ops $885k
DN-665 SANTA CRUZ AREA: Loyal, stable, multi-generational patient base. FFS. 1460sf w/ 4 ops $540k
DN-693 SAN JOSE Facility: Attractive & spacious! Faces one of the city’s major thoroughfares. 1080sf w/ 4 ops $95k
DN-713 CASTRO VALLEY Lease: Well maintained, attractive, “Move-In Ready” dental office. 1500sf w/ 5 ops Call for details!
DG-723 SAN JOSE: The practice exceeds $1.2mil in collections annually! 1,450 sf w/ 5 ops. $850k

NORTHERN CALIFORNIA

EG-685 LINCOLN/ROCKET: Perfect location in amazing community! Retail Shopping Center w/ 4 ops $570k
EG-716 ELK GROVE: Remarkable potential for growth w/ attention to marketing & increased office hours! 1200sf w/ 3 ops $270k
EG-722 ROSEVILLE: On track to collect $1.5M in 2017 with increased profit compared to last year! Price Reduced even though collections are increasing! 1919sf w/ 4 ops $1.05M
EG-727 SACRAMENTO: Steady Income from HMO. Increase office hours & begin advertising to watch the collections skyrocket! 1100sf w/ 3 ops $220k
EG-744 SACRAMENTO: Well established, highly esteemed Sacramento Practice 1320sf w/ 3 ops $250k
EN-626 CARMICHAEL: Lifestyle you just can’t beat! HMO 1250sf w/ 3 ops $300k
EN-628 ORANGEVALE: Great place to work, play & live. HMO 1310sf w/ 4 ops + 1 add’l $375k
EN-654 CITRUS HEIGHTS: Well established & loaded with 30+ years of goodwill! 1300sf, 3 ops + 2 add’l. $150k
EN-660 ROSEVILLE: Highly-esteemed, well-respected, fee-for-service practice w/ loyal patient base. 2950sf w/ 5 ops $995k
EN-664 SACRAMENTO Facility: Great corner location, excellent visibility & easy access! 2300sf w/ 4 ops $55k
EN-702 SACRAMENTO: Long-established practice w/ emphasis on preventative vs reactive dentistry! 1600sf w 4 ops + 1add’l. $495k
EN-708 SACRAMENTO: Family-oriented practice with appreciative & loyal patient base. 1600sf w 4 ops + 1add’l. $150k
EN-747 CITRUS HEIGHTS Facility: Attractive, popular Retail Shopping Center! 2200sf w/5 ops + 6 add’l. $150k
FC-650 FORT BRAGG: Family-oriented practice. 5 ops in 2000sf, 6 npts/mo $350k for the Practice & $400k for the Real Estate

NORTHERN CALIFORNIA CONTINUED

FC-677 FORT BRAGG: Beautiful, FFS Practice, 4 ops +1 add’l, in 2375sf, Gross $1M+/yr $500k
GC-472 ORLAND: Live & practice in charming small town community. 1000sf w/ 2 ops. Seller Retiring $160k
GG-454 PARADISE: 2550sf w/ 9 ops. 40 yrs goodwill! Amazing Opportunity! $525k
GN-606 BUTTE COUNTY: Hesitate & you’ll miss out on this one-of-a-kind opportunity! 1700sf w/ 4 ops $125k
GN-667 OROVILLE: Great place to work & play! Constant growth attracting an influx of residents! 1000sf w/ 3 ops $295k
GN-668 BUTTE COUNTY: Remodeled in 2010! Well-maintained, long-established professional complex. 1200sf w/ 2 ops $95k
GN-717 YUBA CITY: Seller Retiring. All reasonable offers considered. Building available for purchase! 2400sf w/ 5 ops $475k
GN-746 YUBA CITY: State-of-the-Art Equipped! Includes the latest technology in CBCT Imaging. Real Estate also available! 1600sf w/ 3 ops +1 add’l. Practice $480k/ Real Estate TBD.
HN-213 ALTURAS: Well managed w/consistent revenues! Collected ~$760 in 2016! 2200sf w/ 3 ops + 1 add’l. $195k
HN-280 NORTHEAST CA: Only Practice in Town! 900sf w/ 2 ops $60k
HN-618 SIERRA FOOTHILLS: Seller Retiring! Huge opportunity for growth by increasing office hours! 750sf w/ 2 ops $95k

