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Dentistry as an Endurance Sport

Kerry K. Carney, DDS, CDE

What if we thought of our professional lives as an endurance sport instead of a business career? What if the goal was to stay mentally and physically strong and healthy? What if our primary driver was to remain happy and to continue to enjoy helping our patients achieve and maintain oral health over the decades that span the course of a career in dentistry?

Many practice management courses spend a lot of time trying various strategies to maximize production: the shortest possible time to return on investment, the most efficient scheduling to meet production goals, the minimizing of nonproductive downtime. If the office is open, it should be producing at maximal potential. Time is money and overhead is high, so increasing speed and proficiency is an obvious goal in order to achieve success. If monetary success is the measure then we run, run, run (or, as my husband likes to say, “Work, work only!”).

When I was in dental school, one of our clinical instructors advised us to never run our practices continuously at 80 miles an hour. He said we would need to be able to accelerate our speed when necessary but we needed to maintain a sustainable pace, one that would not wear us out. I was reminded of that advice when I was doing some physical therapy recently.

For a career in dentistry to be experienced as an endurance sport, we need to look at all the pieces that go into that sport and how we can train to excel in that sport. A day in the office requires physical fitness. Dentistry has habitual positions that can lead to stress on some parts of our bodies and weaken others.

In the first half of the century, the dentist’s habitual position was standing, now the seated position is usually preferred. An occupational hazard then was varicose veins, now it is hemorrhoids. We all have taken ergonomic classes that reinforce the need for good posture and healthy body mechanics. However, it only takes the restoration of the distal aspect of an upper third molar in a patient with restricted opening to know that the dentist will assume whatever contorted position necessary to complete the procedure.

We buy the aids: head-mounted lights, stools with armrests and loupes for improved vision, but if dentistry is an endurance sport, we need to be thinking about how to help the body strengthen the muscles that are not engaged in that habitual position. We need to learn how to stretch and lengthen those muscles that are always flexed in the dentist’s habitual work position.

In dental school, we spend a lot of time on head and neck anatomy, but recently I have had to spend a lot of time understanding the workings of the muscles of the back, legs and body core. I have learned that the health of the psoas muscle is critical in those who, like dentists, sit for a living. If dentistry is an endurance sport, we should dedicate time to understanding our muscular-skeletal interactions and train to keep our bodies fit and ready for the fatiguing, straining rigors of everyday practice.

The nutritional aspect of training for an endurance sport has to be considered as well. In dental school, we learned basics about nutrition and its effects on the dentition. But what about the nutritional guidelines for dentist athletes? Maybe we should have spent some time studying what we should be eating. What individualized diet would provide us the appropriate quantity and quality of calories for our level of activity? When should we be eating and what should we be eating to stay alert and relaxed? Caffeinated afternoon pick-me-ups are probably not be the best answer for endurance training.

Every sport has a psychological aspect. Psychology for the dentist athlete is complicated. The dentist needs the ability to focus on the fine motor skills for performing surgical and therapeutic interventions but everyday dentistry requires a lot more than that. The dentist needs the kind of team training to be able to work smoothly and efficiently with office staff and colleagues.

As dentist athletes, we have the added complication of patient interaction to consider in our mental training. In order to be able to endure and be happy, we have to learn how to compartmentalize some aspects of our interactions with patients and staff. Our patients trust us to care for them. We must be able to empathize with our patients. Even when our patients are transported to us on vehicles of pain and fear, we cannot get wound up in psychodramas of their own making.

Some people are natural athletes and some are natural psychosocial athletes. For some of us, the psychological aspects of dentistry are what trip us up, burn us out and cause us to have a short and/
or unhappy career in dentistry. This psychosocial component of training is frequently undervalued, overlooked and underdeveloped. We need to train for a mental fitness that can sustain us along the emotional and psychological obstacle course that must be negotiated day after day.

Every training program has to incorporate rest and relaxation and dentistry as an endurance sport is no different. It is too easy to think we are indispensable and irreplaceable. The dentist athlete needs to build into the training schedule time to sleep, rest, relax and recreate.

A career in dentistry is not a sprint. Dentistry is surely an endurance sport and one that is practiced not in loneliness, like the long-distance runner, but with companions and friends. It is a veritable steeplechase day after day. With some luck and effective training, it is an endurance sport we should be able to look forward to enjoying every day for the long haul.

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The Journal welcomes letters

We reserve the right to edit all communications. Letters should discuss an item published in the Journal within the last two months or matters of general interest to our readership. Letters must be no more than 500 words and cite no more than five references. No illustrations will be accepted. Letters should be submitted at editorialmanager.com/jcaldentassoc. By sending the letter, the author certifies that neither the letter nor one with substantially similar content under the writer’s authorship has been published or is being considered for publication elsewhere, and the author acknowledges and agrees that the letter and all rights with regard to the letter become the property of CDA.
Mental Health Dialogue

I recently had the opportunity to glance through the April 2016 issue of the Journal. As a licensed psychologist who has worked with dentists for a number of years, I was particularly interested in the student editorial by Christian Piers titled “A Light for Dark Places: Mental Wellness in Dental School.” It seems to me that Mr. Piers courageously attempted to open a dialogue about mental health and suicide for dental students. He, in my opinion, correctly points out that “suicide is complicated.”

While his editorial indicates that there have been several research studies attempting to establish some connection between an elevated suicide rate and dentists, there is also a substantial body of work indicating no strong correlation can be made based on the existing data. For the most part, this is due to issues of statistical sample size in those studies that support this idea. The prevailing theory seems to be that dentists are exposed to high levels of stress leading to increased levels of depression.

At one point in my practice, I came to realize that approximately one-third of my clients were dentists, leading me to contact a friend who is also a dentist. His lack of surprise when I presented this fact surprised me! He was unable to identify a possible reason other than commenting that he believed dentistry attracted a certain type of individual and that the “profession is hard on people, physically and mentally.” Over my years of working clinically with dentists, there has not been a single case of one of my dentist client’s presenting complaint being depression. Indeed, following my routine initial intake assessment I have yet to determine a primary diagnosis of depression for a dentist.

I do see some common themes in dentists with whom I work. A review of the psychology literature in dentistry supports my anecdotal findings. There are specific personality traits needed to enter into the field of dentistry. The field seems to demand a heightened level of attention to detail, and for some, this can reach the level of clinical significance described in perfectionism. Dentistry also appears to require a heightened level of competitiveness to succeed in training and private practice. And, at its zenith, heightened competitiveness can lead to social isolation. Couple these traits with the rigors of dental school or managing a private practice and it is conceivable that some may become periodically overwhelmed.

I share support with the editorial regarding self-care and access to mental health services. Additionally, there may be a need to address any stigma that may be associated with an individual seeking these services. Finally, I would assert that if my anecdotal findings can be supported through appropriate research, this might prove to be an invaluable aid for dental schools. Having this information would enable them to identify individuals who may be prone to the overwhelming sense of helplessness and/or hopelessness leading to suicide, thereby having an opportunity to intervene prior to any harmful action being taken by the individual.

DON J. TALLEY, PHD, NCC
Santa Rosa, Calif.

CDA Well-Being

With great interest I read in the April 2016 issue of Journal Christian Piers’ student editorial dealing with traumatic and tragic loss. His writing was so provocative that I had to read it in its entirety in spite of students and other obligations calling me. I am more than pleased to read his perspective on a subject dentists often avoid discussing. Opening up the topic is much healthier than burying it and avoiding it. How sad to learn of the tragic loss of this beautiful student leader.

Part of the helpful solution is the CDA Well-Being program that was wisely set up years ago. The local dental societies assist the Well-Being Committee in action. Dental school is difficult; dental practice is stressful. The Well-Being program aids people who need extra help in dealing with stress. It is CDA’s helping arm where confidentially colleagues with history and/or experience in this area provide guidance and help to those calling CDA’s help line (for regional numbers, go to cda.org/Portals/0/pdfs/cda_wellbeing_brochure.pdf). Any number of issues, including chemical dependency, depression, sexual, financial and eating disorders, can be addressed. There are many helpful resources to whom we refer those in need. As a Well-Being Committee member in San Diego for many years, former committee chair and CDA regional director, I assure you the rewards we experience in seeing the miracle of a colleague getting better and healing is intense, even emotionally impactful at times, when a colleague’s life is saved. The value of CDA Well-Being in assistance given cannot be measured.
Thank you, Mr. Piers, for the excellent and well-written way you handled a difficult topic.

I commend and admire you. You will do very well and will be an asset to our profession wherever you give your talents.

RONALD E. FRITZ, DDS
Rancho Santa Fe, Calif.

Dental Problems

I have read the editorial in the May issue of the Journal entitled “A Meaningful Glance at California’s Oral Health Care System.” It is curious that California’s high-income adults have more dental problems than the poor. Based on the information provided in the article and my own experience, I would like to venture some possible reasons. As said in the article, California has many more dentists per 100,000 population than the national average, which means competition among California dentists is fierce. In the meantime, Medicaid (Medi-Cal in California) pays among the lowest reimbursements in California. Both factors lead the majority of California dentists to focus on treating the wealthy and/or well-insured patients, who are actually a minority compared to the vast numbers of low-income and welfare patients. Consequently, these dentists tend to perform expensive, aggressive and unnecessary treatments, persuading and enticing their patients with heavy advertisements. Unfortunately, such dental treatments frequently CAUSE problems. During my 10 years of working for public clinics, I often saw previously insured patients come in with serious problems caused by elective cosmetic treatments (these patients had been dropped by their dentists because they lost their insurance). Now, I own a private office in a low-income neighborhood, treating 80 percent Medi-Cal patients. Receiving $40 for a filling and $41 for a wisdom tooth extraction while trying to operate a “for-profit” business, I understand the financial strain just too well.

Anna Dong, DDS
Pittsburg, Calif.
Impressions

The nub:
1. All change is within reach.
2. No one changes the system or others without being changed themselves.
3. Change is not sustainable without infusions of energy from outside.

David W. Chambers, EdM, MBA, PhD, is professor of dental education at the University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, and editor of the Journal of the American College of Dentists.

Mrs. Jellyby and the Hot Rock

David W. Chambers, EdM, MBA, PhD

“Think globally, act locally.” The first part of this popular phrase seems a bit vague; the second part is trivial. If your friend said he had decided to only hug the people he could actually reach, you would wonder whether he really got the concept.

Perhaps the greatest send-up of this concept is Charles Dickens’s classic Bleak House. In the chapter on telescopic philanthropy, we are introduced to the Jellybys, a magnificently dysfunctional family of a voiceless father and an uncertain number of kids, all ill clothed, sick and infantile. One daughter is an alcoholic and another elopes to escape serving as her mother’s slave. Only Mrs. Jellyby has it together. She is the doyen of the East London Branch Aid Ramification, a charitable group devoted to bringing Christianity and Western economic civility to the Borrioboola-Gha people in Africa by getting London soft hearts to donate funds for a coffee plantation and a factory to turn piano legs. Mrs. J. is the center of a network of communication that involves getting and sending hundreds of letters a day and distributing bundles of flyers for the cause. She is a force for global change. But the oldest daughter says, “It’s disgraceful. The whole house is disgraceful. The children are disgraceful. I am disgraceful.”

Now for the hot rock. Imagine dropping one in the shallow end of a friend’s swimming pool. A minute or two later, you will find that the temperature of the rock and all the water is the same. Perhaps immeasurably higher, but that certainly will not last. This is the second law of thermodynamics, also called entropy. In any closed system that allows interchange, the trend is inexorably toward homogeneity. This is one of those laws that cannot be broken. But we can get around it. The easy, short-term dodge is to build barriers that divide the hot from the cold, separating the haves from the have-nots. The more productive means is to make the system open — to expect and encourage new energy, hope and resources from outside the system.

Both accommodations have their shortcomings. Building personally profitable protective enclaves will add stress to the system and it will eventually collapse. Trying harder from within must be continually maintained and will never lead to large sustainable change. The effect of the hot rock (the heat coming from outside) will be greatest and most lasting on the water immediately contacting the rock. A smaller change will be caused by that hotter water that warms the water immediately next to it, but cools some in the process. The water at the deep end of the pool remains unaffected. The most effective counselors for Alcoholics Anonymous, domestic abuse, drug addiction, community development and good oral hygiene are those who live close enough to touch them.
While the ADA Code turns 150 this year, it is an evolving document and by its very nature cannot be a complete articulation of all ethical obligations. The ADA Code is the result of an ongoing dialogue between the dental profession and society, and as such, is subject to continuous review.

For more, see the ADA Code at ada.org/ethics.
**Dental Prescribing of Antibiotics on the Rise**

New research analyzing data on outpatient prescriptions from 1996 to 2013 has found a 62.2 percent increase in the rate of prescribing of antibiotics by dentists, according to a study in *The Journal of the American Dental Association*.

For their study, the authors obtained anonymized, line-listed data on outpatient prescriptions from a centralized, population-based prescription database. According to the report, the researchers’ analyses used Anatomical Therapeutic Classification standard codes and defined daily dose (DDD) values. The authors normalized prescribing rates to the population and expressed the rates in DDDs per 1,000 inhabitants per day (DID).

From 1996 to 2013, while overall antibiotic use declined from 18.24 DID to 15.91 DID, and physician prescribing declined from 17.25 DID to 14.11 DID, dental prescribing increased from 0.98 DID to 1.59 DID (62.2 percent), and its proportionate contribution increased from 6.7 percent to 11.3 percent of antibiotic prescriptions. The rate of prescribing increased the most for dental patients 60 years or older, the authors wrote.

“Communication from dentists in Canada and the United States identified the following explanatory themes: unnecessary prescriptions for periapical abscess and irreversible pulpitis; increased prescribing associated with dental implants and their complications; slow adoption of guidelines calling for less perioperative antibiotic coverage for patients with valvular heart disease and prosthetic joints; emphasis on cosmetic practices reducing the surgical skill set of average dentists; underinsurance practices driving antibiotics to be a substitute for surgery; the aging population; and more dental registrants per capita,” the authors wrote.

Antibiotic prescribing should be reviewed to make sure that practitioners are compliant with guidelines, according to the researchers, who noted that in doing so, most practitioners can find opportunities to prescribe less often and for shorter durations.

For more, see the research published in *The Journal of the American Dental Association*, May 2016, vol. 147, issue 5, pp. 320-327.

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**Cancer Gene Spectrin May Predict Survival Outcomes of Mouth Cancers**

Loyola researchers have identified a tumor gene that may help to predict survival outcomes in patients with cancer of the mouth and tongue. If the gene spectrin (involved in the formation of cell membranes) is expressed, patients are 4.6 times more likely to die at any given time, according to a study by researchers at Loyola Medicine and Loyola University Chicago Stritch School of Medicine.

If the cancer gene is expressed, a patient may require more aggressive treatment, such as radiation and possibly chemotherapy in addition to surgery. Conversely, if the gene is unexpressed, the patient might be able to safely forgo aggressive treatment and undergo surgery alone.

Using the Gene Expression Barcode, researchers examined publically available genetic data from 54 tumor samples. The samples were taken from patients who had HPV-negative squamous cell carcinoma in the mouth.

Even when researchers controlled for cancer stage and other factors, patients with the expressed spectrin gene still were significantly more likely to die than those in which the gene was turned off. This finding suggests that the spectrin gene may provide more information about survival than cancer stage alone.

The authors caution that the results are preliminary and need to be validated in an independent patient group.

For more, see the study in the journal *Otolaryngology—Head and Neck Surgery*, published online before print April 19, 2016.

Michael J. Zilliox, PhD, director of the Loyola Genomics Facility and lab manager Gina Kuffel. Image courtesy of Loyola University, Chicago.
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Techniques Improve for Determining Male or Female Jawbone

Scientists recently discovered new data that improve techniques for determining whether a jawbone comes from a man or woman. The scientists discovered that the differences that help to distinguish between the jawbone of a male and a female are different if the subject has a meso-, dolicho- or brachyfacial pattern (the three types of anthropometric profiles).

“These results imply that an assessment of the vertical facial pattern of the individual is required before a sexual diagnosis of the mandible is proposed,” the authors wrote.

Scientists at the University of Granada and the National Museum of Natural Science (of the CSIC) have applied a new, more accurate technique in order to analyze the differences in mandible size and shape, which are linked to gender. The new technique will be useful when determining whether a bone comes from a man or a woman.

For their study, the team of researchers analyzed the jawbones of 187 adult subjects (92 men and 95 women) from Granada using lateral teleradiography of the cranium. The size and shape of the jawbones were studied using specific geometric morphometric techniques. The authors reported finding statistically significant differences in the size and shape of the bones between men and women. This sexual dimorphism can be clearly observed in all the patterns, both vertical and sagittal, that were analyzed, according to a news release. The male jawbone is bigger across all subgroups.

For more information, see the study published online ahead of print in the Journal of Comparative Human Biology, Jan. 23, 2016.

Novel Marker of Oral Cancer Discovered

For the first time, researchers have identified a reliable marker (PDGFRβ) to detect carcinoma-associated fibroblasts (CAFs) — which are cells within the tumor that encourage growth and metastasis — in oral cancer tissues, according to new research. With this discovery, anti-PDGFRβ treatment could soon be combined with existing tumor treatments to provide a more effective cancer therapy.

CAFs have been shown to be strongly predictive of disease severity, but their study has been hampered by a difficulty in identifying reliable markers for their isolation from tissue samples.

Researchers identified a set of collagen genes they expected to be largely CAF-specific, namely COL1A1, COL1A2 and COL3A1. Using a large gene expression dataset from the cancer genome atlas comprising hundreds of oral cancer samples, they then looked for additional genes whose expression best associated with the average expression of these three collagen genes. In doing so, they identified several markers, including PDGFRβ, which they confirmed to be CAF-specific using immunostaining assays in oral carcinoma specimens.

“Given the known association of CAFs with poor prognosis in certain cancers, including those of the head and neck, the identification of robust and reliable markers of these cells is necessary to further assess their role in tumor initiation and progression,” explained Maria Trojanowska, PhD, professor of medicine at Boston University School of Medicine, in a news release.

“Overall, this study identified PDGFRβ as a novel marker of stromal activation in OSCC, and further characterized a list of promising candidate CAF markers that may be relevant to other carcinomas,” the authors concluded, adding that their “novel approach provides for a fast and accurate method to identify CAF markers without the need for large-scale immunostaining experiments.”

For more, see the research published in the journal PLOS ONE, April 29, 2016.
Hepatitis C Is Top Infectious Disease Killer in U.S.

Deaths associated with hepatitis C reached an all-time high of 19,659 in 2014, according to new surveillance data released by the Centers for Disease Control and Prevention (CDC). And, a second CDC study showed that annual hepatitis C-related mortality in 2013 surpassed the total combined number of deaths from 60 other infectious diseases reported to the CDC, including HIV, pneumococcal disease and tuberculosis.

The greatest hepatitis C burden falls on baby boomers — those born between 1946 and 1964 — many of whom have unknowingly been living with the infection for many years. According to a study published previously in The Lancet Infectious Diseases, many baby boomers were infected during medical procedures in the years after World War II, when injection and blood transfusion technologies were not as safe as they are today. Without diagnosis and treatment, they increasingly develop liver cancer and other life-threatening hepatitis C-related diseases, and they may unknowingly transmit the disease to others.

The surveillance data also point to a new wave of hepatitis C infections among people who inject drugs. Acute cases of hepatitis C infection have more than doubled since 2010, increasing to 2,194 reported cases in 2014. The new cases were predominantly among young, white individuals with a history of injection drug use, living in rural and suburban areas of the Midwest and Eastern United States.

“Because hepatitis C often has few noticeable symptoms, the number of new cases is likely much higher than what is reported. Due to limited screening and underreporting, we estimate the number of new infections is closer to 30,000 per year,” said John W. Ward, MD, director of CDC’s Division of Viral Hepatitis, in a news release. “We must act now to diagnose and treat hidden infections before they become deadly and to prevent new infections.”


Governor Signs Historic Package of Bills Restricting Tobacco Use

Gov. Jerry Brown has signed a package of five bills that are the most significant set of tobacco restrictions in years. Brown signed:

- SB 5 X2 by Sen. Mark Leno classifies e-cigarettes as tobacco products. This will make them subject to smoke-free laws, age restrictions and other rules governing tobacco products.
- SB 7 X2 by Sen. Ed Hernandez and Assemblymember Jim Wood, DDS, raises the age to buy tobacco products from 18 to 21.
- AB 7 X2 by Assemblymember Mark Stone closes loopholes in the state’s smoke-free workplace laws.
- AB 9 X2 by Assemblymembers Tony Thurmond and Adrin Nazarian requires all schools to be tobacco-free.
- AB 11 X2 by Assemblymember Nazarian establishes a tobacco licensing fee program under the state Board of Equalization.

“These bills are the most significant set of tobacco restrictions in years and we thank the governor and Legislature for taking this historic step,” CDA President Ken Wallis, DDS, said. “Dentists see the devastating effects of tobacco use every day and we are very pleased that California has taken bold steps to protect our residents from these deadly products.”

California becomes the second state, after Hawaii, to raise the smoking age to 21. More than 100 cities, including San Francisco, have also passed this policy. The state also joins a growing number of states regulating e-cigarettes like tobacco products in order to combat the exploding use of e-cigarettes by teens.
Introduction

Since the time of Koch and Pasteur, medical microbiologists have focused narrowly on finding the specific pathogen that causes a particular disease. Guided by Koch’s Postulates, investigators proved causation of many diseases including polio and diphtheria. However, not all infectious diseases are caused by a single organism. This is particularly true of diseases involving biofilms where the community can be composed of several hundred species. The bacteria most associated with causing caries, Streptococcus mutans, and periodontal disease, Porphyromonas gingivalis, are not exogenous pathogens like Vibrio cholera or Corynebacterium diphtheriae, but rather common members of the oral microbiota that are present at low numbers in most people without causing disease. The bacteria in the oral cavity include those associated with health and those that, under the right conditions, may cause disease. These bacteria interact with each other, with host tissues and the immune and inflammatory response systems. Investigators have realized that to fully understand human health and disease, we need to understand not just the few suspected pathogens, but rather all members of the microbiota. Microbiome is the term used for the entire microbial community, including pathogens, commensals and mutualists. The National Institutes of Health recognized the importance of studying the human microbiome and launched the Human Microbiome Project (HMP) in 2008. The microbiomes of oral, gut, nasal, vaginal and skin body sites were examined in 300 healthy individuals to lay a foundation for future microbiome investigations. Information and scientific methods developed in the HMP are now routinely used in studies of the oral cavity and oral diseases. In this issue of the Journal, we present four articles examining different aspects of the oral microbiome.
Dental Calculus and the Evolution of the Oral Microbiome

How has the human oral microbiome changed over the past 7,000 years? Did the change from hunter-gather to farmer affect our oral microbiome? Have organisms like Porphyromonas gingivalis and Streptococcus mutans been part of our oral microbiomes for millennia or have they recently been acquired? If only we could go back hundreds or thousands of years and obtain oral plaque samples, we could answer these, and many other, questions. It turns out that there are time capsules available to us that can take us back thousands of years — the dental calculus on the teeth of ancient skulls and mandibles. Fortuitously, as calculus forms, it traps human and microbial DNA. In the article on the next page, Christina Warinner, PhD, describes her studies of ancient calculus and provides insight into human health and disease and our evolving microbiome from prehistoric times to the present day. The fields of anthropology and molecular biology have joined and are giving us a much better understanding of human evolution, biology and human history.

Subgingival Microbiome Shifts and Community Dynamics in Periodontal Diseases

Our understanding of periodontal disease has changed markedly in the past 50 years. We have gone from being able to identify only about 100 oral bacteria using cultivation methods to being able to precisely and rapidly identify the more than 700 bacterial species that comprise the oral microbiome. We have not only identified key pathogens, but have come to recognize the importance of commensals and those species associated with health. In the article on page 421, Patricia Diaz DDS, PhD, and coauthors Anilei Hoare, PhD, and Bo-Young Hong, PhD, describe shifts in the subgingival microbiome in periodontal diseases. Transitions from health to disease and from gingivitis to periodontitis involve microbial successions and adaptations to changing conditions and host responses in the gingival sulcus.

Understanding Caries From the Oral Microbiome Perspective

Caries is a disease strongly influenced by intake of dietary sugars and carbohydrates. Despite advice from dentists and public health officials, many Americans and their children continue to ingest excessive amounts of sugars and develop widespread carries. Fluoridated water and application of topical fluoride has significantly reduced caries in the majority of the population, but caries still remains high in disadvantaged populations. The article on page 437 by Anne Tanner, BDS, PhD; Christine Kressirer, PhD; and Lina Faller, PhD, reviews the microbiota in caries and how our understanding of the caries process has evolved to include the roles that beneficial bacteria play in mitigating acid produced in response to sugar exposure. Caries is now recognized as involving not only Streptococcus mutans, but also many acidogenic and aciduric species and the concomitant loss of many beneficial species. Microbiome studies using molecular tools now allow investigators to follow microbial shifts and analyze changes in metabolism with high precision. This greater understanding may lead to therapies that shape the composition of the microbiome and ultimately reduce caries.

Uncultured Members of the Oral Microbiome

Until 16S rRNA molecular methods were developed for identification of all bacteria, we knew only about those that could be cultivated. In the 1990s molecular methods revealed that more than half of the 700 bacterial species in the oral cavity had not yet been cultivated. Over the past 15 years, through the concerted efforts of many oral microbiologists, 68 percent of oral bacterial species in the mouth have been cultured. This contrasts with far lower percentages of bacteria cultured from other body sites such as the gut and skin. When a bacterium is cultured, it can be fully characterized, used in in vivo and in vitro experiments, manipulated by knocking out or replacing genes and formally named. In the article on page 447, William Wade, BSc, PhD; Hayley Thompson, BSc, PhD; Alexandra Rybalka, BSc, PhD; and Sonia Vartoukian BDS, FDS, PhD, describe efforts to culture all members of the oral microbiome, explain why some bacteria are very difficult to culture and describe which groups of bacteria remain uncultivated.
Dental Calculus and the Evolution of the Human Oral Microbiome

Christina Warinner, PhD

ABSTRACT Characterizing the evolution of the oral microbiome is a challenging, but increasingly feasible, task. Recently, dental calculus has been shown to preserve ancient biomolecules from the oral microbiota, host tissues and diet for tens of thousands of years. As such, it provides a unique window into the ancestral oral microbiome. This article reviews recent advancements in ancient dental calculus research and emerging insights into the evolution and ecology of the human oral microbiome.

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The human oral cavity is home to a diverse ecology of microorganisms, collectively known as the oral microbiome. Composed of more than six hundred taxa, the oral microbiota plays a central role in dental health, and increasingly it has been shown to influence extraoral organs and tissues, as well as general health. Investigating the evolution of this rich microbial ecology is challenging, but it is critical for understanding the origins and prevalence of oral and systemic disorders as diverse as dental caries, periodontal disease, rheumatoid arthritis, cardiovascular disease, respiratory illness and a range of infectious diseases.

Research on what constitutes a healthy or normal oral microbiome has expanded dramatically over the past decade, in large part due to the advent of high-throughput DNA sequencing and major public funding initiatives, such as the National Institutes of Health’s Human Microbiome Project (HMP). However, the fact that the oral microbiome will cause dental disease in a majority of individuals during their lifetimes has been argued to suggest that even the “healthy” or “normal” oral microbiome today is already in an altered dysbiotic state and likely has been for some time.

To better understand the oral microbiome and its associations to both dental disease and other so-called “diseases of civilization,” it is useful to investigate how our vulnerability to disease is related to human evolutionary history. Evolutionary medicine, a field that integrates both medicine and evolutionary biology, provides a framework for rethinking conventional models of oral health and disease, and biological anthropology,
a field that encompasses primatology, paleoanthropology, bioarchaeology and human biology, provides a context in which to characterize the ancestral state of the human microbiome. Together, these fields provide pathways to better understanding the oral microbiome, and ultimately to restoring and better maintaining oral health in the future.

One of the most exciting recent discoveries in biological anthropology has come from an unlikely quarter — dental calculus (FIGURE 1). Since at least the 1980s, it has been known that archaeological dental calculus contains preserved cellular structures of oral bacteria, but it was only recently discovered that it is also a robust and long-term reservoir of well-preserved DNA and proteins. Advances in ancient DNA and paleoprotein technologies now allow detailed characterization of these ancient biomolecules, enabling direct comparisons between ancient and modern oral microbial communities. Host proteins preserved within ancient dental calculus additionally provide insights into past microbial virulence and host immune response, and food particles entrapped within dental calculus offer unprecedented insights into the diets of past populations.

This article provides an overview of recent ancient dental calculus research and discusses four areas in which the investigation of ancient oral microbiota is poised to make significant contributions to an evolutionary perspective on human health and disease: dental caries; periodontal disease, rheumatoid arthritis and cardiovascular disease; respiratory infections and meningitis; and major infectious diseases.

Dental Calculus and Ancient Biomolecules

Dental calculus is a complex, mineralized bacterial biofilm that forms from dental plaque on the surfaces of teeth. It is found in all known past and present human populations, and it is nearly ubiquitous among adults without active dental hygiene. The amount of dental calculus buildup on the dentition varies widely among populations and may result from a complex combination of factors related to subsistence, dietary abrasiveness, dental hygiene practices and genetic predisposition. However, prior to modern dentistry it is not uncommon to observe heavy calculus deposits in excess of 100 mg, especially from post-Neolithic periods (FIGURE 1).

Importantly for archaeology, dental calculus preserves over time at least as well as bone and dentine, and it has even been found on the teeth of Neanderthals and australopithecines, in addition to humans. Among primates, the oldest known calculus to date was reported on the dentition of a Miocene orangutan ancestor and dates to roughly twelve million years ago (FIGURE 2). Dental calculus is thus noteworthy for its availability in the fossil record throughout the entirety of human evolution.
Dental calculus formation occurs when dental plaque undergoes periodic mineralization events, although the timing and triggers for this process are not well understood. During mineralization, calcium phosphate ions from saliva and gingival crevicular fluid precipitate within the dental plaque matrix, at once killing the microbiota and calcifying the microbial cells and other debris in situ (FIGURE 3A). Organic structures preserve well in this environment (FIGURE 3B), and bacterial cell walls with functional cell surface protein epitopes, as well as delicate dietary microfossils such as starch granules, have been found to remain intact for more than 10,000 years. Genetic material also survives within dental calculus (FIGURE 3C), in part because the hydroxyapatite mineral within dental calculus strongly binds DNA. Genetic analyses of archaeological dental calculus have found that it typically contains ten- to thousandfold more DNA than bone or dentine from the same individual, making it the richest source of ancient DNA yet identified in the archaeological record.

A number of recent studies have explored the potential of dental calculus to reconstruct aspects of ancient health and disease (FIGURE 2) and have shown it to be an area of great potential. Made possible by dramatic advances in ancient DNA and paleoprotein technologies over the past decade (FIGURE 4), these studies have uncovered specific aspects of individuals’ health states, diets, ancestry and even craft activities using a combination of genetic, proteomic and microscopic analyses of dental calculus. This type of specificity and the ability to address issues that typically leave no lasting mark in the macroarcheological record provide opportunities to address questions that were previously thought to be unanswerable. By using these new and emerging techniques, dental calculus can be used to address fundamental questions about the evolution of human oral health.

**Oral Microbiota and Disease — Targets for Discovery**

**Dental Caries**

Dental diseases, such as caries and periodontal disease, are among the most prevalent diseases affecting industrialized societies. In the 1960s, the average number of decayed, missing and filled teeth (DMFT index) among 12-year-old children in Western Europe was greater than 5, and by age 15 the average DMFT exceeded 10. Thus, a European child reaching
adulthood in the mid-20th century could expect one-third of his or her dentition to be compromised by dental decay. From the 19th century to the mid-20th century, dental decay was perceived as so inevitable that complete dental extraction and replacement with dentures became a popular gift for young women and brides in Western Europe and parts of North America. Extensive dental public health interventions and the introduction of fluoridated dentifrices over the past 50 years have significantly reduced caries rates, but even today dental caries affect more than 40 percent of children and 90 percent of adults in the United States.

Dental caries are easily observed in the archaeological record, and they have been systematically studied for more than a century. Extensive data have now been collected on paleoanthropological and archaeological populations around the world, spanning time periods from the Pleistocene to the 19th century. It is clear from these studies that diet is the major driver of caries frequency. Although caries are observed during all time periods, and are, in fact, also present in nonhuman primates, caries frequencies vary remarkably through time and space. The most salient patterns relate to the transition between foraging (hunting and gathering) and agriculture, and later to the widespread availability of refined flour and sugar, especially sucrose, during the 18th century.

Numerous studies have documented increases in caries frequency with the onset of agriculture. The Eastern Woodlands of North America is perhaps the most intensively studied region, and analysis of caries frequency at 180 sites reveals that during the 2,000 years preceding the introduction of maize agriculture the percentage of teeth affected by dental caries was approximately 2 to 5 percent. After the introduction of maize ca. 500 CE, caries frequencies increased steadily from 14 percent during the Late Woodland period to 18 percent during the late Prehistoric period to 22 percent during the early Contact period (FIGURE 5). Although not all regions saw such sharp increases, a general trend toward increased caries frequency in agricultural populations is observed globally, with relatively few exceptions.

The origin of today’s extreme levels of dental decay, however, lies not with agriculture, but with the introduction of refined flours and sugars during the Early Modern period (ca. 1450-1800 CE; FIGURE 2). In Western societies, tooth decay was so severe during this time that smiling is all but absent from European and American portraiture of the period. Since then, consumption of refined carbohydrates has been met with increasingly aggressive oral hygiene regimens and prophylactic dental care as necessary measures to prevent premature tooth loss. The need to continuously engage in such behavior has been argued to be a sign that even the “healthy” oral microbiome today is in an altered state of dysbiosis.

One question that emerges with these observations is to what degree the oral microbiota of these populations changed in step with subsistence practices. Recent research on the gut microbiome has identified major changes in microbial ecology associated with foraging, agricultural and industrial lifestyles, but less is known about the oral microbiome of traditional societies. A recent study of ancient oral microbial communities spanning the past 8,000 years reported evidence for slight, phylum-level microbial shifts correlated with the onset of agriculture and industrialization; deeper sequencing and a larger sample size in future studies is anticipated to clarify these associations and provide greater taxonomic resolution during these transitions.

Direct investigation of ancient carious lesions using ancient DNA techniques has identified the presence of the cariopathogen Streptococcus mutans, but reconstructing the full polymicrobial community contributing to such lesions is challenging because dentine is highly susceptible to postmortem alteration by environmental microbes. By contrast, dental calculus is much better preserved. Genetic sequences from S. mutans have been detected in the dental calculus of diverse populations, but interestingly
it has not been detected in samples prior to the Bronze Age (ca. 2200-1000 BCE)\(^{18}\) (FIGURE 2). Recent phylogenetic analysis of modern \textit{S. mutans} strains has estimated that \textit{S. mutans} underwent an exponential expansion ca. 10,000 years ago (95 percent confidence interval: 3,268–14,344 years ago), suggesting an association with the onset of agriculture in the Near East.\(^{57}\) Future ancient DNA investigations of dental calculus using whole-genome capture enrichment technologies\(^{32}\) may be able to reveal the natural history of \textit{S. mutans} and determine the major drivers of its evolution and functional role within the oral cavity of humans.

Periodontal Disease, Rheumatoid Arthritis and Cardiovascular Disease

Periodontal diseases affect up to 90 percent of the worldwide population,\(^{9}\) and moderate-to-severe chronic periodontitis is estimated to affect 13 percent of U.S. adults older than age 30,\(^{38}\) although new diagnostic criteria suggest that this may be a gross underestimate.\(^{59}\) In addition to dental morbidities, periodontitis is also associated with increased risk of a wide range of so-called “diseases of civilization,” including type II diabetes, obesity, rheumatoid arthritis, cardiovascular disease, stroke and pulmonary disease.\(^{50,65}\) This association may reflect a partially causal relationship. For example, \textit{Porphyromonas gingivalis}, an oral bacterium strongly associated with periodontitis,\(^{66}\) has recently been implicated in the development and progression of rheumatoid arthritis.\(^{67,68}\) and treatment of periodontitis has been found to alleviate rheumatoid arthritis symptoms.\(^{69,70}\) Periodontitis is also associated with a 19 percent increase in the risk of cardiovascular disease,\(^{71}\) and a recent study of cardiovascular specimens found that >80 percent of diseased heart valves and >90 percent of aortic aneurisms have been infected with cariogenic or pathogenic periodontal bacteria.\(^{62}\)

Although there is ample evidence for periodontitis in the archaeological record, periodontal diseases are very poorly studied. The most widely used laboratory guide for osteological analysis, \textit{Standards for Data Collection From Human Skeletal Remains},\(^{72}\) contains recording metrics for age estimation from dental development and wear, enamel hypoplasia measurement, caries scoring and dental calculus quantification, but no information on how to record or measure periodontal disease in archaeological dentitions. As a result, it is rarely noted in archaeological reports. When periodontal disease is mentioned, the descriptions are usually qualitative and limited to small case reports.\(^{12}\) At present there have been no large-scale, systematic studies of periodontal disease prevalence in the past, leaving many gaps in our understanding of the antiquity, prevalence and significance of periodontal disease in human evolutionary history. Although clinical metrics\(^{73}\) are difficult to apply to archaeological specimens, molecular characterization of periodontal disease is increasingly feasible. Recent conceptual changes in periodontology over the past 50 years now point to disrupted microbial communities and host-mediated inflammatory tissue destruction as the proximate causes of periodontal disease,\(^{74,75}\) and new molecular approaches, such as metagenomics and metaproteomics, allow culture-free investigation and comparison of oral microbiota and host immune response from both clinical and archaeological dental samples (FIGURE 2). For example, a recent study of medieval dental calculus found that periopathogens common today were also associated with suspected periodontal disease cases nearly a thousand years ago.\(^{39}\) Frequencies of
the periodontal pathogens *P. gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Filifactor alocis* were found to be highly elevated in dental calculus collected from archaeological dentitions with generalized moderate or severe attachment loss. Additionally, virulence factors expressed by these taxa and host innate immune proteins were found at high abundance among the proteins recovered from these samples, strongly suggesting periodontitis-associated inflammation. In dental calculus from one individual, genetic sequences for *T. forsythia* were so abundant that a near complete ancient genome for this pathogen could be reconstructed. The ancient strain was found to harbor the same 14 virulence proteins found in the periodontal pathogens. In dental calculus from one individual, genetic sequences for *T. forsythia* were so abundant that a near complete ancient genome for this pathogen could be reconstructed. The ancient strain was found to harbor the same 14 virulence proteins found in modern *T. forsythia*, but it lacked several mobile elements, including a putative antibiotic resistance gene, *tetQ*, found today in the *T. forsythia* reference strain.

Two additional studies have begun to look at temporal changes in periodontal pathogens. In a study of ancient Chilean and Argentinian dental calculus, *P. gingivalis* was detected in most time periods from 2500 BCE to the present, and in a study of ancient Europeans, *P. gingivalis* was detected in all major periods dating back to the Mesolithic, ca. 5550 BCE-3450 BCE (Figure 2). Interestingly, the latter study found that *P. gingivalis* frequencies were lower in Mesolithic hunter-gatherers than in post-Neolithic farmers, a finding consistent with observations that hunter-gatherer societies in the recent past and today appear to have lower rates of periodontal disease than agricultural and industrialized societies.11,76 However, further research is necessary to ensure that this finding is not simply an artifact of more advanced DNA decay in older samples.

The investigation of *P. gingivalis* in archaeological dental calculus may also have implications for the study of rheumatoid arthritis. In 1990, rheumatologist Bruce Rothschild, MD, proposed a radical hypothesis — that rheumatoid arthritis is a vector-transmitted infectious disease that originated in central North America and spread to the Old World after European contact in the region ca. 1750.76,79 Among the evidence he assembled, he noted that the first documented case of rheumatoid arthritis in Europe dates to 1800, while cases in North America are known from archaeological remains up to 6,500 years old, and that Native Americans today have unusually high rates of the autoimmune disease (more than fivefold higher than the U.S. general population). Although aspects of this hypothesis are now quite dated,80 the recent link made between rheumatoid arthritis and *P. gingivalis*77,68 has rekindled interest in a possible microbial origin for this disease. By using ancient DNA from dental calculus along with osteological data, it may be possible to unravel the historic biogeography of virulent *P. gingivalis* strains and their relationship to autoimmunity.

Finally, the recent link made between cardiovascular disease and periodontal and cariogenic taxa raises questions as to the antiquity of oral involvement in atherosclerotic plaques. Cardiovascular disease is conventionally viewed as a disease of modernity, but archaeological evidence now confirms its presence in diverse ancient cultures, from the elites of ancient empires to prehistoric hunter-gatherers. Calcific atherosclerosis has been identified, first by autopsy and later by X-ray and computed tomography (CT), in the coronary and peripheral arteries of mummies originating from ancient cultures in Egypt, the Alps, Peru, the American Southwest, the Aleutian Islands and Korea.81,84 Moreover, radiological scans suggest that the prevalence of atherosclerosis was relatively high in prehistory, and, in the case of Egypt, no significant difference was found in the incidence or prevalence of atherosclerotic plaques between today and 3,500 years ago.

A collaboration of cardiologists, radiologists, molecular biologists and archaeologists known as the Horus study team has been at the forefront of ancient atherosclerosis research since 2008.83 Because many of the risk factors associated with cardiovascular disease today do not apply to ancient populations, they have proposed that the high rates of atherosclerosis in the past may have resulted from chronic systemic inflammation caused by long-term infection by gastrointestinal parasites or repeated exposure to microbial or viral pathogens;83,86 however, in the absence of direct evidence for such infections, they note that other yet-to-be discovered risk factors may also play a role. Poor oral health may be just such a risk factor. Untreated caries, heavy calculus deposits and alveolar recession suggest both dysbiotic oral microbial communities and sustained inflammation were prevalent in many ancient populations. A promising future direction in this research would be to genetically test ancient calcific atherosclerotic plaques directly for the presence of oral taxa. Systematic CT scanning has identified atherosclerotic plaques in more than 50 ancient mummies from around the world, and biopsies of these plaques could be analyzed and
compared to microbial profiles generated from dental calculus collected from the same individuals. Taxonomic matches would be strong evidence for a long-term role of oral involvement in the initiation of cardiovascular disease.

Respiratory Infections and Meningitis

The oral microbiome is the natural reservoir for a large number of pathobionts (endogenous potential pathogens), including Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes, Corynebacterium diphtheriae, Bordetella pertussis and Neisseria meningitidis, which cause a wide range of acute and potentially life-threatening respiratory and meningeval infections, especially in children. Importantly, asymptomatic carriage of these microbes is relatively prevalent, although it varies widely among populations. For example, reported carriage rates of S. pneumoniae range from 1 percent in parts of Scandinavia to more than 80 percent in parts of France and Gambia, while H. influenzae carriage rates range from 3 percent in Sweden to 88 percent in Costa Rica, and carriage rates for M. catarrhalis, a common cause of middle ear infections, range from 2 percent in parts of Sweden to 82 percent in parts of the Netherlands.87 In Europe, N. meningitidis is typically carried by an average of 24 percent of teenagers and 8 to 13 percent of adults,88 and nearly one-third of American schoolchildren carry S. pyogenes at any given time.89 For pathogens with widespread vaccine programs, such as Corynebacterium diphtheriae and Bordetella pertussis, carriage rates are generally low,90-94 but were presumably once much higher. In addition to these pathobionts, even commensal oral taxa pose serious health risks among the elderly and immunocompromised. Aspiration of common oropharyngeal taxa can result in aspiration pneumonia,95,96 the leading cause of death and the second most common cause of hospitalization among nursing home patients.96 Additionally, it is common for the elderly to acquire extrarocal pathobionts, such as Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli, in their dental plaque, which also contribute to aspiration pneumonia.2

To date, multiple pathobionts have been identified in archaeological dental calculus, including S. pneumoniae, H. influenzae, S. pyogenes, C. diphtheriae and N. meningitidis.92 Additionally, genetic sequences consistent with Bordetella parapertussis, an organism responsible for persistent cough in children that is similar to pertussis,93 and several species of Moraxella were also identified. These pathobionts were detected in the dental calculus of two individuals excavated from the medieval St. Petri cemetery in Dalheim, Germany (ca. 1100 CE). Although there is no evidence that these organisms were causing disease in these individuals, they nevertheless provide the first direct evidence of respiratory pathobiont carriage in the oral cavity before the 20th century.92

Only a century ago, respiratory infections were the leading causes of death in the U.S.,94 and studies of periostal rib lesions in skeletal collections99 and soft tissue changes in South American mummies103-105 suggest that pneumonia and/or other respiratory infections were a major cause of human mortality in prehistory.97 The discovery that dental calculus is a reliable source of genetic material from common respiratory pathobionts opens up the possibility of conducting epidemiological studies of past carriage rates, and also presents the opportunity to investigate the evolution of these taxa through time.