CENTRAL & SOUTHERN CALIFORNIA

IC-468 SAN JOAQUIN VALLEY: High-end restorative practice! 6 ops in 2500+sf office. Call for Details! $425k
IG-687 TURLOCK: Established quality practice - remarkable opportunity! 2000sf w/ 5 ops $298k
KC-678 LOMPOC & SANTA MARIA: Live & practice along the central coast. Plenty of room for growth, Call for Details! $240k

SPECIALTY PRACTICES

AC-748 SAN FRANCISCO Perio: Practice in this prestigious building in desirable central location. 3 ops, 980sf $800k
BC-709 HAYWARD Ortho: Provides personalized care to wonderful patient base. 5-8nt exams/mo, 4 chairs/bay, 1948sf $215k
IC-543 CENTRAL VALLEY Ortho: 1650sf w/ 5 chairs in open bay & plumbed for 2 add’l. Strong referrals and PT base $125k

“Ask the Broker” can now be found at www.westernpracticesales.com
Dental Dictionary (Free, Farlex Inc.)

Dental practitioners, staff and patients periodically use references when trying to find answers to questions they might have regarding treatment and procedures. Oftentimes, the first thing they do when looking for this information is perform a web search. However, not all sources of information on the web may be accurate. Dental Dictionary is a free reference app that contains information from trusted sources on thousands of terms related to dentistry and across other disciplines to assist in providing answers to these questions. Coupled with simple search tools and other useful features, members of the dental team can obtain the information they need conveniently from one place.

Dental Dictionary has a simple home screen with a search field where users can enter any search term. Predictive results appear as the term is entered and users can then select one from the results. The definition and any information related to the term are prominently displayed along with links to other related terms. An audio icon appears next to terms that have pronunciation recordings that users can playback. Some terms contain photos, diagrams and radiographs in addition to the text information displayed. From time to time, users may know only some part of the term they need to look up. For those situations, users can choose to do a simple search, which takes a search entry and looks for a match that starts with, ends with, contains or has any portion of the partial term entered. Favorite terms can be bookmarked by tapping on the star icon for quick reference in the future. Other features such as emailing, texting and posting searches are available by tapping on the share sheets icon.

With a wide array of dental information from trusted sources available in one simple-to-use mobile application, Dental Dictionary is a worthwhile addition to the tools available for any dental team, including students and patients alike. Although its feature set is not extensive, it has just enough to be an easy and useful tool for anyone to use.

— Hubert Chan, DDS

No Battery Needed for Cellphone Prototype

The battery life of cellphones has long been a struggle. Most people run to their chargers by the end of the day and those with older phones retreat to their chargers multiple times a day. Researchers at the University of Washington have developed a prototype cellphone that doesn’t need a battery, consuming just a few micro-watts of power. The phone operates on power that is harvested from RF signals transmitted by a basestation 31 feet (9.4 m) away. (Essentially, it has to be close to a computer to work.) The phone can communicate with a basestation that is 50 feet (15.2 m) away. Researchers built the phone using components that are largely available commercially. To prove the phone’s strength, the researchers also performed a Skype call over a cellular network. The design of the phone can sense speech, actuate the earphones and switch between uplink and downlink communications, all in real time. For more information, visit dl.acm.org.

— Blake Ellington, Tech Trends editor

Millennials Consume Music in Multiple Apps, Radio

Consuming music is one of the main activities people participate in on the internet. A recent Nielsen study dived into this a little more, especially as it related to millennials. The study, which looked into how people between the ages of 18 and 34 utilize communication apps and stream music, found that close to 60 percent rely on two or more apps to stream music (39 percent for those who are 35 and older). The study also found that millennials use a lot more apps than older generations. Somewhat surprising is the fact that people between the ages of 18 and 24 remain loyal to the radio, averaging more than 10 hours of listening per week. Millennials as a whole listen to the radio on a weekly basis (93 percent).

— Blake Ellington, Tech Trends editor
Keep the **CDA Presents** energy alive.

Discover ongoing opportunities to learn, connect and grow:

- See and share the best moments from the convention in our Social Hub
- Catch up on classes you missed online through our on-demand library
- Check with our exhibitors for continuing deals on dental innovations
- Mark your team’s calendar for the convention dates next year

Experience the nation’s leading dental convention.
See what’s next at cdapresents.com.

**Anaheim, CA**  
May 17–19, 2018  

**San Francisco, CA**  
Sept. 6–8, 2018
With the Uveneer direct composite template system, you can quickly and easily create natural-looking direct composite veneers in one visit.

This system isn’t only useful for anterior restorations. The Uveneer template system can also be used for shade selection and to create mock-ups and temporaries during porcelain veneer fabrication.