Dental calculus may also help to date the origin of infectious diseases that have recently emerged from the oral cavity, such as bacterial meningitis and gonorrhea. Phylogenetic studies of Neisseria, a genus of bacteria that colonize mucosal surfaces in animals, indicate that N. meningitidis and N. gonorrhoeae belong to a recently diverged pathogenic clade in humans that is most closely related to the common nasopharynx commensal Neisseria lactamica;103-105 however, the timing and context of this divergence are not well understood. Both N. meningitidis and N. gonorrhoeae are obligate human taxa, indicating that this divergence must have occurred since the chimp-human split approximately 6 million years ago, and whole-genome comparisons with other Neisseria species suggest that N. meningitidis may have undergone a population bottleneck and acquired its virulence genes for polysaccharide capsule synthesis very recently, perhaps only a few centuries ago.105 Non-specific endocranial meningeal reactions are often found in skeletons,106 and with the recent identification of putative N. meningitidis genetic sequences in archaeological dental calculus, this hypothesis has become testable. Future ancient DNA investigations using whole-genome capture enrichment technologies107 hold great promise for resolving the origins and evolution of N. meningitidis, a pathobiont whose mortality rate from bacterial meningitis and septicemia continues to exceed 10 percent even in developed nations.108
Major Infectious Diseases

Finally, although not true members of the oral microbiome, several opportunistic and obligate pathogens can be found transiently within dental plaque, buccal mucosa and saliva. These include the causative agents of tuberculosis (Mycobacterium tuberculosis), leprosy (Mycobacterium leprae), plague (Yersinia pestis), syphilis (Treponema pallidum), gastritis (Helicobacter pylori) and smallpox (Variola virus), among others. Mycobacterium tuberculosis, for example, is present in sputum and regularly comes into contact with the oral cavity throughout the entire course of the disease. In addition to sputum, M. tuberculosis has also been detected in 92 percent of dental plaque samples from infected patients using polymerase chain reaction (PCR)-based techniques. Leprosy involves the oral cavity in up to 60 percent of cases, and multiple oral structures may develop lesions and ulcers, including the hard and soft palate, the gingiva, tongue, lips and buccal mucosa. During outbreaks of bubonic plague, Y. pestis that escapes the lymphatic system and infects the lungs causes pneumonic plague, a highly infectious form of the disease that results in lethal fulminant pneumonia. T. pallidum is known to cause oral lesions during the secondary phase of syphilis infection, which may last for many years, and this provides ample opportunity for passive or active incorporation and preservation in calcifying dental plaque biofilms. Finally, H. pylori is readily found in the saliva and dental plaque of infected individuals, and smallpox causes oropharyngeal lesions.

Infectious diseases have played a major role in shaping human history, and many pathogens continue to present serious challenges to public health. Little is known, however, about the origins or evolutionary history of most human infectious agents. Ancient DNA research has contributed greatly to what is known about the origins of a handful of pathogens, including M. tuberculosis, Yersinia pestis, H. pylori and T. pallidum and it has been used to confirm the presence of several additional pathogens in ancient infections, including M. leprae, T. pallidum and Variola virus.

However, genetic detection rates for most ancient pathogens are low, even in remains with overt and relatively diagnostic paleopathology indicators. Antemortem tissue destruction likely enhances postmortem decay, which may contribute to poor preservation of pathogen DNA within infected bone, and examples of well-preserved soft tissue are relatively rare outside of a few geographic regions. Dental calculus presents a promising alternative for screening ancient skeletons for infectious pathogens. Because dental calculus calcifies during life, it does not undergo the same decomposition processes as the rest of the body, and it is nearly ubiquitous in skeletal collections.

Because dental calculus calcifies during life, it does not undergo the same decomposition processes as the rest of the body, and it is nearly ubiquitous in skeletal collections.

Using bone samples, including treponemal diseases such as venereal syphilis, which is of particular importance given its historical and clinical significance, as well as its past intractability to ancient DNA analysis. Although none of the above pathogens has yet been identified from archaeological dental calculus, the fact that so many infectious agents transiently inhabit the oral cavity during disease progression makes future detection of pathogens from dental calculus at least plausible. If successful, such analyses could greatly expand our understanding of human pathogen evolution.

Conclusion

The incorporation of genetic material from commensals, pathobiont and pathogenic microorganisms into dental plaque, and later dental calculus, presents a rare opportunity to study the evolution of the human oral microbiome and associated diseases in archaeological skeletal collections spanning thousands of years. Great progress has already been made in developing the tools and technologies necessary to extract genomic and proteomic information from ancient dental calculus, and clinical research on the oral microbiome is laying the theoretical foundations for making this information relevant in today’s dental practices and hospitals. Through collaborations between oral health science and ancient dental calculus research, we can leverage knowledge of the ancestral oral microbiome to improve human health today.
34. Leech MT, Bartold P. The association between rheumatid
Subgingival Microbiome Shifts and Community Dynamics in Periodontal Diseases

Patricia I. Diaz, DDS, PhD; Anilei Hoare, PhD; and Bo-Young Hong, PhD

ABSTRACT High-throughput 16S rRNA gene sequencing has allowed the characterization of subgingival microbiome shifts from health to periodontitis identifying health-associated, periodontitis-associated and core species, which preserve their proportions from health to disease. The development of gingivitis is also characterized by distinct shifts. Microbiome shifts resemble microbial successions and result from interspecies interactions and community adaptation to the changing environment as inflammation ensues. Gingivitis-associated and core species are proposed as likely mediators of microbiome transitions.

Periodontitis is one of the most prevalent chronic conditions in humans with 46 percent of U.S. adults suffering from the disease and about 9 percent presenting with a severe form.1 Because the oral cavity is easily accessed, a large body of work exists trying to uncover the etiopathology of this condition. Several lines of evidence indicate that bacteria are necessary for the development of inflammation in the periodontal tissues. This evidence includes the classic experimental gingivitis studies in humans by Löe et al., evidence of the resolution of inflammation after periodontal treatment involving mechanical debridement and animal models showing lower levels of bone loss in germ-free and antibiotic-treated animals.2,3 Tissue destruction, however, is mediated by the host and it is, therefore, the interplay between the subgingival community of microorganisms and local immune responses that ultimately drives bone and connective tissue attachment loss.4

The subgingival microbiome, that is, the community of microorganisms that inhabits the subgingival environment, has been the subject of investigation for many decades. Research comparing the subgingival microbiota under different periodontal conditions has been conducted using various techniques. Most notable historic studies prior to the advent of 16S ribosomal RNA (rRNA) gene sequencing are summarized at the top of Table 1. These studies were of exceptional quality and were performed with what, at the time, were state-of-the-art methods to characterize the oral microbiota. The techniques available at the time, however, did not allow a global...
### TABLE 1

**Classic Studies of the Subgingival Microbiome in Different Periodontal Conditions Prior to the High-Throughput Sequencing Era**

<table>
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<tr>
<th>Reference</th>
<th>Technique</th>
<th>Key findings</th>
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| Listgarten, 1976<sup>5</sup> | Compared plaque from different periodontal conditions using light and electron microscopy. | - Differences in plaque thickness and composition between health, gingivitis and chronic periodontitis.  
- Health: Gram-positive cocci predominate.  
- Gingivitis: Coccoid, filamentous, flagellated and spirochaete forms found; mixture of Gram-positive and Gram-negative.  
- Periodontitis: A distinct bacterial population with Gram-negative, flagellated and presumably motile forms, rich in spirochetes and bristle brush formations. |
| Armitage et al., 1982<sup>6</sup> | Compared plaque from different periodontal conditions using dark field microscopy. | - Plaque load of spirochaetes positively correlated with periodontal disease severity. |
| Moore and Moore, 1994<sup>8</sup> | Compared plaque from different periodontal conditions via cultivation. | - Five hundred and nine taxa identified, 368 of which were detected more than once.  
- Complexity of subgingival communities increases from health to disease.  
- *Actinomyces* spp., *Veillonella parvula*, *Streptococcus* spp., *Capnocytophaga gingivalis*, *Eubacterium saburreum*, *Aggregatibacter* spp., *Leptotrichia* D16, *Neisseria* spp. and *Rothia dentocariosa* were increased in health.  
- *Fusobacterium nucleatum*, *Filifactor alocis*, *Eubacterium* spp., *Campylobacter* spp., *Dialister pneumosintes*, *Prevotella* spp., *Peptostreptococcus* spp., *Porphyromonas gingivalis*, spirochaetes and a *Mycoplasma* sp. increased with disease severity (health to gingivitis to periodontitis). |
| Socransky et al., 1998<sup>7</sup> | Evaluated 40 species in subjects with and without periodontitis via checkerboard DNA-DNA hybridization. | - Identification of five complexes of tightly associated species.  
- The red complex (*Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola*) showed a strong positive correlation with periodontitis severity. |

**Studies based on 16S rRNA gene sequencing via Sanger methods**

<table>
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<th>Reference</th>
<th>Technique</th>
<th>Key findings</th>
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| Kroes et al., 1999<sup>47</sup> | Evaluated the subgingival microbiome via 16S rRNA gene cloning and Sanger sequencing and via cultivation in one subject with gingivitis. | - First study to demonstrate the utility of 16S rRNA gene sequencing in subgingival microbiota characterization.  
- 77 species/phylotypes identified, 48 percent of which were novel.  
- More novel phylotypes identified via 16S rRNA gene sequencing than by cultivation. |
| Paster et al., 2001<sup>11</sup> | Evaluated the subgingival microbiome via 16S rRNA gene cloning and Sanger sequencing in subjects with different periodontal conditions. | - First study to provide a detailed molecular characterization of the subgingival microbiome in several subjects.  
- A total of 347 species/phylotypes identified, 215 of which were novel, with the number of species in subgingival plaque estimated to be ~415. |
| Kumar et al., 2005<sup>12</sup> | Evaluated the subgingival microbiome via 16S rRNA gene cloning and Sanger sequencing in health and periodontitis. | - A total of 274 species/phylotypes identified.  
- First controlled study comparing health and chronic periodontitis via 16S rRNA gene sequencing.  
- Identified species/phylotypes associated with health: *Veillonella* sp., *Campylobacter gracilis*, *Campylobacter showae*, *Abiotrophia* adiacens, *Eubacterium* saburreum, *Gemella* sp., *Streptococcus sanguis*, *Streptococcus mutans*, *Capnocytophaga gingivalis*, *Rothia dentocariosa*, *Eubacterium* sp. and *Selenomonas* sp. |
view of the microbial composition of a given sample together with high-resolution taxonomy and high-throughput sample processing. Microscopic techniques such as those used by Listgarten et al.\(^5\) and Armitage et al.\(^6\) did not permit taxonomic classification of species. The DNA-DNA checkerboard study by Socransky et al.\(^7\) was performed with a technique that allowed high-throughput processing of samples and, therefore, the study includes many subjects. However, the microbiota evaluation was limited to 40 species. The comprehensive cultivation study of Moore and Moore\(^8\) was limited by laborious laboratory procedures and lack of culturability of a great proportion of the subgingival microflora. Despite the discussed limitations, these studies revealed a great proportion of the subgingival microbial diversity and community dynamics and were crucial to our current understanding of periodontitis etiopathology. All of these investigations agree that periodontal disease is associated with shifts in the composition of the community of microorganisms at the subgingival crevices in comparison to health.

16S rRNA Gene Sequencing: A New Era in Periodontal Microbiology

The field of microbiology took a turn after the advent of 16S rRNA gene sequencing as the method of choice to infer phylogenetic relatedness among bacterial species.\(^9,10\) rRNAs are essential components of the protein synthesizing machinery in all cells and, therefore, their gene sequences have highly conserved (similar) regions across species (Figure 1). These conserved regions are utilized to design “universal” PCR primers capable of recognizing segments of the 16S rRNA gene sequence of most, if not all, bacterial species. Apart from conserved regions, there are also several hypervariable

**FIGURE 1.** Steps required for the characterization of the subgingival plaque microbiome via 16S rRNA gene sequencing. Species are represented by different colors. Conserved regions in the 16S rRNA gene are depicted in gray and are used for design of universal primers. Hypervariable regions (V1 to V9) are depicted in black and are used for identification of species. Arrow labeled as “a” shows steps required prior to the advent of high-throughput sequencing. The different 16S rRNA genes from all species in the sample were amplified via PCR with universal primers. Individual 16S rRNA gene amplicons were cloned and multiplied in a host such as *Escherichia coli* to obtain a separate preparation of each cloned 16S rRNA gene. Cloned 16S rRNA genes were then sequenced via Sanger methods and the sequences compared to a reference database for their identification. Arrow labeled as “b” shows how high-throughput sequencing has simplified the process by allowing direct sequencing of PCR amplicons. Up until now, the most widely available sequencing technologies did not allow sequencing of the full-length 16S rRNA gene and, therefore, our group targets the first 350 base pairs containing the hypervariable regions V1 and V2, which perform very well discriminating oral species from each other. Universal primers flanking this region are designed as fusion primers incorporating also a spacer (orange), an identifier (blue) and an adapter (dark gray) sequence.\(^47\) All 16S rRNA gene molecules amplified in the PCR reaction are then simultaneously sequenced. Moreover, the addition of identifier sequence tags allows sequencing of many samples simultaneously.
### TABLE 2

Studies of the Subgingival Microbiome in Different Periodontal Conditions Using High-Throughput Sequencing of the 16S rRNA Gene

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<th>Reference</th>
<th>Methods</th>
<th>Key findings</th>
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<td><strong>Health versus periodontitis</strong></td>
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| Griffen et al., 2012[^14]  | 29 periodontally healthy controls and 29 subjects with chronic periodontitis (shallow and deep sites were sampled). | ■ 16 phylo, 106 genera and 596 species identified. 81 percent of sequences could be mapped to cultivated species.  
■ Community profiles were different in health and disease with higher diversity in periodontitis.  
■ 123 species-level phylotypes were enriched in periodontitis, while 53 were enriched in health.  
■ Shallow pockets of periodontitis subjects showed preponderance of disease-associated organisms.  
■ The major health-associated species are suppressed but not lost in periodontitis. |
| Abusleme et al., 2013[^15] | 22 subjects with chronic periodontitis and 10 healthy controls. Periodontitis subjects sampled at two sites with 5 mm probing depth (one with bleeding). Total load and load of specific genera measured via real time qPCR. | ■ Communities in health and periodontitis differed with higher diversity in periodontitis.  
■ 46 species-level phylotypes were enriched in periodontitis and 14 were enriched in health.  
■ Defined core subgingival species as those present in a majority of subjects and at equal relative abundance in health and disease. F. nucleatum is the most abundant core species.  
■ Bleeding was associated with higher bacterial load.  
■ Shifts from health to periodontitis resemble ecological succession without replacement of health-associated species. |
| Hong et al., 2015[^17]     | 34 subjects with chronic periodontitis compared to 79 healthy subjects sequenced by the Human Microbiome Project. Diabetics and subjects with chronic kidney disease included in the periodontitis group. | ■ Communities in health and periodontitis differed.  
■ No demographic or medical characteristics of periodontitis subjects were associated with specific microbial profiles.  
■ Two types of microbiome profiles seen in periodontitis (clusters A and B), with the cluster B community showing a positive correlation with periodontitis extent.  
■ Two types of microbiome profiles seen in health (clusters L and S). |
■ 18 species-level phylotypes enriched in periodontitis and five enriched in health.  
■ Microbial diversity was not significantly different between health and periodontitis. |
| **Periodontitis versus gingivitis versus health** |                                                                         |                                                                                      |
| Park et al., 2015[^37]     | 12 healthy subjects, 10 subjects with gingivitis and 10 subjects with periodontitis. | ■ Distinct communities in health, gingivitis and periodontitis. |
| **Health versus gingivitis** |                                                                         |                                                                                      |
| Kisler et al., 2013[^35]   | 20 healthy subjects abstained from oral hygiene. Sampled at baseline and after one and two weeks. | ■ Increased community diversity and significant shifts in microbiome structure after two weeks of oral hygiene abstinence.  
■ Identified species-level phylotypes positively and negatively correlated with gingivitis (bleeding on probing). |
| Huang et al., 2014[^36]    | 50 subjects underwent controlled transitions from naturally occurring gingivitis (NG) to healthy gingiva (baseline) to experimental gingivitis (EG). | ■ Temporal shifts in community structure seen along the progression from NG to baseline to EG.  
■ 15 genera could distinguish healthy and gingivitis samples with 74 percent accuracy.  
■ Shotgun metagenomic sequencing showed a functional shift with gingivitis-enriched functions such as flagellar biosynthesis.  
■ Two host types, I and II, with distinct susceptibility to gingivitis were identified. Genera such as Selenomonas, Peptostreptococcus and Veillonellaceae, among others, enriched in type-II hosts. |
regions (different across species) along the 1500 base pairs that form the 16S rRNA gene, which can be used as signatures to discriminate one species from another. Thus, 16S rRNA gene sequencing became the tool of choice to determine the global composition of the bacterial community in a given plaque sample. However, obtaining the 16S rRNA gene sequences of a bacterial community containing multiple species was initially a laborious process as it involved cloning of 16S rRNA gene amplicons into plasmid vectors with their subsequent replication in Escherichia coli to obtain a pure preparation of each 16S rRNA amplified molecule. Each amplified, cloned and purified 16S rRNA gene type was then sequenced by Sanger methods and its sequence compared to a database of archived 16S rRNA gene sequences to find a species match or the closest relative (“a” in Figure 1).

The pioneer studies that investigated the subgingival microbiome in health and under different periodontal conditions via 16S rRNA gene cloning and Sanger sequencing are summarized at the bottom of Table 1. These studies provided for the first time a global view of the subgingival microbiota that was independent of the cultivation requirements of species. For instance, Paster et al.11 found 347 species-level phyotypes in all subgingival plaque samples analyzed, 215 of which were novel. Among the novel sequences were some from phyla such as Synergistetes and TM7, which at the time had no cultivable oral representant. Moreover, about 60 different Treponema species were identified revealing a great variety of species from this difficult to cultivate genus. Using similar methodology, Kumar et al.12 statistically compared the microbiota in health and periodontitis identifying taxa strongly associated with either condition (Table 1). However, due to the labor-intensive cloning process, these studies were still not high throughput and lacked the sequencing depth to cover most subgingival diversity within samples.

High-Throughput Sequencing and the Road to Commoditization of Microbiome Evaluation

The development of 16S rRNA gene sequencing opened a new era in periodontal microbiology by allowing a global view of the bacterial species in a sample, but it was not until the advent of high-throughput sequencing methods that the technique became the new gold standard. Massively parallel sequencing techniques circumvent the cloning step and allow direct sequencing of 16S rRNA gene amplicons obtained from a polymicrobial sample (“b” in Figure 1). Furthermore, the addition of a sequence tag to each sample during the initial PCR amplification makes it possible to combine different biological samples in the same sequencing run. It is, therefore, now feasible to simultaneously characterize the subgingival microbiome composition of many samples, at a relatively low cost, in a short time period and obtaining thousands of sequences per sample to guarantee detection of most species present. However, 16S rRNA gene sequencing would not have become the method of choice for oral microbiota characterization if not for the existence of curated reference databases such as the Human Oral Microbiome Database (HOMD),13 which allows the classification of most sequences in a dataset to the species level. Thus, the process of evaluating subgingival communities via high-throughput 16S rRNA gene sequencing has become accurate, cost-effective and accessible to most researchers.

Subgingival Microbiome in Health and Periodontitis as Revealed by High-Throughput 16S rRNA Gene Sequencing

Table 2 summarizes a series of pioneer studies of the subgingival microbiome conducted via high-throughput sequencing of 16S rRNA gene amplicons. The studies by Griffen et al.,14 Abusleme et al.,15 Kirst et al.16 and Hong et al.17 constitute the most comprehensive characterizations to date of the shifts in microbiome composition from health to periodontitis. Although sampling techniques and laboratory methods somewhat differ across studies, all manuscripts report profound shifts in community structure (i.e., species proportions) from health to disease. Reassuringly, the reported shifts agree with pre-16S rRNA gene sequencing studies showing a trend toward higher levels of Gram-negative microorganisms in periodontitis and a clear association of the red complex species (Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola) described by Socransky et al.7 with disease.14,15 Furthermore, high-throughput sequencing studies confirmed, as was previously seen via cultivation,8 that periodontitis is associated with an increase in the relative abundance of a diverse
Abiotrophia defectiva
Acidovorax sp. 98-63833
Acinetobacter junii
Achromobacter sp. HOT183
Achromobacter massiliensis
Achromobacter naeslundii
Achromobacter odontolyticus
Achromobacter sp. HOT169
Achromobacter sp. HOT170
Achromobacter sp. HOT171
Achromobacter sp. HOT173
Achromobacter sp. HOT175
Achromobacter sp. HOT177
Achromobacter sp. HOT180
Achromobacter wolwensii
Bergeyella sp. HOT322
Brachybacterium rhizosorus
Burkholderia cepacia
Capnocytophaga gingivalis
Capnocytophaga leadbetteri
Capnocytophaga sutrigena
Comamonadaceae nbo379c11c1
Comamonadaceae VE2A04
Corynebacterium durum
Corynebacterium matruchoti
Eikenella corrodens
Fusobacterium nucleatum ss polymorphum
Fusobacterium nucleatum ss viridans
Fusobacterium morbillorum
Granulicatella acrifaciens
Haemophilus P3D1 620
Haemophilus parahaemolyticus
Haemophilus parasputigena
Klebsiella oralis
Lautropia AP009
Lautropia mirabilis
Leptotrichia sp. HOT212
Leptotrichia sp. HOT225
Moraxella osloensis
Neisseria elongata
Porphyromonas catoniae
Porphyromonas sp. HOT279
Propionibacterium propionicum
Rothia aerea
Rothia dentocariosa
Schwartzia sp. HOT155
Selenomonas noxia
Selenomonas sp. HOT138
Streptococcus B66
Streptococcus cristatus
Streptococcus gordoni
Streptococcus intermedius
Streptococcus mitis bv2
Streptococcus mitis
Streptococcus sanguinis
Streptococcus sp. HOT58
Streptococcus sp. HOT64
unclassified Xanthomonadaceae
Veillonella parvula

**FIGURE 2.** Health-associated, core and periodontitis-associated species of the subgingival microbiome. Green and red shapes show species with significantly different relative abundances in health and periodontitis. Data to construct this figure were extracted from our own studies15,17 and the studies of Griffen et al.14 and Kint et al.16 Species supported by the four included high-throughput sequencing studies are underlined. Gray shape shows core species, which are those detected in a majority of the subjects surveyed and that did not change in relative abundance from health to periodontitis, according to Abusleme et al.15 and Hong et al.17 The number of studies that support the association of a species with each group is indicated as a superscript [maximum of four studies for green and red shapes and maximum of two studies from gray shape]. In case of a disagreement, the species name was placed in both groups and the number of studies that support its placement in either group indicated.
range of taxa, most of which remain understudied from all biological aspects. Figure 2 compiles data from the four high-throughput sequencing studies mentioned above and shows the health-associated and periodontitis-associated members of the subgingival microbiome, that is, the species that increase or decrease in relative abundance according to clinical status. Most studies usually detect more periodontitis-associated than health-associated species, as periodontitis communities are more diverse due to a more even distribution, in terms of percentages, of the species that form the community. It is evident from Figure 2 that the communities associated with health and periodontitis are complex entities and that a thorough understanding of this disease would require a profound study of each community member and its interplay with other species and the host.

Studies by our group also focused on identifying those species that appear in most subjects and do not change in their proportions from health to disease.\textsuperscript{15,17} We named these taxa, depicted in Figure 2, the “core species” of the subgingival microbiome. Core species are bound to be metabolically versatile as they are capable of thriving under the nutritional and environmental conditions present in both health and periodontitis. Most important, core species are probably capable of synergistic interactions with health- and disease-associated species as they successfully grow with both groups. Also, they are able to grow well under various types of community arrangements, as they are very prevalent despite variations in community composition from subject to subject. Because of this versatility, we hypothesized that core species act as metabolic cornerstones for the whole community and that their presence is likely important in the microbiome shifts from health to periodontitis.\textsuperscript{15,17} Interestingly, the most abundant core species in both of our studies\textsuperscript{15,17} was \textit{Fusobacterium nucleatum}, a Gram-negative anaerobe with a demonstrated ability to interact physically via coaggregation with a diverse range of oral species.\textsuperscript{18} \textit{F. nucleatum} has also been shown to metabolically support the growth of periodontitis-associated taxa in several in vitro investigations. In a continuous-culture aerated polymicrobial community, 

F. nucleatum had a positive influence on the biomass of the Gram-negative anaerobes \textit{P. gingivalis} and \textit{Prevotella nigrescens}.\textsuperscript{19} Moreover, \textit{F. nucleatum} can easily adapt to aerated conditions by metabolizing oxygen via enzymatic activities such as that of NADH oxidase, thereby reducing the environment to anaerobic levels in which \textit{P. gingivalis} can thrive.\textsuperscript{20} \textit{F. nucleatum} also generates CO\textsubscript{2}, which is subsequently metabolized by \textit{P. gingivalis}.\textsuperscript{20} The development of targeted strategies to control subgingival plaque maturation may benefit by focusing on interspecies interactions involving core members, as they may have an essential role facilitating microbial succesions.

Although there are species strongly associated with health or disease, it is also important to highlight that health-associated species can be detected in periodontitis and periodontitis-associated species in health, albeit in low proportions and at a lower frequency.\textsuperscript{14,15} Therefore, periodontitis is associated with shifts in the species that numerically dominate subgingival communities rather than caused by de novo colonization. Evidence of the indigenous nature of periodontitis-associated species was previously provided by more targeted analyses, which showed that colonization of periodontitis-associated species occurs early in life.\textsuperscript{21,22}

A better understanding of microbiome shifts from health to periodontitis needs to consider not only changes in species proportions, but also changes in biomass. Techniques such as 16S rRNA gene sequencing can only reveal shifts in the relative proportions of species but do not measure changes in biomass of specific taxa. To understand the evolution of the subgingival microbiome from this perspective, in one of our studies we measured changes in the total bacterial load from health to periodontitis using real-time quantitative PCR.\textsuperscript{15} We found that after a single pass of a curette, 1,000 times more bacterial cells could be recovered from periodontitis sites (with probing depths (PD) equal to 5 mm) in comparison to sites in healthy subjects (with PD <4 mm). These measurements were in close agreement with those previously reported by Moore and Moore using a cultivation approach.\textsuperscript{8} This 3-log increase in biomass could not solely be explained by the larger surface area of periodontal pockets, but is probably the result of a combination of continued biofilm accretion due to lack of disruption by adequate hygiene and of increased community growth capacity. The flow rate of gingival crevicular fluid (GCF) into the sulcus has been shown to gradually increase from health to gingivitis and to periodontitis.\textsuperscript{23,24} Since
subgingival communities rely on GCF as a nutritional source, communities in periodontitis may achieve higher biomass due to the greater availability of inflammation-derived nutrients. The shifts in composition of periodontitis-associated communities may also facilitate synergistic nutrient acquisition activities. Enrichment cultivation studies in serum and evaluations of the growth of periodontitis-associated taxa in the presence of serum proteins suggest that increases in GCF flow rate are likely to promote the enrichment of proteinase-rich taxa, which would in turn release peptides that could be utilized by themselves and by other species in the community.25-28

Our group has also measured changes in the total load of specific genera from health to periodontitis.15 The health-associated genus *Actinomyces* was shown not to change its total biomass from health to disease. Therefore, its decreased relative proportion in periodontitis is the consequence of lack of growth as the whole community matures and increases in biomass. *Actinomyces* are, therefore, outcompeted during microbiome shifts. In contrast, the total number of cells of the core genus *Veillonella*, which in our study did not change in terms of relative proportions from health to periodontitis, was shown to be higher in disease. The dynamics of microbiome evolution from health to periodontitis in terms of total number of bacteria present at each stage are shown in **FIGURE 3**. To construct this figure, we calculated the sum of health-associated, core or periodontitis-associated species under the healthy or the periodontitis states, using relative abundance tables from our published studies.15,17 We then assumed a 3-log change in total biomass from health to periodontitis and, therefore, decrease in terms of their proportion. It should be noted that although the load of certain species such as *Actinomyces* may not change as shown by qPCR, other health-associated species such as streptococci are likely to proliferate with the whole community,15 and, therefore, the total load of health-associated species also increases from health to periodontitis. In contrast, **FIGURE 3** shows that core species increase their biomass from health to disease in a much more dramatic way than health-associated species, thereby preserving their proportion within the community. The most drastic increase in biomass, however, occurs for periodontitis-associated species, which show a ~4-log increase becoming dominant in disease. This figure makes it evident that not only periodontitis-associated, but also health-associated and core species, could have an important role during periodontitis in terms of their interaction with the immune system as their load is still significant in disease.

16S rRNA gene sequencing studies also revealed that microbiome shifts occur in the whole mouth rather than at specific tooth sites. Griffen et al.14 showed that the communities of healthy sites in subjects with periodontitis are depleted of health-associated taxa and have increased proportions of periodontitis-associated species when compared to sites in periodontitis-free individuals. An obvious clinical implication from this finding is that treatment of periodontitis requires a global, whole mouth approach.

Taking all these data together, it can be concluded that the subgingival microbiome community evolves from health to periodontitis by a process of microbial succession, with the emergence of newly dominant species, but without replacement of pioneer...
health-associated species, as we have previously proposed.\textsuperscript{15} TABLE 3 presents a summary of key points regarding current knowledge on microbiome shifts from health to periodontitis.

### TABLE 3

**Key Points Summarizing Microbiome Shifts From Health to Periodontitis**

- Community biomass increases by at least 3-log from health to periodontitis.
- Microbiome diversity and, therefore, community complexity increases in periodontitis due to a more even distribution of species.
- Health-associated species are those that occupy ~60 percent of the biomass in health and only ~10 percent of the biomass in periodontitis.
- Periodontitis-associated species increase their biomass ~4-log from health to periodontitis. They occupy ~5 percent of the biomass in health and ~50 percent of the biomass in periodontitis.
- Microbiome shifts are not localized but involve to some degree all subgingival sites in the mouth of an individual.
- Microbiome shifts associated with gingivitis are distinct to those in periodontitis, with gingivitis possibly representing a transitional stage from health to disease.

Beyond 16S rRNA Sequencing: Understanding Changes in Community Function From Health to Periodontitis via Metatranscriptomics

Subgingival communities can also be characterized via shotgun DNA sequencing, which provides an overall view of all genes or via RNA sequencing, which reveals all RNA transcripts in a given community. A metatranscriptomic study (sequencing of all RNA transcripts in a community) permits evaluation of community composition based solely on taxa that are metabolically active. It also allows examination of changes in the metabolic activities of specific species or metabolic changes in the community as a whole. Metatranscriptomics has been used to compare the subgingival microbiome in health and periodontitis.\textsuperscript{29} This evaluation revealed that periodontitis communities have augmented biological processes related to flagellar motility, peptide transport, iron acquisition, beta-lactam degradation, lipid A biosynthesis and cellular stress responses. Overexpression of these processes in periodontitis did not only occur in periodontitis-associated species, but health-associated and core species also contributed to such increased functions, in accordance with the concept that the whole community responds to the changes in environmental conditions that accompany pocket formation.\textsuperscript{29} One of the most widely upregulated functions among species during periodontitis was iron acquisition, suggesting that the ability to compete for this nutrient may be an important determinant of which species are ultimately able to thrive as biomass accumulates. Another widely upregulated function in periodontitis was response to oxidative stress, which could be a consequence of increased neutrophil presence in disease, as these cells utilize oxygen radical generation as a killing mechanism. Alternatively, increased oxidative stress could also result from greater abundance in periodontal pockets of proteinase-rich species with the subsequent degradation of iron-containing serum proteins such as transferrin. Proteinases stimulate bacterial growth via the release of peptides and iron, but have also been shown to contribute to oxygen radical generation when targeting iron-containing proteins.\textsuperscript{30}

In another study, Jorth et al.\textsuperscript{31} compared the metatranscriptome of shallow and deep pockets in subjects with aggressive periodontitis, showing that a larger fraction of the microbial population is metabolically active in deep pockets, implying greater nutrient availability and increased community productivity in disease. Also importantly, although communities from different subjects harbored different species, the community metabolic activities were conserved among subjects, showing there is redundancy in terms of the species that can perform specific metabolic functions within a community. This study also showed that although the core species *F. nucleatum* did not vary in abundance in shallow and deep sites, it upregulated lysine fermentation to butyrate in the deeper pockets.\textsuperscript{31} This switch to butyrate suggests a change from a more oxygenated to a more anaerobic environment as pocket depth increased, in agreement with our previous work which showed *F. nucleatum* switches its fermentation end products from acetate under oxygenated conditions to butyrate in a more reduced environment.\textsuperscript{31}

A recent study by Yost et al.\textsuperscript{33} evaluated the subgingival metatranscriptome during chronic periodontitis progression comparing subject-matched progressing and non-progressing sites at two time points. This study revealed that communities from nonprogressing sites were very stable in terms of their composition and metabolic activities, contrary to communities from sites that lost attachment. Streptococci and other health-associated species such as Actinomyces became less metabolically active during progression, while *Prevotella* spp., *Pseudomonas* spp. and the archaea *Methanobrevibacter smithii*, among others became more active. With respect to the metabolic activities of the communities, the gene ontology (GO) terms of pathogenesis, response to oxidative stress, ferrous iron transport, protein secretion and growth, among others, became enriched in sites that broke down. The most striking finding of this study, however, was the large
differences in the metabolic activities of communities from baseline samples (before attachment loss occurred) of progressing and nonprogressing sites. A comparison of the species that were active in baseline samples showed that a great number of species, belonging to all groups as defined in this review, were more active in baselines of sites that progressed. With respect to the community metabolic activities, biological processes such as lipid A and peptidoglycan biosynthesis, response to oxidative stress, flagellar motility and amino acid, iron and potassium transport were enriched in the baselines of sites that lost attachment. These results suggest that since the community activities were different prior to detectable tissue destruction, the local microenvironment may also have been different with increased inflammation at these sites. Also, interspecies interactions and community arrangements in these communities
may have been distinct allowing greater overall community metabolism.

Taking it all together, metatranscriptomic analyses of the subgingival microbiome suggest that the microbial community adapts to the changing nutritional and environmental conditions in the gingival crevices. Because biomass increases with disease, probably due to greater availability of carbon sources originating from GCF, it is expected that competition for the scarcest resources also becomes fierce and those species able to adapt and capture the limiting nutrients (e.g., iron, which is a compound of low bioavailability) are those that ultimately thrive. Metatranscriptomic studies, therefore, highlight the ecological nature of microbiome shifts and the importance of understanding community adaptation to environmental pressures.

Subgingival Microbiome Shifts in Gingivitis

Gingivitis is defined as the presence of inflammation in the gingival tissues without loss of connective tissue attachment and bone resorption. Because gingivitis is thought to be a required step in the transition from health to periodontitis, oral microbiologists have also focused on understanding microbiome shifts under this condition. Furthermore, because gingivitis can be induced in humans by oral hygiene abstention and the condition resolves after resumption of plaque control, the human experimental model of gingivitis has been repeatedly characterized. 34-36

Classic studies performed via cultivation assessing plaque evolution at the gingival margin during oral hygiene abstention show a shift from a dominance of Gram-positive cocci during the initial days of plaque accumulation to an increase in Gram-negative morphotypes including rods, filaments and spirochaetes after two to three weeks. 34 Significantly, these shifts correlate with the appearance of clinical signs of gingivitis. Recent studies using high-throughput sequencing of 16S rRNA genes in conjunction with experimental plaque accumulation models agree with the classic cultivation studies but define, with greater accuracy, the molecular taxonomy of shifts during gingivitis onset. 35,36 Figure 4 depicts the species associated with the transition from health to gingivitis according to the studies of Kistler et al. 35 and Huang et al. 36 It is apparent from this figure that the microbiome becomes more complex in gingivitis with enrichment of a great variety of taxa, in accordance with an increase in diversity as reported by high-throughput sequencing. 35,36 Moreover, most of the species depleted in gingivitis are characterized by their aerobic or facultative anaerobic metabolism, while most enriched species are anaerobes, which suggests the formation of anaerobic microniches during biofilm accretion. Most species enriched in gingivitis are also Gram-negative. However, the species enriched in gingivitis are not the same as those associated with periodontitis. In fact, out of the 69 gingivitis-associated taxa shown in Figure 4, only 14 species are also periodontitis-associated. Gingivitis-associated species are also not the same as core species, with only Catonella morbi present in both groups (Figures 2 and 4). Gingivitis-associated species, however, appeared among those we designated as variable species when health and periodontitis were compared (Figure 3). These species were not strongly associated with health or with periodontitis and were not consistently detected as they were not enriched under either condition. Therefore, the microbiome shift associated with gingival inflammation is unique and distinct to the health-associated microbiome and to the shift associated with tissue destruction.

Proposing Gingivitis-Associated and Core Species as Mediators of Transitions From Health to Periodontitis

Our group recently explored whether subjects belonging to the same clinical category (health or disease) harbored different microbiome subtypes, perhaps reflecting different stages of dysbiosis. 37 We found that communities from healthy subjects tended to cluster into two groups, one large cluster L, with the majority of subjects, and one small cluster, designated S, with only a few subjects. Cluster L samples were enriched for health-associated Streptococcus, Actinomyces, Rothia, Lautropia, Bergeyella, Kingella and Granulicatella species. In contrast, cluster S samples were enriched for species from the genera Bacteroidales[G-2], Capnocytophaga, Campylobacter, Corynebacterium, Fusobacterium, Leptotrichia, Parvimonas, Porphyromonas, Prevotella, Selenomonas, Tannerella and TM7[G-1], the majority of which are gingivitis-associated or core as defined in this review (Figures 2, 4 and 5). Similarly, in Hong et al. 37 we explored the existence of community types within periodontitis finding also a tendency for two clusters to form. One periodontitis cluster (designated cluster A) was enriched for species from the genera Campylobacter, Corynebacterium,
Fusobacterium, Leptotrichia, Prevotella, Tannerella and TM7[G-1] species, many of which are also gingivitis-associated or core species. In contrast, the second periodontitis cluster detected (cluster B) was enriched with the red complex, Filifactor alocis, Treponema spp., Porphyromonas gingivalis, Fretibacterium spp. and other species strongly associated with periodontitis. Cluster B was also associated with greater periodontitis severity (greater number of sites involved) suggesting a later temporal stage in the disease process.17

The data discussed above, although cross-sectional, suggests that the different clusters represent a temporal progression. In FIGURE 5 we have put together a conceptual model of the microbiome shifts from health to periodontitis, based on the microbial clusters found by Hong et al.17 In this model, we propose that the enrichment of gingivitis-associated and core species is an intermediate stage in the health-to-periodontitis microbiome shifts. As shown in FIGURE 5, a great number of species enriched in the health cluster S and in the periodontitis cluster A are also gingivitis-associated, with also some core species. Indeed, whether gingivitis represents a transitional stage between health and periodontitis has been a matter of debate. However, very few studies have been properly designed...
to address this question. One notable exception is the study by Tanner et al., who evaluated progression of periodontitis in subjects with very mild levels of disease in their whole mouth. This study found a positive correlation between baseline levels of clinical gingivitis (as measured by gingival index and bleeding on probing) with mean changes in PD, suggesting gingivitis indeed mediates the health-to-disease transition. In the model proposed in FIGURE 5, each cluster represents a temporal stage with examples of species enriched at each stage shown at the bottom of the figure. The potential interactions between the microbiome and the host driving these transitions are shown at the top of FIGURE 5. In clinical health, the microbiome of most subjects is enriched for health-associated species such as Rothia aeria and Actinomyces spp. (cluster L). Cluster S possibly represents a transitional stage of subclinical dysbiosis, in which the microbiome is shifting toward the gingivitis state. This initial shift is mediated by biofilm accretion due to inadequate oral hygiene as demonstrated by the experimental gingivitis studies. During this phase, the growth of the community would be mostly driven by interspecies interactions because oral taxa depend on each other for growth under the nutritional conditions present in the gingival sulcus. If biofilm accretion is not disrupted, microbial successions occur with the rise of gingivitis-associated species, which are likely to depend on the prior growth of health-associated taxa. The increased biomass and these initial shifts in species abundances would represent a higher load of microorganisms, especially Gram-negative, and their products to which the host responds. The resultant inflammation probably exerts an environmental pressure that selects for species effective at utilizing serum-derived nutritional resources. The species that flourish under gingivitis, however, need to have relative tolerance to oxygen as the sulcus depth is shallow at this stage. In this respect, gingivitis-associated anaerobes such as F. nucleatum and Prevotella melaninogenica have been shown to have increased tolerance to oxygen than periodontitis-associated species such as P. gingivalis and Treponema spp., which flourish at a later stage once pockets form. During the health-to-gingivitis transition the community also needs to adapt to the new inflammatory environment. With greater biomass comes increased competition and, therefore, interspecies interactions, some of them antagonistic, would continue to influence which species succeed as shifts occur. The host defenses are also likely to exert great environmental pressure at this stage selecting for those species more resistant to the actions of antimicrobial host effectors such as complement and iron-sequestering lactoferrin, which have been shown to be increased in gingivitis. FIGURE 5 then proposes that gingivitis communities are likely to set the stage for a subsequent shift characterized by the rise of periodontitis-associated taxa. The events that lead to the transition from gingivitis to periodontitis are less clear but it seems subversion and dysregulation of the immune responses are likely to mediate tissue destruction. After initial periodontal breakdown, periodontitis-associated species increase in biomass, but gingivitis-associated core species are still important community components. Profound dysbiosis is detected once pockets have formed with a final microbiome transition in which red complex species, Filifactor alocis, Fretibacterium spp., among others, outcompete other taxa. As more sites are compromised in a subject, the greater the chances of detecting these profoundly dysbiotic communities representing the end-stage of microbiome shifts. As in health and gingivitis, interspecies interactions are probably essential to arrive to the stage of final dysbiosis and maintain the stability of these communities.

In clinical health, the microbiome of most subjects is enriched for health-associated species such as Rothia aeria and Actinomyces spp. (cluster L).

Intraspecies Populations Shifts Are Also Likely Important in Health-to-Disease Transitions

Microorganisms belonging to the same species exhibit a certain degree of genetic diversity and, therefore, a species constitutes a heterogeneous population. Rapid succession of bacterial types within a species has been seen to occur during the initial stages of biofilm formation in the oral cavity. It is expected that during microbiome shifts, intraspecies populations will also undergo selection as some cells will be better able to adapt to certain environments depending on their attributes. The presence of a capsule in certain P. gingivalis strains may represent a good example of a virulence attribute mediating intraspecies shifts. Genotypes similar to encapsulated P. gingivalis strains seem to predominate in periodontal pockets, while non-encapsulated genotypes predominate in health. The presence of a capsule has been shown to confer P. gingivalis the ability to avoid neutrophil killing...
and, therefore, encapsulated *P. gingivalis* are better at subverting immune surveillance. However, encapsulated *P. gingivalis* strains are not efficient at forming biofilms, which may explain why the encapsulated genotypes are not the predominant ones found prior to pocket formation, when retention via biofilm formation would be important. Thus, in the healthy shallow sulcus, non-encapsulated *P. gingivalis* may have an advantage over encapsulated types in terms of retention. Non-encapsulated types, however, stimulate host responses in a much more effective way and are, therefore, contained by the immune system. Once a pocket forms, non-encapsulated genotypes can more easily thrive in the sulcus as part of the loosely adherent plaque, while avoiding phagocytes. The *P. gingivalis* case exemplifies why the study of intraspecies population shifts is also essential, although little attention has been paid to it.

Conclusion

High-throughput sequencing of the 16S rRNA gene has allowed a broad view of the subgingival microbiome providing a better understanding of shifts associated with the development of periodontitis. Microbiome shifts seem to be the result of microbial successions and community adaptation to the changing environmental conditions at the gingival sulcus. Important environmental determinants of the species dominant at each stage are likely to be the nutrients available and the oxygen levels in the biofilm and the sulcus. However, a thorough understanding of the responses of subgingival communities to such environmental pressures and a delineation of the microbial genomic determinants of success at each stage are needed. Moreover, microbiome shifts are likely to depend on interspecies metabolic interactions. Communities in health are probably needed for gingivitis-associated taxa to flourish, while gingivitis-associated and core taxa are likely to set the stage for the rise of periodontitis-associated species. The metabolic interactions mediating these successions, however, have not been clearly defined. It can be concluded that the development of novel strategies to treat or prevent periodontitis needs to take into account environmental modification and interspecies dependencies. The identification of species with key metabolic roles in the transitional communities may represent a step forward as these species could represent better therapeutic targets than periodontitis-associated species such as the red complex, whose dominance seems to be the ultimate result of tissue destruction.

**ACKNOWLEDGMENT**

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Understanding Caries From the Oral Microbiome Perspective

Anne C.R. Tanner, BDS, PhD; Christine A. Kressirer, PhD; and Lina L. Faller, PhD

ABSTRACT Dental caries is a major disease of the oral cavity with profound clinical significance. Caries results from a transition of a healthy oral microbiome into an acidogenic community of decreased microbial diversity in response to excessive dietary sugar intake. Microbiological cultivation, molecular identification, gene expression and metabolomic analyses show the importance of the entire microbial community in understanding the role of the microbiome in the pathology of caries.
the next year with the key goals of determining the microbial composition of the microbiome in 15 body sites in men and 18 in women from about 300 healthy adults and to determine the complete genomes of 3,000 human-associated bacterial species. More than 200 papers have been published describing the results of the HMP and a major summary of its findings was published in 2012. Body sites studied included nine from the oral cavity: subgingival, supragingival, keratinized gingivae, hard palate, tongue dorsum, buccal mucosa, palatine tonsils, throat and saliva. A major finding from these studies is that each of the oral sites has a slightly different microbiome. Information on oral bacteria, their taxonomy and genomic information is publicly available in the Human Oral Microbiome Database (homd.org).

Clinical Significance
Dental caries is a worldwide health concern with a very uneven distribution of affected individuals. Caries disproportionately affects socially and economically disadvantaged populations. In the United States, costs for dental services were $84.4 billion in 2014 (National Health Expenditures, 2014), of which, caries restorative care comprised a substantial component. While dental caries affects all ages, it can be particularly devastating when it affects the primary teeth in young children. 

Starting in the 1980s, the means for identifying and classifying bacteria underwent a radical change from using cultural methods to using molecular methods. Molecular methods are based on gene sequences to characterize and identify bacteria, most frequently using the 16S rRNA gene that codes for a smaller subunit of the ribosome. This approach was pioneered by Carl Woese who recognized the importance of the 16S rRNA gene for determining phylogeny and used it to revise the tree of life for all living organisms. In every environment examined, molecular methods revealed a vast array of bacteria that had not been previously identified or described. Many of the newly recognized bacteria had not been identified using the prevailing methods of strain identification using biochemical tests, or had not been cultivated and so were invisible in previous cultivation-based studies. Examination of the species present in the oral cavity using 16S rRNA methodology was pioneered by the Relman and Dewhirst/Paster laboratories. The oral cavity is now known to have approximately 700 species identified using 16S rRNA methods. These studies have provided a solid understanding of which bacteria are present in the entire oral cavity and which are present at specific sites as described below for dental caries.

Based on cultural analysis, the caries-associated biofilm was shown to include a wide range of bacterial species, with principal suspected caries pathogens including acidogenic streptococci, particularly S. mutans, Actinomyces, Bifidobacterium and Lactobacillus species. In older adults caries is frequently associated with exposed root surfaces resulting from gingival recession and from medication-induced low salivary flow. This can compromise an individual’s ability to eat leading to malnutrition and expediting mortality in the elderly.

Study of Bacteria Associated With Caries

Early Culture Studies
Prior to the 1980s, bacteria were identified in culture studies by growing them in petri dishes on bacterial media and characterizing phenotypic traits such as Gram stain reaction, motility, aerobic or anaerobic growth, fermentation of various carbohydrate, end products of fermentation and enzyme activities. Early oral microbiology studies in humans, including those of Loesche and Syed, Hardie and Bowden and Gibbons and van Houte who isolated and named more than 100 oral species that set the stage for studies on the microbiological cause of caries. The approaches used to associate bacteria with caries were those of Koch and Pasteur, trying to link caries to a single pathogen. The initial link between bacteria, notably Streptococcus mutans, Lactobacillus and Actinomyces species and dental caries, came from observations in experimental animal models from investigators that included Jordan, Fitzgerald and Keyes, Bowden, van Houte and Caulfield. Cultivation studies linked S. mutans with caries in humans and demonstrated transmission of S. mutans in humans from mother to child. Investigators showed an increased risk of caries the younger a child was infected.

Molecular Methods Expand the Caries-Associated Microbiota

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Types of Caries and Their Microbiota

There are many ways to subdivide types or stages of caries, but here we will highlight three types: White spot lesions or early caries in the enamel, dentin caries where the lesion has penetrated the enamel into the dentin, and root caries, which occurs on exposed root surfaces of cementum or dentin. Figure 1 illustrates these types of caries and lists the major bacteria associated with these lesions.

White Spot Lesions

White spot lesions (WSLs) are noncavitated precariously lesions in enamel and represent an early stage of tooth demineralization. Due to mineral loss, WSLs have an opaque and chalky-white appearance when compared with the healthy enamel surrounding them. WSLs are an early sign of a site at risk for caries lesion development in both primary and secondary dentitions. Cultivation and molecular studies have identified the major species involved, which are shown in panel A of Figure 1. It is a consistent observation that S. mutans is not detected in all white spot, or indeed all other types of carious lesions, whether assayed by cultivation or molecular methods. Other consortia of acidogenic bacteria including nonmutans streptococci and Actinomyces species are, however, present when S. mutans is not detected. These studies have also shown that bacterial species diversity is reduced in caries compared with healthy sites, suggesting an acidic environment suppresses acid-sensitive bacteria.

Veillonella species are commonly observed in initial lesions and it has been suggested that they support growth of streptococci by using lactic acid as an energy source and facilitating the growth of acidogenic species. Even acid-tolerant species show reduced growth at pH levels below pH 4.5 and benefit from members of the microbial community that help maintain a more neutral pH environment.

Dentin Caries

As caries extends into and eventually through the enamel, it reaches into dentin and dentinal tubules. As the dentin breaks down a cavity is formed that, without intervention, will ultimately progress into the pulp and nerve fibers. The key culture studies

**FIGURE 1.** Principal species in caries-associated microbiomes. The major species detected by culture (C) or molecular (M) approaches. The diagrams illustrate position of lesions into enamel (white), dentin (orange), pulp (red): A = white spot initial lesions, B = dentin caries, C = root caries. Source information from references cited in the text.
of dentin caries in adults are from Wade and Hunter and in children from severe ECC from Beighton and Tanner laboratories. Molecular studies have included focus on Lactobacillus and Prevotella species in adults and childhood caries. The dentin microbiota is characterized by a more anaerobic microbiome than caries-free sites or initial lesions. In addition to S. mutans there are increasing proportions of acidogenic acid-tolerant gram positive rod species in Actinomyces, the Bifidobacterium family (Bifidobacterium, Scardovia, etc.) and Lactobacillus species. Increased detection of S. mutans in dentinal caries in adolescents with abundant biofilm and extensive caries compared with adolescents with routine dental care suggests that S. mutans infection may be population dependent. Increasing proportions of the highly acid-tolerant Bifidobacterium/Scardovia and Lactobacillus taxa in advanced lesions fit with the expanded ecological plaque hypothesis described below. The dentin microbiota also includes significant proportions of more acid sensitive, but acid-producing species in Prevotella, Capnocytophaga and Selenomonas. Caries deep in dentin may not be at an extremely low pH suggesting that these Gram-negative anaerobes can play an active role in caries. The key species identified in these studies are shown in panel B of FIGURE 1.

Root Caries

Root caries affects dentin exposed from gingival recession or periodontal surgery in adults and the elderly. Risk factors include reduced salivary flow from medications or disease (Sjögren’s syndrome), sugar-sweetened, syrupy medications and frequent intakes of cariogenic foods. Gram-positive rods have been associated with root caries as well as yeast and Streptococcus species (panel C in FIGURE 1). It has been observed that the microbial composition of carious lesions vary between subjects more so than in WSLs and dentinal caries, thus there is a less characteristic microbiota consistently observed. The major species associated with root caries are listed in panel C of FIGURE 1 and includes many of the same species found in dentinal caries.

Culture Compared With Molecular Analyses

Comparisons between molecular methods and anaerobic culture showed many similarities in the taxa identified, but also some key differences. Molecular analyses allow for greater depth of samples and more species can be detected, especially fastidious and difficult-to-grow species and species not yet cultured. Cultural methods, however, enhanced detection of other species.
including *S. mutans*, *Actinomyces* and *Bifidobacterium/Scardovia* species,\(^{45,77}\) The reasons these species were missed in many molecular studies is that the bacterial cells are difficult to break open for DNA release and their DNA is not recognized by standard primer and adapters used for 16S rRNA amplifications\(^{42,58}\) and pyrosequencing.\(^{59}\) Many of the “not yet cultured species” inferred by molecular methods were found as isolates in investigator’s strain collections when these collections were characterized using 16S rRNA sequencing.\(^{42,45}\) Thus, findings from molecular and cultural methods have proved complementary in identifying the bacteria associated with caries.

Caries Models

Models of the roles of bacteria in caries development including nonspecific, specific\(^{60}\) and ecological plaque hypotheses\(^{63}\) have been based on data from culture studies. The extended ecological hypothesis of Takahashi and Nyvad incorporated three stages in caries development.\(^{50}\) The first dynamic stability stage had a dominance of nonmutans streptococci and *Actinomyces* that produced mild and infrequent acidification that was not associated with net tooth mineral loss. The second acidogenic stage followed acid-induced adaptation or selection to those nonmutans streptococci and *Actinomyces* species that could lower the pH. This led to moderate and more frequent acid formation likely associated with initial enamel or dentin (root caries) demineralization. The final aciduric phase was characterized by increased acid-induced adaptation or selection with a microbial increase in mutans streptococci and other aciduric bacteria. Clinically, this translated to tooth mineral loss, lesion formation and progression. This model incorporates the concept that various stages of dental caries are associated with different microbial communities.

While some interspecies interactions can enhance virulence in an environment,\(^{52-63}\) cooperation among species may also reduce pathogenicity. The concept of Koch’s postulates focusing on one species for one disease has been reevaluated in the era of microbial community-based diseases. Byrd and Segre suggest that the presence of pathogens may not necessarily equate to disease because of colonization resistance.\(^{66}\) In this scenario, the community confers an ameliorating impact on pathogenicity. In caries, acidity produced by *S. mutans*, for example, can be counterbalanced by ammonia production from arginine deiminase or urease activity from other species.\(^{67}\) Thus, while investigators might aim to define the most likely and most frequently active pathogens, their detection may not definitely indicate disease. This is illustrated by the observations that despite strong associations of *S. mutans* to ECC by culture,\(^{29,68}\) and of *S. mutans* and *S. sobrinus* to ECC using PCR methods,\(^{69}\) the mutans streptococci have not proved universally reliable in caries risk assessment.\(^{6}\)

**Example of Caries Microbiome Community Changing With Progression**

**FIGURE 2** illustrates the changes that occur in the oral biofilm community in subjects with caries at different stages of disease, sampled to represent caries progression. The data for this figure came from studies that combined culture and molecular approaches to examine severe ECC progression\(^{37,38}\) (FIGURE 2). Advanced dentin lesions were found to harbor a new *Bifidobacterium* species (an early report of *Scardovia wiggsiae* in caries), *S. mutans* and veillonellae\(^{48}\) in addition to previously uncultivated species. In another study using the same clinical design, *Actinomyces* and nonmutans streptococci with higher levels of *S. mutans* were identified in association with increasing lesion depth.\(^{37}\) Species in higher levels in dentin than *S. mutans* included *Bifidobacterium dentium*, nonmutans streptococci, and Veillonella, *Atopobium*, *Propionebacterium* and *Lactobacillus* species. In children’s permanent teeth, dentin-colonizing species included *S. mutans*, *Lactobacillus* spp. *Propionebacterium* and *Atopobium* species in contrast to bifidobacteria in primary teeth.\(^{37}\) In adults, carious lesion development was also associated with a shift in microbiome composition with increasing proportions of *S. mutans*, several *Lactobacillus* species, *Scardovia inopinata* and *Rothia dentocariosa*.\(^{70}\) These studies indicated differences in microbiota with lesion progression from caries-free sites to WSL and to cavities, with increasing detection of *S. mutans*, lactobacilli and bifidobacteria with disease, although *S. mutans* was not found in all carious sites. These data illustrate that in the caries process, the entire microbiome is reshaped with changes in the relative proportion of essentially all species.
The effect of pH on the dentin microbiome was recently explored in a study of the association between lesion activity, pH and depth.52 The results of this study are shown in FIGURE 3. The three panels present the changes in microbial profile looking at phylum-, genus- and species-level identifications. Increased Lactobacillus levels, but not S. mutans or S. sobrinus, correlated with a lower pH (FIGURE 3). Lactobacilli were associated with pH 4.5-5.0 (p = 0.0003) whereas Prevotella species were detected at higher levels at pH 5.5-6 (p = 0.042).52 Higher bacterial counts were observed in surface and superficial dentinal layers compared to deeper dentinal layers, whereas surface and shallow lesion layers were more acidic than deeper in dentin.52 Lower microbial diversity was observed with increased dentin acidity.71 The
more acidic dentin had high proportions of *Lactobacillus* and *Atoptobium* (in Actinobacteria) species whereas in less acidic lesions the microbiome was more diverse. This finding is consistent with the observation that the caries microbiota can be less diverse than that of caries-free sites, suggesting suppression of the acid sensitive bacteria in the low pH of active lesions. Different pH profiles from surface to deeper layers of dentin was observed between individuals, which likely reflects different microbial complexes associated with lesions of varying activity. The pH of active lesions at pH 5.5 ± 0.3 was lower than that of arrested lesions pH 6.1 ± 0.02 (p < 0.05) in keeping with the acid theory of dental caries.

Understanding Not Only Who’s There, But What They Are Doing

The Human Microbiome Project was interested in finding out not only which bacteria were present at various body sites, but also to begin to understand what they were doing at the molecular level. Answering this biological question is the next major step in understanding the microbiomes role in human health and disease. The major way researchers look at what cells (either bacterial or host) are doing is to look at gene expression or which proteins the cells are actively making. This type of study is called transcriptomics and is performed by sequencing the messenger RNA. In all cells, genes are coded in the DNA. Genes that the cell needs are transcribed from DNA into messenger RNA. The RNA message is translated on the ribosome where amino acids are assembled into proteins. Metatranscriptomics is simply looking at the transcriptome of many organisms at a time, such as all the bacteria in a plaque sample. Metabolomics is used to determine the metabolic activity of the microbiome and is another approach to examine what the bacteria are doing. These new meta-approaches as applied to dental caries were reviewed in depth by Nyvad, Crielaard, Mira, Takahashi and Beighton.

The association between low pH and caries was established by Stefan in 1944 and repeated for initial caries in van Houte’s laboratory. Metagenomic analysis indicated increased mixed-acid fermentations in caries compared with caries-free biofilms. In contrast, there was more enzyme activity in caries-free than caries subjects suggesting that acid production from caries-associated microbiomes could be destroying enzyme activity needed to maintain health. Two critical biofilm enzymes associated with health are urease and arginine deiminase that produce ammonia and can raise the biofilm pH. Clinically, caries-free children and adults had higher arginine deiminase levels than those with caries. Using a combination of metabolomics and metatranscriptomics several new pathways were found for counterbalancing low pH and data suggested that ammonia production from arginine deiminase might be active in initial lesions, whereas urease activity might balance the acidic microbiota of more advanced disease. Study of metabolomics in twins noted higher levels of arginine and lysine in caries-free compared with caries children. These studies indicate the importance of knowing microbiome activity as well as composition and the value of studying health in addition to disease particularly in relation to ammonia production to counteract dietary-associated pH drops in the oral microbiome.

Analysis of the Spatial Organization of Plaque

Cultural and molecular methods have indicated which bacteria are present in carious lesions but not their spatial organization. Microscopy can show the spatial arrangement of bacteria, but usually without identification of the bacteria imaged. Fluorescence in situ hybridization (FISH) uses fluorescently labeled gene probes to identify specific bacteria. In Figure 4, FISH confocal microscopy was used to examine bacteria in association with occlusal caries. The microbiome in cavities included *Lactobacillus* and *Bifidobacterium* species and both these acidogenic, acid tolerant bacterial groups were observed invading tubules in keeping with the microbiota identified from dentin caries. *S. mutans* was detected in all carious sites, whereas bifidobacteria were observed only in active lesions further confirming the importance of these species in caries pathogenicity. Other species examined were not directly associated with lesion sites, for example *Fusobacterium* species were observed in a zone between lesion-associated biofilm and a looser surface biofilm mass. This observation concurs with the hypothesis that fusobacteria can bridge tooth adherent and other bacteria in gingival biofilms. *Veillonella* species and *Streptococcus mitis* were observed in surface biofilm layers suggesting that they did not have a direct cariogenic role in lesions. Overall, these observations concur with the major species detected by molecular and cultural assays. This
FIGURES 4. Bacteria in situ in carious lesions. A–L are images of biofilm, including caries-associate biofilms on occlusal surfaces, stained with situ hybridization (FISH) probes. The red stain is general bacterial stain. Specific species or genus probes are for Streptococcus yellow-green (A–E, J); Veillonella purple-magenta (A); Fusobacterium purple-magenta (B); S. mitis turquoise (C); Actinomyces purple-magenta (D); Lactobacillus purple-magenta (F, J, L); Bifidobacterium purple-magenta (G, K); S. mutans yellow-green (H, J). From: Dige I, Gronkjaer L, Nyvad B. Molecular studies of the structural ecology of natural occlusal caries. Caries Res 2014;48:451-460.
microscopy study provides a significant addition to caries microbiology in that specific bacteria were being observed in lesions. The study was, however, limited by the relatively few probes used compared to the full diversity of the caries microbiome.

Recently, methods have been developed to label up to 28 different bacterial species or higher taxa simultaneously in the oral microbiome. The plaque microbiome can lose microbial diversity by sugar-driven acidic conditions, the oral microbiome is sufficiently stressed for the roles of species, whereas there is increasing evidence for the multitudes streptococci and lactobacilli species, whereas there is increasing evidence for the roles of Actinomyces, Bifidobacterium and other Gram-positive rod species as important in the community. Health-associated biofilms normally produce ammonia and other bases, which raise biofilm pH and counteract bacterial driven tooth demineralization. However, if the oral microbiome is sufficiently stressed by sugar-driven acidic conditions, the microbiome can lose microbial diversity and be converted to a pathogenic acidogenic and aciduric microbiota.

We now understand caries as a process involving not just one or a few pathogens, but rather involving the interactions of all the microbes in the oral cavity, including health-associated commensals, with each other, the host tissues and the host response systems. Our newly increased ability to analyze the microbiome composition and function adds the possibility of developing powerful tools for risk assessment and treatment planning.

**Conclusions**

Dental caries manifests in several clinical forms with differences in the composition of the microbiome based on patient age, site of carious lesion and stage in progression. Caries results from the combined activity of the microbial community, which is altered by exposure to local acid production as a result of excessive intake of dietary sugars and carbohydrates. There are well-known and newly recognized species that have important roles in caries. Traditional caries-associated species include the mutants streptococci and lactobacilli species, whereas there is increasing evidence for the multitudes streptococci and lactobacilli species, whereas there is increasing evidence for the roles of Actinomyces, Bifidobacterium and other Gram-positive rod species as important in the community. Health-associated biofilms normally produce ammonia and other bases, which raise biofilm pH and counteract bacterial driven tooth demineralization. However, if the oral microbiome is sufficiently stressed by sugar-driven acidic conditions, the microbiome can lose microbial diversity and be converted to a pathogenic acidogenic and aciduric microbiota.

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Uncultured Members of the Oral Microbiome

William Wade, BSc, PhD; Hayley Thompson, BSc, PhD; Alexandra Rybalka, BSc, PhD; and Sonia Vartoukian, BDS, FDS, PhD

ABSTRACT

Around one-third of oral bacteria cannot be cultured using conventional methods. Some bacteria have specific requirements for nutrients while others may be inhibited by substances in the culture media or produced by other bacteria. Oral bacteria have evolved as part of multispecies biofilms, and many thus require interaction with other bacterial species to grow. In vitro models have been developed that mimic these interactions and have been used to grow previously uncultivated organisms.

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Conflict of Interest Disclosure: None reported.

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Conflict of Interest Disclosure: None reported.

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Conflict of Interest Disclosure: None reported.

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Conflict of Interest Disclosure: None reported.

The human mouth is heavily colonized by microorganisms with all of the different types represented: bacteria, archaea, fungi, protozoa and viruses. This review will focus on the bacteria because of their importance in the common dental diseases, dental caries and periodontal diseases, and because the phenomenon of unculturability has been extensively investigated in bacteria. It has long been realized that not all bacteria that can be seen under the microscope can be cultured in the laboratory. An early estimate of oral bacterial culturability was that only around half could be grown.1 Recent advances in culture have modified this estimate so that it is now considered that around two-thirds of oral bacteria can be cultivated.2 Although uncultivated bacteria cannot be grown on commonly used laboratory media, they clearly compete well in the bacterial communities found in the mouth and many are associated with oral disease. At present, their role in pathogenesis and contribution to antimicrobial resistance is unknown, which is why there is substantial interest in culturing uncultivated bacteria and subjecting them to detailed phenotypic and genomic analysis.

The aim of this review is to list the uncultivated members of the oral microbiome, discuss the reasons why some bacteria are difficult to culture in vitro and describe recent advances in culturing previously uncultivated oral bacteria.

Definition of “Unculturability”

Clearly all bacteria that can be detected on Earth have grown at some time. Culturability is, therefore, a relative term and dependent on the conditions used to encourage growth in a particular experiment. A distinction should also be made between growth in monoculture and as part of a mixed community. The explosion in culture-independent studies has revealed huge numbers of novel bacterial taxa, the majority of which cannot be identified as belonging to...
previously cultivated and characterized species. This does not mean, however, that all of these taxa cannot be cultured. Indeed, a comprehensive cultural analysis of the microbiota of severe early childhood caries revealed 45 species-level taxa that, at that time, had not been cultivated. In addition, it is often forgotten that in microbiome surveys, only DNA is detected, not living cells, and the detection of an organism’s DNA does not necessarily mean that the organism was viable at the time of sampling. Additionally, DNA extraction and PCR reagents are frequently contaminated with DNA from environmental bacteria and can make up a significant proportion of amplicon libraries, particularly when samples are taken from sites with low bacterial levels. A practical definition of unculturability will be used for this review. An organism will be regarded as uncultivated if there are no reports that it has been grown in previous cultivation studies.

Uncultured Oral Bacteria

When culture-independent methods were first used to study the composition of the oral microbiota and compared to cultural analyses of the same samples, it was clear that a substantial number of bacterial taxa could not be readily cultured. The Human Oral Microbiome Database (HOMD, homd.org) lists the bacteria found in the mouth. Many species-level taxa have yet to be named and are, therefore, assigned human oral taxon (HOT) numbers. HOMD release 13.2 includes 210 species-level taxa that have yet to be cultured. Many uncultivated taxa belong to genera whose members are predominantly cultivable. Because still relatively few oral bacteria have been cultured and identified by 16S rRNA gene sequence analysis, it is possible that these taxa are cultivable but representative strains have not yet been

![Phylogenetic tree showing uncultivated species-level oral taxa within the phylum Bacteroidetes. Tree prepared by the neighbor-joining method from a distance matrix constructed using the Jukes-Cantor algorithm and an alignment of 998 bases. Sequences representing type species of relevant genera are included for reference and colored red.](image-url)

**FIGURE 1.** Phylogenetic tree showing uncultivated species-level oral taxa within the phylum Bacteroidetes. Tree prepared by the neighbor-joining method from a distance matrix constructed using the Jukes-Cantor algorithm and an alignment of 998 bases. Sequences representing type species of relevant genera are included for reference and colored red.
encountered. Thus, the common genera Actinomyces, Prevotella, Streptococcus and Veillonella all include such taxa.

In contrast, many groups of uncultured taxa cluster in deep branches of the phylogenetic tree with no or few cultivated neighbors. **Figure 1** shows a phylogenetic tree of the uncultivated members of the phylum *Bacteroidetes*. It can be seen that *Bacteroidetes* genera G-3 and G-7 comprise a branch of six uncultured species-level taxa of which *Bacteroidetes* [G-7] HOT-281 is the most commonly detected taxon, while *Bacteroidetes* genera G-4 and G-5 constitute another deep branch with four uncultivated taxa. Similarly, the phylum *Firmicutes* includes a number of deep-branching lineages made up of uncultivated taxa (**Figure 2**). These include a major branch of the *Peptostreptococcaceae* with seven uncultivated taxa from five genera, four uncultivated taxa within the *Ruminococcaceae* and a number of uncultivated representatives of the *Lachnospiraceae*, *Syntrophomonadaceae* and *Veillonellaceae*.

The *Fusobacteria* phylum includes an uncultured branch consisting of *Fusobacteria* [G-1] with two taxa: HOT-210 and HOT-220, and one comprised of uncultivated taxa from five genera, four uncultivated taxa within the *Ruminococcaceae* and a number of uncultivated representatives of the *Lachnospiraceae*, *Syntrophomonadaceae* and *Veillonellaceae*.

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A substantial number of spirochetes have yet to be cultured. All oral
spirochetes belong to the genus Treponema, and of the 49 oral Treponema taxa, only 14 have been cultured. In particular, one branch of 10 taxa has no cultivable representatives: HOT-250-256, HOT-508, HOT-517 and HOT-518. The recently described phylum Synergistetes, which includes a large number of uncultured taxa, has no representatives. The majority of Cluster A taxa have yet to be cultured, while Cluster B includes the recently described species Jonquetella anthrophi and Pyramidobacter piscolens.

Until recently, the phylum Chloroflexi had no cultivated representatives among the oral microbiota, although environmental relatives have been cultivated. Three strains of Anaerolineae bacterium HOT-439, an important taxon thought to serve as a biomarker for periodontitis, have been isolated from subgingival plaque samples and found to grow with the help of Fusobacterium nucleatum. Three oral phyla have no, or very few, cultivated representatives: GN02, TM7 and SR1. Candidate division GN02 was first described to comprise a group of sequences identified in a study of the Guerrero Negro hypersaline microbial mat. Three oral taxa are found: HOT-871, HOT-872 and HOT-873, representing two class-level taxa. Interestingly, four related taxa were identified among the canine oral microbiome. Little is known regarding the genetic potential or functional capability of this group of organisms, although its ubiquity, albeit at low levels, suggests that it deserves to be the target of future studies.

Saiharbibacteria appear to be associated with oral disease, particularly those conditions associated with a mature anaerobic biofilm.

member of the oral microbiome, and typically makes up around 0.01 percent of clone libraries. At this level, it will be challenging to detect individual cells by fluorescence in situ hybridization (FISH) or attempt direct isolation. Three species-level taxa of SR1 have been identified among the canine oral microbiome, but were only detected when a specific Bacteroidetes-TM7-SR1 primer was used.

The TM7 candidate division was named with reference to the Torf, mittlere Schicht, or peat, middle layer, in which it was first detected in a German peat bog. Subsequently, members of this division have been isolated from a wide range of environments including waste water and batch reactor sludges, fresh and sea water, and soil. The name Candidatus Saccharibacteria has recently been proposed for organisms formerly described as TM7.

Saccharibacteria are found in a range of animals. In invertebrates, they have been detected in the microbiota associated with sponges and corals, termites and nematodes. They form part of the intestinal microbiota of mammals and appear to be a consistent member of the mammalian gut microbiome, having been found in mice, cattle, dogs, pigs, elephants, gazelle, bighorn sheep, takin, buffalo, bonobo and gorillas.

In humans, Saccharibacteria have been detected in several habitats, including the intestinal tract, skin, vaginal fluid and oral cavity, although they typically make up less than 1 percent of the community at a given site. Saccharibacteria appear to be associated with oral disease, particularly those conditions associated with a mature anaerobic biofilm. For example, Paster et al. found 34 sequences representing the Saccharibacteria division among 2,522 cloned 16S rRNA genes from the subgingival plaque of healthy subjects and patients with periodontal disease, which were later identified as oral taxa HOT-346, HOT-347, HOT-349, HOT-355 and HOT-356. Of these, only HOT-346 was found in health, while HOT-356 (represented by phylotype I025, now recognized to belong to HOT-356) was associated with periodontitis, a finding confirmed in a study using oligonucleotide probes specific for HOT-356, in which the taxon was found in 50 percent of healthy subjects and 83 percent of patients with periodontitis. The same trend was seen in a polymerase chain reaction (PCR)-based study, with HOT-356 being detected in 91 percent of diseased sites and 71 percent of controls, although the difference was not statistically significant. In refractory periodontitis, Saccharibacteria HOT-346, HOT-356 and HOT-437 were detected in significantly higher proportions than

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in patients whose periodontal treatment was successful or healthy controls in a study using the Human Oral Microbe Identification Microarray (HOMIM).49 Three Saccharibacteria phylotypes were found in oral samples collected from subjects with halitosis and one of them, HOT-352, was significantly associated with the condition.45

The culture of members of the division Saccharibacteria has long been a goal. It was reported that Saccharibacteria had been successfully cultured after 50-day aerobic incubation of low-nutrient solid media, with microcolonies visible to the naked eye.50 No further detail of this isolation has been reported, however. There have been a number of reports of the successful isolation of Saccharibacteria bacteria in mixed culture. For example, microcolonies of Saccharibacteria from soil were obtained using a soil substrate membrane system.51 After seven days incubation, several morphotypes were detected by means of FISH with the TM7-905 probe, although no pure cultures were obtained. Using the same method, Abrams et al.52 reported the isolation of microcolonies that included cells that reacted positively with the TM7 probe, although no pure cultures were obtained. Using the same method, Abrams et al.52 reported the isolation of microcolonies that included cells that reacted positively with the TM7 probe, although no pure cultures were obtained. Rybalka53 found that Saccharibacteria could be isolated in mixed culture with a variety of other species, including Slackia exigua and Atopobium parvulum, but could not be isolated in pure culture or even maintained as a mixture for more than a few subcultures.

The successful isolation of a pure culture of a Saccharibacteria phylotype was reported from a sample of dental plaque but a culture was not deposited with a culture collection.54 A Saccharibacteria strain successfully isolated and maintained from saliva, TM7x, was an extremely small coccus found in an exclusive physical and parasitic relationship with a strain of Actinomyces odontolyticus.55 It would appear then that the Saccharibacteria isolates studied thus far are only found in such close associations with other bacteria. It was further reported that association with TM7x caused the A. odontolyticus host to change its morphology from relative short rods to filaments,56 although the growth phase and natural morphological variation of both partners in the interaction requires further investigation. The TM7x genome was found to be small at 705 kb and completing lacking

\[\text{Strictly anaerobic bacteria coexist with oxygen-consuming and -tolerant species and cooperate to protect each other from atmospheric stress.}\]

in amino acid biosynthesis capability, perhaps explaining its need to parasitize other bacteria. Small genomes are a feature of a number of other Divisions yet to be cultured, including SR1, OD1 and WWE 3,57 suggesting that a limited metabolic repertoire and dependence on association with other organisms may be common features of phylum-level taxa with no or few cultivable representatives.

**Reasons for Unculturability**

If bacteria are able to grow in a particular environment but we are unable to cultivate them in the laboratory, then clearly at a basic level we are unable to reproduce the conditions that they need for growth. Understanding these conditions is key to the cultivation of previously uncultivated organisms.

Atmospheric conditions, particularly the presence or absence of oxygen, as well as the availability of CO2, are obviously extremely important. Some bacteria require specific nutrients for growth. Methanoseta species, for example, are obligately acetotrophic; hence, the addition of acetone to media, which is slowly converted to acetate, will promote the growth of these otherwise slow-growing species.58

Sometimes the medium itself can be toxic. It has been shown that autoclaving agar-containing culture media in the presence of phosphate can generate inhibitory levels of hydrogen peroxide, an effect that can be avoided by replacing agar with gellan gum.59

Oral bacteria typically live as part of a multispecies community in densely packed biofilms. Within the biofilm, there are a variety of gradients of nutrients, signaling molecules and gases, due to the diffusion patterns of these substances and the metabolic activity of neighboring bacteria, such that conditions for individual cells, and groups of cells, can vary markedly.60,61 Despite the mouth being exposed to the atmosphere, about half of oral bacteria are obligate anaerobes. As oral biofilms develop, obligate aerobes and facultative anaerobes rapidly reduce the local oxygen concentration; four days of plaque formation in vivo in two subjects produced a mean redox potential at the tooth surface of –127 mV.62 Strictly anaerobic bacteria coexist with oxygen-consuming and -tolerant species and cooperate to protect each other from atmospheric stress.63

In a similar way, there will be a concentration gradient within the biofilm for nutrients with their concentration decreasing with increasing depth of biofilm, while bacterial metabolic products will be increased. There can be direct interactions between species.
with one using the end products of another for growth. For example, Veillonella species use lactate produced by streptococci as a major carbon source.64

Other possibly important factors in growth-regulating interactions between bacteria are bacterial signaling molecules. Gram-negative bacteria communicate by means of acyl homoserine lactones (AHLs),65 and Gram-positives use small diffusible peptides,66 but both systems are primarily intraspecies. In contrast, autoinducer-2 (AI-2), the product of the luxS gene, has homologues in a wide variety of organisms, both Gram-positive and Gram-negative and has been suggested to act across taxonomic boundaries.62 Bacterial signaling affects a number of functional aspects including expression of the biofilm phenotype, production of virulence factors and growth itself. Bacteria accustomed to growing in biofilms may thus require the presence of exogenous signals for growth. For example, a small, 5-amino acid peptide, thought to be a signaling molecule stimulated the growth of a previously uncultivated Psychrobacter strain.68

Resuscitation-promoting factor (Rpf) is a protein that was identified as being able to revive Micrococcus cells from dormancy.69 Rpf is structurally similar to lysozyme70 and cleaves peptidoglycan. Rpf, therefore, likely to generate peptidoglycan fragments from the cell walls of intact bacteria, which might act as signaling molecules.71 Muropeptide fragments are known to have signaling properties in Bacillus subtilis, where they bind to PrkC, a serine/threonine kinase on the cell surface.72 A specific muropeptide, a disaccharide-tripeptide with a meso-diaminopimelic-acid residue, typically found in Gram-positive bacteria is necessary for this activity. Rpf then may function by producing muropeptides from peptidoglycan with signaling, possibly growth-stimulating properties. Further work is required to investigate if this is a general method of growth regulation.

### Methods for Culture of Uncultured Bacteria

A number of approaches have been taken in attempts to culture previously uncultured bacteria and these are summarized in the **TABLE**. Perhaps the most promising approach is the recognition that bacteria in nature frequently live in multispecies biofilms and are, therefore, in chemical contact with other bacteria. If chemical interactions can be maintained in vitro, then isolation of novel organisms should be possible. For example, D’Onofrio et al.73 grew seawater sediment bacteria in vitro, then isolation of novel organisms was possible. For example, D’Onofrio et al.73 grew seawater sediment bacteria on agar plates in mixed culture at various dilutions. Because disproportionally more colonies were seen on plates that had been heavily inoculated, pairs of colonies growing within 2 cm of each other were subcultured and then grown together. Around 10 percent of these pairs showed evidence of the growth of one organism being dependent on its pair. The growth of many of the dependent isolates was

<table>
<thead>
<tr>
<th>Approach to cultivation</th>
<th>Examples</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media supplementation, customization or modification</td>
<td>Addition of supplements to media e.g., siderophores, N-acyl homoserine lactones or growth factors; design of media selective for specific bacterial taxa using the SMART method; modified media preparation methods/substitution of agar with gellan gum as gelling agent, to limit growth inhibition by hydrogen peroxide.</td>
<td>14, 59, 68, 73, 74, 91, 92</td>
</tr>
<tr>
<td>Modification of growth conditions</td>
<td>Modified temperature, pH, O2 presence/absence, incubation time, gravity.</td>
<td>93, 94</td>
</tr>
<tr>
<td>Modification of sample handling</td>
<td>Dilution-to-extinction to achieve single-cell isolation; small inoculum for reduced microbial competition.</td>
<td>77, 78</td>
</tr>
<tr>
<td>Simulated natural environment</td>
<td>Diffusion chamber incubated within the natural environment allowing passage of growth-stimulatory chemical compounds across a membrane; hollow-fiber membrane chamber for in situ cultivation in the natural environment; l-tip in situ cultivation device permitting inward diffusion of natural chemical factors.</td>
<td>89, 95, 96</td>
</tr>
<tr>
<td>Microfluidic device</td>
<td>Encapsulation of subsets of the microbial community to form microdroplets that are exposed to signals or nutrients from external bacteria.</td>
<td>97, 98</td>
</tr>
<tr>
<td>Community culture and co-culture</td>
<td>Bacterial culture facilitated by chemical components produced by the main bacterial community separated from the target organism by a membrane, transwell insert or a well within the media plate; growth of bacteria in consortia, followed by detection and enrichment of specific bacterial targets using colony hybridization; co-culture of bacterial strains with “helper” species on which they depend for provision of growth factors or for environment modification.</td>
<td>14, 99–103</td>
</tr>
<tr>
<td>High-throughput methods</td>
<td>I chip: A device comprised of hundreds of miniature diffusion chambers, within each of which a single cell is cultured; hollow-fiber membrane chamber device (see above) comprised of 48-96 chamber units.</td>
<td>96,104</td>
</tr>
</tbody>
</table>

**TABLE** Summary of Recently Described Methods for the Cultivation of Difficult-to-Culture Bacteria
stimulated not only by its co-culture partner but also by Escherichia coli. A panel of E. coli mutants was, therefore, constructed and tested to determine the identity of the substance produced by E. coli that was stimulating the growth of the dependent isolates. Enterobactin, a siderophore, was found to be responsible and adding siderophores to culture media allowed a number of novel bacteria to grow.

A novel system has been developed that explores genomic data for information on specific carbon source requirements and antimicrobial resistance of particular bacteria, leading to the development of highly selective media designed by SMART — selective medium-design algorithm restricted by two constraints.74 Using this method, the authors prepared “selective” media for five plant-pathogenic bacteria and demonstrated accurate selection for the target bacterial species among a panel of 18 strains representing 10 species. The use of such systems may provide a rational basis for the development of novel culture media.

A range of compounds with siderophore activity have been screened for their ability to stimulate the growth of oral bacteria that are unable to grow in pure culture.14 Growth of Prevotella HOT-376 was more strongly stimulated by the siderophore pyoverdines-Fe, than by a culture filtrate of its helper Fusobacterium nucleatum (the positive control); to a lesser extent, it was also stimulated by ferric citrate, desferricoprogen, ferrichrome-Fe-free and salicylic acid. Likewise, growth of Fretibacterium fastidiosum of Synergistetes cluster A was consistently stimulated by desferricoprogen, salicylic acid and ferrichrome-Fe-free. Consequently, media used for culture of heavily diluted samples of subgingival plaque, were supplemented with siderophores pyoverdines-Fe or desferricoprogen, or a neat suspension of subgingival plaque, which led to the successful isolation of several previously uncultivated bacterial strains, including Chloroflexi taxon Anaerolineae bacterium HOT-439.14

One method of achieving a pure culture of a slow-growing organism is the dilution-to-extinction method whereby dilution ensures that single cells are placed in a growth medium and have time to grow without being inhibited by other bacteria.75,76 A high-throughput version of the method was successful in cultivating a number of novel strains of the seawater organism SAR11 as well as representatives of the abundant, but previously uncultured, SAR116 clade.77 Dilution to extinction would appear to be a method most suited to samples such as seawater where bacterial concentrations are relatively low and the bacterial cells are found primarily in planktonic suspension and interactions between bacteria are, therefore, limited. The oral microbiome, conversely, is primarily made up of dense biofilms where this method may be less generally applicable, although some novel taxa have been recovered using this method.78

Culture-independent surveys have made available 16S rRNA gene sequences for the microbiomes studied. These data can then be used to design specific oligonucleotide probes which can be used in FISH to visualize uncultured bacteria in samples of biomass.79,80 Thus, even though an organism cannot be grown, its morphology can be determined. This has been successfully performed for TM7, Tannerella BU063 and Synergistetes cluster A.10,21,18 An alternative labeling method is to use antibodies if it is possible to select an appropriate specific antibody. Because antibody labeling, unlike DNA probing, is nonlethal, it can be possible to obtain viable cells for culture, after sorting as described above; in this way fluorescent antibodies have been used to obtain viable cells after sorting.82 Flow cytometry can be used to isolate single cells from mixtures from which whole genome sequences can be obtained; this approach has been successfully used to sequence genomes of the health-associated taxon related to Tannerella forsythia, HOT-286 (BU063).83

Mixed primary cultures frequently include representatives of bacterial species not yet cultured in isolation. Colony hybridization is a useful method for determining the location of specific taxa on solid media.84 By membrane blotting and the use of specific probes, target organisms can be localized to specific regions of replica plates, allowing their subculture and enrichment. This method was used to culture the first representative of Synergistetes cluster A: Fretibacterium fastidiosum from subgingival plaque in periodontitis.85,86

The isolation of previously uncultivated bacteria may be a multi-stage process. Clearly, the agar plate is an alien environment for bacteria used to living in biofilms associated with mammalian tissues. One approach has been to establish biofilms in vitro, seeded with natural inocula. The Calgary Diolfilm Device, a microplate-based system with plastic pegs coated with hydroxyapatite to mimic the tooth surface, has been successfully used to produce dental
plaque biofilms. Saliva was used as the inoculum and biofilms with a composition resembling dental plaque could be reproducibly established. Next-generation sequence analysis of the biofilms showed that they included representatives of uncultured oral bacteria and one of these, Lachnospiraceae HOT-500, was successfully isolated following colony hybridization enrichment. Mimicking natural conditions in a similar fashion, Jung and co-workers developed the T-tip method as an in vitro cultivation device using the natural environment as a source not only of the bacterial community, but also of the associated chemical compounds. They cultivated from Baikalian sponges a greater range of bacterial strains using this method than by conventional plating. Bacterial communities can even be cultured in vivo by means of a device where an agar substrate is placed in a chamber separated from the oral environment by a membrane. Bacteria can grow on the agar while in chemical communication with their natural environment. This method was found to be of value for the culture of previously uncultivated oral bacteria and complementary to dilution to extinction and conventional plating.

Future Prospects

A number of approaches for the cultivation of previously uncultivated oral bacteria have been developed and successfully used to isolate representative strains. Progress has been slow, however, with only a small number of new species cultivated. Efforts should be directed toward developing high-throughput methods of detecting the growth of novel organisms. The relative ease in obtaining genome sequences both of individual isolates and from shotgun metagenomic analysis of communities should provide information to guide the provision of nutrient substrates and potential growth-promoting signaling molecules. The culture of an organism remains the key factor in determining its characteristics, including the production of virulence factors and resistance to antimicrobials.

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Some practice owners are unaware that many insurance companies, including The Dentists Insurance Company, have the right to inspect malfunctioning equipment in order to determine the cause of failure. Under most policies, some causes are covered, while some are not. The burden of proof to establish that the cause of the loss is covered rests with the insured.

“It is a policy requirement that we are provided an opportunity to inspect the broken equipment, and along with this requirement is the policyholder’s responsibility to preserve the property in question,” said Sheila Davis, assistant vice president, Claims and Risk Management Claims, TDIC. “This is essential so that we can determine whether the loss is covered. Disposal of property without our go-ahead could affect your claim.”

In 2015, TDIC had a total of 446 property claims with an average value of $30,000-$50,000, not including loss of income. Most claims were due to water damage, typically resulting from the failure of a water supply line to dental equipment.

A common scenario is this: Unbeknownst to the practice owner, there is a point of weakness in the water system. Perhaps there is a loose compression fitting, a worn valve or a tiny hole in a piece of flexible tubing. At night, or over a weekend, when the water...
is not being used, the water pressure builds and the dam bursts, flooding the office.

When faced with a situation like this, practice owners need to follow certain protocols. Because of the complex nature of dental equipment, they should preserve not only the entire mechanism, but the failed parts as well.

“Each of these can usually be examined to determine why the failure occurred and which part failed,” Davis said. “But if the equipment is disposed of, then the opportunity to determine how and why the failure occurred is lost.”

Some practice owners erroneously assume the repair technician’s report can be used to obtain this information. But the reality is, most of these “reports” are just invoices; they often lack the details needed to make a determination of cause.

“We need to know exactly how the equipment malfunctioned and why it failed. Tech reports don’t generally disclose this,” Davis said.

In addition, the opportunity to recover the amounts paid in the claim from the responsible party may be lost if the cause of the damage is disposed of. For example, should an insurance carrier determine the loss was caused by a manufacturer’s defect, the manufacturer would have a right to inspect the equipment independently. If there is no equipment to inspect, it is difficult, if not impossible, to hold the at-fault party accountable.

In some cases, practice owners don’t want the equipment taking up precious office space, nor do they know what to do with the broken equipment once a claim is in process. But more often than not, technicians will be happy to return for the equipment in a few days, after the insurance representative has had a look.

“For any type of equipment breakdown, it’s better to err on the side of caution and keep the equipment. In most cases, we can send out someone to inspect the equipment or failed component the same day or the following day,” Davis said.

In one recent case, a dentist experienced the failure of her vacuum. Knowing she couldn’t afford to close her practice during the claims process, she replaced it, storing the broken one onsite. TDIC was able to get an inspector out to her practice right away, and she was able to continue seeing patients while her claim was being processed.

“We understand that you can’t afford to have downtime,” Davis said. “But by calling us in tandem with calling a technician, and by preserving your old equipment, you can ensure your claim will be processed smoothly.”

Experiencing an equipment breakdown is an unfortunate reality of the dental profession. As a practice owner, the steps you take during this time can mean the difference between a smooth recovery or a complicated one. By following a few simple protocols, you can get back to business quickly and painlessly.

TDIC’s Risk Management Advice Line at 800.733.0634 is staffed with trained analysts who can answer property insurance and other questions related to dental practice.
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6109 NORTH LAKE TAHOE “Best-of-the-best!” Solid foundation anchored by 8.5 days of Hygiene. Consistent $1 Million per year performer. Beautiful office with unsurpassed views.

6108 SANTA ROSA 3-day practice collected $320,000. Two days of hygiene, 3-ops and 500+ active patients.

6107 EUREKA 100% out of network with the insurance industry. Produced $918,000 and collected $895,000 on 20-hour week. 7+ days of Hygiene.

6106 FOLSOM AREA No rush and no chaos here. Staff is a Dream Team. Beautiful facility. 2015 collected $640,000. Unique opportunity seeks Dentist looking for something special.

6105 MODESTO Collected $430,000+ on 3-day week. 3-days of Hygiene. 5-ops. Central location. Successor should open 4th day.


6103 SAN FRANCISCO’S UNION SQUARE Opportunity to acquire highly regarded practice with condo. Beautiful 5-ops, digital and paperless. 6th op available. 2015 collected $658,000.

6102 SAN RAFAEL 2-ops. 3-day week. 2015 collected $259,000. 2014 saw $332,000. Full price $150,000.

6100 SANTA CLARA Phenomenal launching pad for Ambitious Successor. Fantastic location, 5-op facility. Management not taking advantage of what is possible even though 2015 collected $758,000 with Profits of $323,000. Positioned to be $1 Million+ year performer immediately!

6099 FAIRFIELD Collected $500,000 in 2015. 3-days of Hygiene. 4-ops with digital and radiography.

6098 WEST PETALUMA Petaluma is now the hip business center of the North Bay! Collected $468,000 with Profits of $199,000. 3-days of Hygiene with 4th day starting September.

6096 NORTH FRESNO’S ST. AGNES MEDICAL VILLAGE 4-days of hygiene. Collected $150,000 in 2015. 4-ops with 3 upgraded. Full price $150,000.

6094 PERIO PRACTICE - SAN FRANCISCO BAY AREA Shall appeal to Perio desiring high-end practice in very desirable area. 2015 Produced $1.25 Million, collected $1.25 Million and realized Profits of $690,000 on 3-day week.

6089 MOUNT SHasta Small town living-renowned for outdoor lifestyle. Escape from Rat Race and corporate intrusion. 3-day week collected $900,000. Strong Profits.

6070 VISALIA This practice is well positioned for its next caretaker. Strong Hygiene Department, beautiful facility, well equipped. Digital throughout. Collected $727,000 on part-time schedule in 2015. Extend hours and be busier. Best location!

ANTELOPE VALLEY Has grossed $1.8 Million. Fantastic location. 60,000 autos pass by per day. 8 ops. Partnership for $250,000 or buy all.

ARCADIA Facility only. 3-ops equipped. $65,000 or $95,000 with Ortho.

BAKERSFIELD AREA 5-ops, next to McDonalds. 1,800 sq.ft. includes building. Grosses $40,000/month. Full Price with building $350,000.

BAKERSFIELD Established 55 years. 5-ops in 3,000 sq. ft. Will do $1 Million. Full Price $300,000. Building available for $350,000.

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INDIO 4,000 sq.ft. dental building. Full Price $650,000.

LADERA RANCH GROSSING $650,000. Shopping center location.

LAGUNA NIGUEL Location, location, location! 4-ops with Panorex. Full Price $185,000.

LA JOLLA Established 20-years. 3-ops. Grossed $150,000. Super opportunity with immediate growth. Full Price $150,000.

LAWNDALE Hi identity. 2 ops. Full price $125,000.

LOS ANGELES HMO GROSSING $1.2 Million. 5-ops. Full Price $1.2 Million.

LOS ANGELES HMO Does $4 Million. Full Price $1 Million.

NORCO – CORONA Will do $1.5 Million. 8-ops. Exquisite. Full Price $1.2 Million.

NORWALK Fantastic high identity location. 5 ops. Full Price $250,000.

ORAL SURGERY PRACTICE – LOS ANGELES Established 40 years. ORANGE Beautiful 10 operatory office ready for merger.

PASADENA Established 60 years. 7-ops. Always $1+ Million. Full Price $600,000.

REDLANDS Shopping center. Grosses $350,000. Full Price $250,000.

RIVERSIDE Facility only. 4 ops. Full Price $50,000.

SOUTH ORANGE COUNTY BEACH CITY Grosses $650,000. 4 ops. Beautiful!

PERIO PRACTICE - PRESTIGIOUS BEACH CITY Established 40 years.

TORRANCE Established 12 years. 5 star building. 3-ops. GROSSING $250,000. Full Price $195,000.

TUSTIN Dental building. Full Price $1.5 Million.

VENTURA - OXNARD 5-ops. GROSSING $850,000. High identity. Full Price $685,000.

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The U.S. Department of Health and Human Services (HHS) released in March 2016 a guidance document and FAQ on the Health Insurance Portability and Accountability Act of 1996 (HIPAA) requirements for providing a patient with access to his or her record. This article highlights some of the key points in the guidance, which is found at hhs.gov/hipaa. More Q-and-As are included in the CDA Practice Support resource, Patient Request to Access Records (Records Release) Form and Q-and-As, which are available at cda.org/practicesupport.

HHS provided new information on the following topics:

- Fees, particularly limits on what can be charged.
- Avoidance of unreasonable delay to patient.
- Access in the form or format of the patient’s choosing, including unencrypted email.

What does “right to access record” mean?
It means a health care provider must:

- Allow a patient to inspect his or her record.
- Provide a copy or summary of the record if requested by the patient.
- Transmit a copy of the record to a person or entity of the patient’s choosing. Requests for this type of access must be written.

Must the access request be in writing?
State law requires health care providers to comply with written requests for access, but does not expressly require only written requests. A HIPAA-covered entity may require a request be written and that its own form be used. The requirement to use a written access request must be noted in the covered entity’s Notice of Privacy Practices. A covered entity may offer electronic options for making the request (for example, a web portal or email), but it cannot require the use of those options. Any requirement to use a covered entity’s form may not create a barrier or unreasonably delay a patient from obtaining access.

What is considered to be the patient’s record?
HIPAA gives a patient the right to review or obtain a copy of his or her information maintained in a covered entity’s “designated record set.” The designated record set is that group of records maintained by or for a covered entity that is used, in whole or part, to make decisions about an individual, or that is an entity’s billing and payment records for that individual. The designated record set...
may include information generated by other health care providers that is maintained by the covered entity.

The record includes images, impressions and models if they have been used to make decisions about an individual's treatment.

What may I charge?

The HHS guidance made clear that the fee for access may include only the cost of:

■ Labor to make the requested copy, whether in paper or electronic form.
■ Supplies such as paper or portable electronic media.
■ Postage when the patient requests the copy or summary be mailed.
■ Preparation of an explanation or summary of the record if requested by the patient.

A covered entity may either calculate actual labor costs to fulfill a request or develop a fee schedule based on average labor costs to fulfill a request.

The fee may not include costs associated with verification of the request, documentation, searching for and retrieving the record, maintaining systems, recouping capital for data access, storage or infrastructure, or anything not included in the above paragraph. A per-page fee may not be charged for records maintained electronically. If a dental practice collects fees, it should prepare a document listing the fees and provide it to the patient with the Patient Request to Access Records form.

A covered entity may charge a flat fee for standard requests for electronic copies of electronic records, provided the fee does not exceed $6.50, inclusive of all labor, supplies and postage.

The fee for providing a summary must be agreed to by the patient in advance.

If a patient requires a copy of a portion of his or her record to support an appeal regarding eligibility for a public benefit program, such as Dental-Cal, the copy shall be provided by the dental office at no charge. The patient is entitled to no more than one copy free of charge, but may not be limited in the number of requests for copies.

Dental practices that are not HIPAA-covered entities must follow the state's rules and may charge no more than:

■ Twenty-five cents per page for copying paper documents.
■ Fifty cents per page from microfilm.
■ Actual cost for duplicating X-rays, photos, models, impressions, etc.
■ Actual postage cost.

In addition, such a dental practice may charge a fee based on reasonable clerical costs incurred in locating and making the records available for inspection.

What are acceptable methods of verifying the access request from a patient or patient's representative?

All dental practices must take reasonable steps to verify the identity of the person making the request for access. There is no one required method of verification. A patient may not be required to be present to make an access request. Methods of verifying identity include:

■ Checking identification of the individual making the request in person.
■ The emailed request was sent from the same address as the one collected from the patient at the first appointment.
■ Signature and information on a written request matches that in the record.

The patient is requesting an electronic copy, but I keep paper records. Am I required to provide an electronic copy?

If the dental practice is a HIPAA-covered entity, the answer is yes. In its March 2016 guidance, HHS clarified several issues related to the form and format of copies. Generally speaking, a covered entity must comply with a patient's request for a specific form and format unless it is not readily producible. Examples of form and format are paper, film, electronic/pdf, electronic/jpg and electronic/DICOM or .dcm.

If the form and format requested by the patient is not readily producible by the covered entity, both parties should agree on an acceptable format.

A dental practice that is not a HIPAA-covered entity is not required to provide electronic copies.

We always use a secure method to send patient information electronically. A patient is requesting we send his information to him via unencrypted email. What do we need to do to comply with the patient’s request?

A dental practice must (1) advise the patient of the risks of unsecure electronic transmission of information and (2) the patient must consent to the unsecure electronic transmission of information before the dental practice can send the information via unencrypted email.

CONTINUES ON 466
4103 SAN FRANCISCO GP
Vibrant downtown location in historic high-rise bldg. Retiring doctor offering 30+ years of goodwill. 4.5 days of hygiene, 1,500+ active patients (all fee-for-service). Beautiful, spacious shared facility in approx. 2,500 sq. ft. Seller has use of 4 ops (3 fully equipped and 1 overflow op). 2015 GR $796K. 2014 GR $768. Average adjusted net income $274K+ Asking $599K.

4085 SANTA ROSA GP & BUILDING
Practice and real estate are offered for sale in a well-established condominiumized medical/dental complex conveniently located in the heart of Santa Rosa, near Memorial Hospital. This 1,200 sq. ft. single story office comes equipped, furnished and ready for you to continue your professional career with established and new patients (approx. 750 active patients). Seller is retiring after almost 20 years but will assist for a smooth transition. Average Gross Receipts of $256K with adj. net of approx. $110K. Asking price $160K for the practice, and $270K for the real estate.

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 Dексis sensors, & Cerec Omnicam & MCXL units. Loyal & stable pt. base in charming community, w/ a small town feel. Perfect for a dentist who wants to escape the grind and live along the coastline. Avg. GR $1.4M+, 2016 on schedule for $1.5M+. Seller willing to help for smooth transition. Asking $1,041,000.

4091 HOLLISTER GP & PEDIATRIC
Country living at its best — small town community feel with affordable housing, in quaint bedroom community to Silicon Valley. General and Pediatric practice located in corner professional building on well-travelled street near Hazel Hawkins Hospital. Fully-equipped 1,600 sq. ft. office with 2 enclosed adult ops and 3 open pedo ops. Great opportunity for a turn key practice with trained staff and approximately 700 active patients. 2014 GR $228K. Seller is relocating out of the area, but will help for a smooth transition. Asking price only $125K.

4114 CONCORD GP
Retiring owner offering a well-established, Concord practice, in desirable community with 30+ years of goodwill. Strategic location with growth potential. 3 fully-equipped ops. in 836 sq. ft. facility. 5 year Avg. GR $360K+ with 2 doctor days. Owner available for a smooth transition. Asking $224K.

4115 WALNUT CREEK GP
Walnut Creek practice in gorgeous facility with recent leasehold improvements plus new and upgraded equipment. Practice has 30+ years of goodwill. Looking for a mature, experienced practitioner for a loyal and mature patient base. Located in commercial center with several amenities and marketing opportunities. Doctor works 2 days per week. Owner available for a smooth transition. Asking $432K.

4096 MENDOCINO COUNTY GP
Seller offering well est. 48 year practice. Located in outdoorsman’s paradise. Just 2 hours North of SF surrounded by redwood forest, vineyards and mountains. 950 sq. ft. office in single level building w/ 4 fully equipped ops. 2014 GR $565. Asking $300K.

4110 SANTA ROSA GP
Don’t miss this opportunity — absolutely gorgeous, state of the art office located within two major thoroughfares in the heart of Santa Rosa. Practice generating $2.1M+ in GR on 5 dr. days/week with 9 days of hygiene. 2015 Adj net over $569K. 3,000+ active patients, 9 fully equipped ops in spacious 2,950 sq. ft. Equipment includes digital x-rays, Sirona-D Cone Beam system, Cerec Blucam and Omnicam. Asking $1,436,000.

4093 SAN JOAQUIN VALLEY ORTHO
Established over 35 years with a solid reputation, near several referral sources in seller owned building. 2,500 sq. ft. office with 7 chair open bay in professional center on a well-travelled street with many retailers. Avg. Gross Receipts $763K. Seller retiring and willing to help for smooth transition. Asking $561K. The building is available to purchase as well for $608K.

4086 SILICON VALLEY PERIO
Well-established Perio practice in prime San Jose location with referral sources nearby. Located in a commercial & residential mix neighborhood with a large daytime business draw. Approx. 1,100 sq. ft. office with 4 fully-equipped ops. Well trained dedicated staff, seller retiring and willing to help for smooth transition. 2014 GR $482K+, 2015 on schedule for $539K+ as of August. Asking $295K.

4065 LAKE COUNTY GP
Seller retiring from general practice located in a slower paced, relaxed community. Plenty of hunting and fishing and out door activities for the enthusiast. Approximately, 1,600 square foot office with 4 fully-equipped operatories. Over 2,000 active patients, average $697K+ in Gross Receipts with an overhead of just 56%, and 4 doctor days per week. Asking $463K.

4105 STANISLAUS COUNTY GP
The Bay Area is getting over crowded. Get away to a less demanding commuter friendly town. Seller retiring from practice est. over 30 years ago with loyal patient base in charming community with historic small town feel. 3 fully-equipped operatories. 2014 GR $647K+ w/approx. 50% overhead. Seller willing to help for smooth transition. Asking $428K.

UPCOMING:

4116 SACRAMENTO COUNTY ORTHO

Contact:
Mike Carroll Pamela Carroll-Gardiner

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I want to transmit a patient’s information to a specialty dentist via unencrypted email — do I need to get the patient’s authorization to do so?

HIPAA allows a covered entity to share patient information with another covered entity without the patient’s authorization if the purpose of sharing the information is the patient’s treatment. HIPAA requires this information sharing be done securely, so a patient’s authorization to share the information via insecure methods is insuffi cient to waive the covered entity’s obligation. To be able to send it via unencrypted email requires the patient make an access request and direct you to send the information to the specialist via unencrypted email.

The patient requests that I mail the copy to an individual. May I ask the patient to pick up the copy instead?

No, you may not. Such a request may be viewed as a barrier to the patient’s right to access his or her record.

A new patient has requested a copy of his records from his former dentist but the dentist is refusing to provide them. What can the patient do?

Suggest that the patient submit to the other practice a written request for records plus a copy of the CDA Oral Health Fact Sheet on Patient Records or, if it is not a California practice, the HHS March 2016 guideline. If the other practice does not comply with the request, the patient can file a written complaint with the dental board and with the Department of Health and Human Services.

Who else may have a patient’s information, and under what circumstances?

Review the resource Uses and Disclosures of Patient Health Information found on cda.org/practicesupport. Requests from others for patient information for purposes not permitted without patient authorization by HIPAA or California Confi dentiality of Medical Information Act (CMIA) (California Civil Code section 56 et seq.) must be submitted on a valid authorization form that meets CMIA and HIPAA requirements. A Consent Form for Use and Disclosure of Patient Health Information is available on cda.org/practicesupport.

Situations for which a dental practice may want to use the consent form are:

- To obtain an adult child’s consent to share detailed information as many times as needed during a time period with the parents who are the payers.
- To market products or services to a patient.
- To participate in research.

Regulatory Compliance appears monthly and features resources about laws and regulations that impact dental practices. Visit cda.org/practicesupport for more than 600 practice support resources, including practice management, employment practices, dental benefi t plans and regulatory compliance.
Undertreatment, an Ethical Issue

Henrik Hansen, DDS

Most dentists may not think that undertreatment can be an ethical issue, but it can be very significant.

Let me relay a true story that was told to me in dental school. A dentist was doing an exam for his mother when he noticed a small lesion on her tongue. It didn’t look too awful and he knew if he sent her for a biopsy, it would be painful. The dentist decided to watch it, and watch it he did. Each time he saw the lesion, it got a little bit bigger, but not too bad in his eyes. However, by the time he decided enough was enough and sent her to an oral surgeon, the lesion was a full-blown carcinoma, and his mother ended up with a significant portion of her tongue removed. Luckily, it had not metastasized, but his mother was left with significant morbidity and her quality of life was diminished.

Having just completed six years on CDA’s Council on Peer Review, I have analyzed hundreds of cases from single crowns to full-mouth rehabilitation. It is common knowledge that dentists diagnose and treatment plan differently based on their training, experience and philosophy, and this is as it should be. However, Peer Review also sees treatment plans that just don’t make sense and are outside the normal range. The most common undertreatment complaints are undiagnosed periodontal disease and decay.

Referring to the CDA Code of Ethics, it’s clear that under treatment violates the ethical principles of veracity (being honest and telling the truth), beneficence (doing good for our patients) and integrity (behaving with honor and decency). When we look at the ethical principle of nonmaleficence (minimizing harm and maximizing benefit), things can become more difficult. We see patients every day with old restorations that appear serviceable and are asymptomatic. However, we know that some of the restorations will have fractures and/or decay underneath that can lead to severe consequences if not removed. On the other hand, removing the restoration may make an asymptomatic tooth “hot,” leading to further complications. What should you do? The most reasonable approach is to do a thorough exam and inform the patient of your best clinical judgment. Involve patients in the decision-making process by fully informing them in language that they can clearly understand, thus ensuring the ethical principle of autonomy.

The cause for overtreatment is usually economic, but undertreatment can be more complex. With the clarity of hindsight, I can see that when I graduated from dental school 38 years ago, I thought I was being conservative in my treatment planning; but the reality was that I had a fear of failure, and the less you do, the less you fail. Other examples of undertreatment include not adequately informing the patient about the importance of radiographs when they refuse. Being too rushed, chemically, emotionally or age impaired could result in a less-than-acceptable exam and treatment plan.

It is a difficult and uncomfortable situation for a subsequent treating dentist to inform a patient that there are problems that should have been addressed, but weren’t. This can be a very tricky discussion to have with a patient. Dentists have an ethical obligation to be honest, but not to inflame the patient by speculation or denigrating the previous dentist. It is also good practice to contact the previous dentist and have an honest discussion regarding your findings to provide him or her the opportunity to respond, as you don’t know all the facts.

We have a legal and ethical duty to our patients to diagnose and treatment plan putting their best interests first, using our best professional judgment and presenting the information in a clear and forthright manner. Patients expect and deserve no less.

Dr. Hansen is currently a member of the CDA Judicial Council, a California Delegate to the ADA House of Delegates and Vice Regent representing California for the International College of Dentists. Dr. Hansen previously served as chair of the CDA Council on Peer Review, was a trustee member of the CDA Board of Trustees, is a former member of the ADA Council on Dental Benefit Programs and is the Immediate Past Chairman of the American College of Dentists, Northern California Section. Dr. Hansen received his dental degree from the University of California, San Francisco, School of Dentistry and maintains a private practice in Fairfield, Calif.

For further guidance, contact your local ethics committee or Britney Ryan, CDA judicial council manager, at 800.232.7645.
Smoke Free (David Crane, Free)

Dental professionals are considered the first line of defense against oral cancers that may be directly caused by tobacco use. Patient education and help with establishing attainable goals have been among the most effective means to help kick the habit. Smoke Free is an app with the ultimate purpose of helping smokers quit by providing relevant and useful feedback. After entering customized information, which includes how much a cigarette pack costs, how many cigarettes are in a pack, how many cigarettes are smoked per day and how long after waking up is the first smoke in the morning, smokers can set a target quit date. After the quit date, the app displays a dashboard of motivational statistics such as the amount of money saved, number of minutes smoke free and how many cigarettes not smoked. The dashboard statistics rotate to also display money saved over a year, number of minutes of life regained and the number of cravings resisted. The app also contains a diary and a series of progress panels that show positive and interactive information to help smokers get through the most difficult times to accomplish their goal. For example, users can create a shopping list and purchase items with the money saved by not smoking. Users can tap on a button from the dashboard to immediately get encouraging help when their cravings arise. An in-app upgrade to the “Pro” version is available, which enables features such as dashboard and progress panel customizations as well as daily missions to help smokers further their chances of successfully quitting. Users can share their progress with anyone directly from the app. Notifications can be enabled for journal and mission time reminders, in addition to awarding badges for major accomplishments. Simple to use and packed with features, Smoke Free is an excellent resource for smokers who are serious in the fight to end their addictions. Complete with a plethora of personal positive information and motivational interactive activities to keep users engaged in their battle to quit smoking, this app is a powerful tool that dental professionals can direct their patients to.

— Hubert Chan, DDS

Consumers Expect Small Businesses to Have a Website

A website is the No. 1 expectation from consumers when they choose small businesses, more so than on-air advertising, loyalty programs or a Facebook page. This according to a study of 800 consumers conducted by Time Warner Cable titled “Small Business Technology Impact Study.” The study found that 36 percent of consumers may not shop at a business that doesn’t have a website. On top of this, 70 percent of the respondents said that having the owners’ photo with a story about the business on the website is effective. These can serve as particularly useful statistics for dentists who are considering launching a website and a social media presence. If dentists were to follow the lead of this study, they would build their website out before firing up their Facebook page.

— Blake Ellington, Tech Trends editor

Chrome Browsing Outpacing Safari Browsing

Something is changing in the world of mobile and desktop browsing. According to a recent Adobe Digital Index (ADI) study, the Chrome browser is gaining more traction with users than the iOS-based browser, Safari. Apple users who traditionally have stuck to Safari for their web browsing have to download the Chrome browser, which is a sign that it is becoming the preferred option. Adobe Analytics show that smartphone use of Chrome increased 75 percent over the last year while Safari only saw gains of 33 percent. While the study showed that those who have an iPhone or iPad still lean toward using Safari, the gap is shrinking between the iOS-based browser and Google-based Chrome browser.

— Blake Ellington, Tech Trends editor
Bay Area

**AC-335 San Francisco**: Great Practice! 2100sf, 8ops in desirable location of SF. Call for Details $475k

**AC-566 San Francisco**: Spectacular views of Washington Square. 3ops +2 add'l plumbed in 1400sf office $325k

**AG-564 San Francisco**: Over 25 yrs goodwill. Large 5,600+ sf w/ 9 ops near Land's End $2.225M

**AG-576 San Francisco**: Part time practice w/ Amazing Growth Potential. Perfect for 1-3 DDS 4 ops 1.400 sf $550k

**AN-514 San Francisco** Facility: Located in the bustling financial district! 1,007 sf w/ 4 ops. Reduced to $125k!

**AN-565 San Francisco**: This remarkable opportunity could be your “dream come true”! 2,067 sf w/ 6 ops. $1.05M

**BC-361 Oakland**: Established for over 23+ years! 2,200 sf w/ 7 ops. Seller is retiring. Now Only: $330k

**BC-509 Hayward Facility**: Facility Only, 800 sf, 3ops w/ xray in each op. Call for Details $60k

**BC-520 Hayward Facility**: Located in Downtown, 1500 sf, 4 equipped ops, X-Rays in 3 ops. Call for Details $65k

**BC-432 Pittsburg**: Own this family-oriented Practice! 1,640 sf w/ 6 ops. Seller is Retiring $350k

**BC-549 Lamorinda Area Facility**: Excellent Location! Highly Visible, 900sf w/3 ops +1 plumbed add'l. Reduced $75k

**BN-504 Richmond**: Established Practice and Real Estate! 1,450 sf w/ 2 ops + 2 add'l $100k / RE $700k

**BN-505 Concord Facility**: The essence of comfort and functionality. 800 sf w/ 3 ops. Now Only: $30k!

**CC-557 St. Helena**: Live and Practice in beautiful Wine Country, 5ops in 1842sf, single-story bldg. $890k

**CG-537 Marin County**: Rare Opportunity in upscale, highly desirable area. State of the art office. 2400 sf w/ 7 ops $1.1M

Bay Area Continued

**CG-548 Rohner Park**: Award winning office by Jim Pride! Collections of $749k in 2015. 1250 sf w/ 4 ops $545k

**CN-482 Santa Rosa**: Buy with or without equipment. ~ 20 yrs goodwill. $35k ($45k with equipment)

**DC-476 Dublin**: Shared Facility. Great for Specialist - Endo, Pedo or Ortho. 1100 sf w/ 2 ops+1 add'l $125k

**DC-561 San Jose**: Eastside Area, Seasoned Staff, Loyal Pts, 6 ops +1 add'l, 2400sf, 10npts/mo $300k

**DG-576 Sunnyvale**: 900sf w/3 ops & Priced to Sell at only $195k - Call for Details!

**DN-542 Fremont**: TWO AMAZING PRACTICES. Call for Details! $1.4M

**DN-497 Pleasanton**: Great Location! Excellent Financial district! 1,007 sf w/4 ops. Reduced! $95k

**EG-560 Carmichael**: Share Facility. Great for Specialty Practice! Ly - Endo, Pedo or Ortho. 1100 sf w/ 2 ops+1 add'l $295k

**EN-558 Davis**: now Only $260k

**EG-546 Chico**: Spacious & Beautifully designed! 3,400 sf w/ 5 ops + 4 add'l $650k

**GG-386 Redding**: Award winning office by Jim Pride! Collections of $749k in 2015. 1250 sf w/4 ops $545k

**GT-550 Salinas**: 3,000 sf w/ 7 ops and collecting over $2.225M. Priced at only $1.4M

Northern California

**EC-525 Sacramento**: Great Location! Excellent Visibility! 1,500 sf w/ 3ops, 10-15 new pts/mo $220k

**EC-531 Greater Sacramento**: Practice and Real Estate for Sale! 1,750sf w/4ops +1 add'l, 8npts/mo $800k

**EN-464 Rocklin**: Don't miss out on this remarkable opportunity! 2,150 sf w/ 4 ops. Now Only: $130k

**IN-554 Turlock**: Speciality Practice $535k Real Estate $750k

**IN-506 Turlock**: Exclusive Practice $150k

**GN-244 Oroville**: Speciality Practice $535k Real Estate $750k

**GC-472 Orland**: Great Practice! 2300sf, 8 ops. Seller is retiring. $480k Real Estate Also Available!

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**FP-572 Modesto**: supplementing Practice $535k Real Estate $750k

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NORTHERN CALIFORNIA CONTINUED

EG-521 FOLSOM Facility: Stands out above the rest! Don’t Miss this one! 1,200 sf w/ 3 ops. Well Equipped! $50k
EG-526 CARMichael: Relocating and leaving 30yrs Goodwill behind! 1,350 sf w/ 4ops & opt to grow! REDUCED: $350k
EG-556 SACRAMENTO: Near CSUS Campus. Long-term 2nd generation office. 935 sf w/ 4 ops $389k
EN-34 ROSEVILLE: Location, Location, Location! Turn-key...just needs you! 2,000 sf w/4 ops. $45k
EG-560 CARMichael: Focusing on the philosophy of treating patients as family! 1,200 sf w/ 3 ops + 1 add’l. $130k
EN-558 DAVIS: Designed for maximum office efficiency and patient flow! 1,487 sf w/ 4 ops + 1 add’l. $650k
EN-573 SACRAMENTO: The goal and focus of this practice is to provide excellent service! 1,075 sf w/ 2 ops. $93.1k
FC-334 NORTHERN CA: Emphasis on prevention. 1,200 sf w/ 4 ops $480k / Real Estate Also Available!
FC-415 FT. BRAGG: Excellent Practice! Dr. avgs 18+ pts/day & 20+ npts/mo, 1,800 sf w/ 5 ops + 1 hyg. Op $425k
FC-489 CLEARLAKE: Located on “4-Corners” of Hwy 53, 4ops in shared 3600sf facility. $470k / 50% interest in RE Also Available
FN-527 TRINITY COUNTY: Be the only dentist in town! “Pride Institute” designed! 2350sf w/5 ops +1 add’l. $250k
GC-472 ORLAND: Live & Practice in charming small town community. 1,000 sf w/2ops. Seller Retiring. $160k
GG-386 REDDING: Amazing Practice. Lease or Buy Real Estate! 2,860 sf w/ 4 ops. Plumbed for 2 add’l!! ONLY $260k
GG-453 CHICO: 5,000 sf w/7 ops Perfect for 1 or more dentists! $325k
GG-454 PARADISE: 2,550 sf w/ 9 ops. 40 yrs goodwill! Amazing Opportunity! $525k
GN-244 OROVILLE: Must See! Gorgeous, Spacious. 2,500 sf w/5 ops! Collections over $450k in 2013. Only $315k
GN-399 REDDING: Loyal patient base and relaxed workweek schedule. 1,440 sf w/3 ops. $150k
GN-507 CHICO: It just doesn’t get any better than this! 3,000 sf w/7ops. Practice $535k Real Estate $750k
GN-546 CHICO AREA: Well-known for offering quality dentistry with sedation. 2,600sf w/4 ops. $350k
HC-461 SONORA: In the beautiful Sierra Foothills, 4ops, 1350sf, freestanding bldg. Practice $700k & RE Also Available!
HN-213 ALTURAS: This well managed practice continues to have consistent revenues! 2,200 sf w/ 3 ops + 1 add’l $115k
HN-280 NO EAST CA: Only Practice in Town 900 sf w/ 2 ops $60k
HN-290 PLACERVILLE: Excellent Merger Op! FFS. 1,400 sf w/ 4 ops $210k
HN-539 Central Sierra/Tuolumne Co: The perfect Merger Op in a rural Sierra Community! 2,000 sf w/ 5 ops. $175k

CENTRAL VALLEY

IC-468 SAN JOAQUIN VLY: High-End Restore Practice! 2500+sf, 6 ops, Price Reduced. All offers considered! $350k
IC-572 MODESTO: In desirable Dental/Medical Professional building of town, 3ops in 1300sf office. $160k
IN-474 STOCKTON: Too good to be true? Absolutely not!  1,600 sf w/ 3 ops $95k
IN-506 TURLOCK: Practice in the heart of the Central Valley! 2,000 sf w/ 5ops + 1 add’l. $425k
IN-512 MERCED: This immaculate practice is an absolute jewel! 1,200 sf w/ 4ops + 1 add’l. Now Only: $110k
IN-554 TURLOCK: A small town feel but with “big city” amenities! 1,900 sf w/5 ops. $795k
IC-541 FRESNO Facility: 1,210 square feet and consists of 2 fully equipped ops and plumbed for add’l op Call for Details!
JG-491 FRESNO: Well-established. 40-50 new Pt/mo. 1,452 sf w/ 4 fully equipped ops. REDUCED! $395k
IN-551 COALINGA AREA: Serving this community of working families! Paperless Practice. 1,200 sf w/ 3 ops. REDUCED! $395k!

SPECIALTY PRACTICES

BC-544 ALAMEDA COUNTY Pedo: 1,056sf w/ 4 chairs in growing, revitalized community, Seller Retiring $225k
BG-517 NORTH EAST BAY Endo: 2,750 sf w/ 8 ops! Strong Practice! $500k
CC-346 SO MARIN CO Perio: Beautiful 1,142 sf w/ 3 ops. No reasonable offer will be refused! Reduced $150k
CG-424 NAPA Prosth: Office has Digital X-ray & NEW 3D Imaging Unit! Ready for Experienced, high-end Prosthodontist! On track to collect just under $1m $690k
DC-459 SF PENINSULA Perio: 50% Partnership Buy In! Call for Details! $580k
FN-536 LAKE COUNTY Pedo: Focusing on Prevent dental problems before they begin! 1,750 sf w/3 ops. Now Only: $275k
IC-543 CENTRAL VALLEY Ortho: 1,650 sf w/ 5 chair bays & plumbed for 2 add’l, Strong Refs & Satisfied Pts Base $180k
IC-540 FRESNO Sleep Appn: Motivated Seller retiring! Step right in and make yours! Call for Details!

“Ask the Broker” can now be found at www.westernpracticesales.com
The following Dr. Bob column was originally printed in the February 1996 issue of the Journal.

The human body. This God-given temple of the soul. Treasure it from birth, nourish it with oat bran and wheat germ, stoke it with vitamins and minerals, baste it with Oil of Olay, this marvelous machine. Give it a massage and aerobic exercises, treat it to Nautilus and every other body-enhancing gadget and potion from the fertile minds of man and Elizabeth Arden, then protect it from every kind of stress you can and you know what? — that sucker will die anyway.

It’s ironic, learning that Ponce de Leon, after years of searching for the Fountain of Youth, finally finds it just off I-95 and approaches the attendant for permission to bathe. “No problem,” says the Seminole-in-charge (sic), handing him a towel. “Fifty pesos and don’t forget to take off your …”

But Ponce, eager to enjoy the fruits of eternal youth, jumps in still clad in his armor and plummet to the bottom like a safe.

Well, what did you expect? When you signed up for dental school, they didn’t mention the nature of the work? C’mon, everybody knows about the postural and visual defects that set in about the second semester and go downhill from there. You didn’t think for a minute, moron-like, that you were going to sit in a high-back
leather chair issuing orders, giving dictation and doing three-martini lunches, did you?

No, it’s our lot in life to give new meaning to the biblical phrase “laying on of hands.” To do this requires that we get fit and remain so even if it means eventually questioning the validity of the whole concept.

Initiated by dentists still young and naive enough to think they could reverse this deterioration, the ongoing fitness craze that has gripped this country for the past couple of decades shows no sign of abating. Orthopedic offices throughout the nation are littered with shin splints, torn tendons and sorely abused bodies. What we’ve got to do is sort out the things that will cripple or main us, thus making us ex-dentists, and seek those things that will give us a better chance of fulfilling our destinies.

The following information will not help a bit:

Right off the bat, so to speak, that eliminates baseball, as it is one of the sports invoking the use of hands, our most productive appendages, or at least the ones we’re most interested in here. Lose the use of your hands and what’s left for you? — nothing but the lecture circuit, a scam that’s already been thought of by hundreds of your colleagues to the point where in a few years there will be nobody left to do the actual work; they’re all out lecturing to each other.

So let’s see what might contribute to your fitness program without the danger of forcing you out to tell other dentists how to bleach teeth.

Golf. Golf gets you into the open air, and it’s where patients think you are on Wednesdays, anyway, but that’s about all you can say for golf. To participate, it is necessary to humiliate yourself by wearing ridiculous pants and impractical shoes whose only other use is tenderizing cheaper cuts of meat. You sit on your dental stool, you sit in your golf cart. You try to get a small object into a hole, and you have a level of frustration as high or higher than that in your dental practice.

It became apparent just after the game was invented that running around in the hot sun chasing a little ball was a dumb thing to do. That’s why the scoring was altered to jump from 15 to 40 just to get the game over with as fast as possible.

Golf does not contribute to your fitness but forces you to recite long, boring anecdotes whose tediousness is exceeded only by the tales of your fellow golfers.

Bowling. Forget bowling, too. Using three of your vital fingers to propel a large leaden sphere in an effort to flatten 10 objects just so you can do it again and again to the accompaniment of gawdawful noise makes no sense at all. The requisite costume is an embarrassment from the garish shoes to the sports shirts with embroidered advertising for brake relinings or fast food joints. Bowling would be bad enough if you did it alone, but tradition requires that you do it in concert with a bunch of bozos whose motivation is based entirely on getting a night out.

Tennis. Tennis used to be a gentleman’s game. From the crisp, white, impeccable outfits to the strict adherence to manners and tradition, it seemed to fulfill all the requirements. Even though the dominant forearm risked resembling Popeye’s while the other one remained like Olive Oyl’s, it was still a fairly civilized sport. This has changed. If you’ve watched tennis lately, you know that Andre Agassi, with his sartorial statement is now a role model; a tennis ball traveling at speeds in excess of 120 mph can alter your physical well-being forever; and there is way too much sweating and grunting. Umpires risk getting a biff in the snoot for a bad call, and people have been known to get stabbed. It became apparent just after the game was invented that running around in the hot sun chasing a little ball was a dumb thing to do. That’s why the scoring was altered to jump from 15 to 40 just to get the game over with as fast as possible. Dentists are advised to take up ping pong, which is the adult version of tennis without the sun.

Scuba diving. Younger dentists who miss the excitement of doing wheelies on a motorcycle with no hands, no helmet and no brains or racing around in their fathers’ Oldsmobiles without the benefit of functioning thought processes, frequently take up scuba diving as an antidote to the deadly confinement of the operatory.

Unless you live in Tahiti, Hawaii, the Bahamas or the Grand Caymans, you will experience only grinding regret every time you look into your closet at the jillions of dollars’ worth of wetsuits, regulators, masks, fins, booties, hoods, gloves, depth gauges, tanks and special wrist watches the size of a manhole cover with 89 functions, none of which reveal the correct time, getting dusty from disuse. I realize that to a dedicated scuba diver this is all offset by the sight of a fish going by in its element deep in the murky depths of water two degrees above freezing, but to a rational person, 30 minutes of a Jacques Cousteau rerun should suffice.

Skiing. You would think that a dentist, even a young one without the wisdom that comes from years of listening to patients lie about their flossing habits, would see the idiocy of falling totally out of control down the face of a mountain while wearing 7-foot boards strapped to boots the size of microwave ovens. Boards that he waxes to make him fall even faster, for God’s sake. Well, lots of dentists do this. You can observe these dentists at the resort bars bemoaning their rotator cuffs and spiral fractures. It’s the only sport open to dentists that requires more expensive gear than scuba diving. The cost of stuff needed for a family of four just to get to the falling-down stage of skiing exceeds the S&L bailouts. Skiing does not contribute to fitness, it cancels it.
Jogging. Nobody jogs anymore. I just put that in there so that if you are still jogging based on the misguided idea that it’s doing you some good, you’ll know it’s time to quit. Power walking is where it’s at now. With your $125 special cross-training walking shoes that has Korea giggling all the way to the bank, you’ll discover power walking is to plain strolling what St. Vitus’ Dance is to Barcalounging. If you insist on power walking, do it in the early hours or after dark so that passing motorists won’t lose control with laughter and crash into light poles.

That doesn’t leave us with much. Certainly dentists with IQs superior to paramecia should eschew pumping iron. That branch of insanity will instantly identify any aneurysm you may have in your body, besides running the risk of making you appear to have had badly managed silicone injections inflicted on areas where you don’t even have muscles and never did.

I know it’s too late to save you from the mistake of buying the stationary bicycle, rowing machine, treadmill and the like. The only benefit these machines have ever produced accrued to the chuckling dealer who sold them to you. Leave them in the closet, under the bed with the dust bunnies or in the garage where they have resided peacefully since the second week they were brought home with such high hopes and firm resolutions.

Above all, don’t be too hard on yourself for being such an incredible jerk for getting in such bad shape in the first place. And if you decide to take up one of these “fitness” sports in spite of all the intelligence to the contrary, be sure to check with your physician before you start. You know what kind of great shape he’s in.
What will you discover in San Francisco?

Inspiration. Industry-leading speakers, like laser dentistry expert Peter K. Pang, DDS, will share practice-changing knowledge at CDA Presents. Earn C.E. credits at cutting-edge programs, inspirational lectures and hands-on workshops. Get a front-row seat and fuel your passion for your profession.
Before wearing UltraFit tray in the mouth

With no impressions or custom trays necessary, Opalescence Go is ready to use right out of the package! The comfortable, adaptable UltraFit™ pre-filled tray provides molar-to-molar coverage, and quickly adjusts to any smile.