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New Directions in the Etiology of Dental Caries Disease

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abstract This review explores the multifactorial etiology of dental caries disease. Current theories suggest that a singular focus on mutans streptococci and lactobacillus as the sole causative microbiological agents is no longer a viable strategy in treatment of this prevalent disease. Dental caries is an infectious transmissible disease process where a cariogenic biofilm in the presence of an oral status that is more pathological than protective leads to the demineralization of dental hard tissue.¹

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Recent research also indicates dental caries has genetic components. When examining for beta-defensin-1, a salivary protective protein, there are three polymorphisms (genetic expressions or variations) of this gene. Individuals with one particular genetic polymorphism, the G_{161A} expression, exhibited five times the DMFT scores of the other two genetic variants. Genetic variations associated with taste have also been implicated as a hereditary component influencing dental caries. Previous reports have characterized the influence of genetic variation on taste preferences and dietary habits. Statistically, significant associations were seen in TAS_{2R} (bitter) and TAS_{1R} (sweet) for caries risk and/or protection. Children with the TAS_{2R} gene object to the bitter taste of many green vegetables, prefer eating sweets, and have demonstrated a significant correlation with increased caries experience.

The Role of Bacteria

Originally thought to be a disease of two primary pathogens, mutans streptococci and lactobacillus, the current biofilm disease model for caries is one of multiple pathogens. Marsh demonstrated in the ecological plaque hypothesis that dental caries is a pH-specific disease. These pathogens are acidogenic and aciduric bacteria that metabolize carbohydrates into acids, resulting in acidic conditions in the oral biofilm. However, it is the acidic pH per se, not the carbohydrate availability, that provides the selection pressure favoring these cariogenic organisms in the biofilm environment. When these acid-producing bacteria dominate the biofilm, the normal balance in the mouth that influences demineralization and remineralization changes to produce prolonged episodes of low pH, resulting in demineralization of the teeth and net mineral loss. Recent biofilm research has introduced the so-called “extended ecological plaque hypothesis” where even

commensal bacteria have demonstrated the ability not only to adapt to live in the acidic environment, but to also develop the ability to produce acid themselves, thus contributing significantly to the disease process.

About 100 different bacteria species have been identified from the human oral microbiome, making the mouth the most microbially diverse environment in the body. However, the picture may be even more complex than that; DNA identification research examining the hypervariable *ccfA* region of the *S* gene sequence, would indicate that there may be 1000 different bacteria in the mouth. Furthermore, when a bacterium replicates its DNA, it produces a specific amount of pyrophosphate, and when examining this metric, this research would anticipate about 1000 distinctly different bacteria in the human oral microbiome.

This research would suggest an even more complex microbial environment and bacterial component in dental caries.

Research also has suggested that dental caries may have systemic effects. While the oral-systemic connection with periodontal disease has gathered a great deal of attention in the past decade, dental caries disease may also have similar consequences. In Nakano et al. reported *Streptococcus mutans* in the coronary arteries and heart valves 10 percent of the time. This suggests that dental caries may play a role in bacteremia and peripheral vascular disease.

In summary, the current biofilm model of dental caries is a complex picture: multiple pathogens, systemic effects, and hereditary components layered on interactions of diet, behavioral, environmental, socioeconomic, and physiological risk factors. Thus, diagnosis for dental caries disease becomes more complex and involves examining these different parameters to get a clearer picture of this disease.

The Role of Saliva

Saliva plays many important roles in the mouth in health and digestion and offers some real potential in evaluating dental caries risk. Saliva is a unique fluid in the body, it is supersaturated with calcium and phosphate, helping maintain the mineral content of teeth, it contains protective proteins and antibodies, enzymes for digestion, lubricants for chewing and swallowing, electrolytes for buffering the pH and many other factors that contrib-

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ute to a healthy balance. As a diagnostic specimen, saliva is readily available, it is easily collected and stored, and it is a noninvasive procedure. There have been more than 1000 proteins or proteomes identified in human saliva, and 10 percent of the plasma proteins associated with different disease processes can also be found in the saliva. As salivary diagnostic technology continues to develop, there will be an opportunity for dental practices to play a major role in point-of-care diagnosis.

Saliva also offers opportunities as a specimen for caries risk assessment and diagnosis. Multiple parameters of saliva have been studied and can be measured. Current tests include saliva flow, both unstimulated (resting) and stimulated; salivary pH, both unstimulated (resting) and stimulated; buffering capacity of the sa-

liva; bacterial pathogen presence, including: mutans streptococci and lactobacillus; plaque cariogenic potential; and, finally, biofilm activity level measured by ATP bioluminescence. The challenge facing dentistry today is finding a valid chairside metric to test for caries risk that is time efficient, cost-effective and also predictive of caries presence and progression.

Salivary Flow (Resting and Stimulated)

Insufficient saliva flow (resting and stimulated) may lead to the subjective complaint of dry mouth, or xerostomia, a condition that jeopardizes the teeth from the lack of buffering ability and reduced availability of calcium phosphate for remineralization. Saliva flow reduces as a natural part of aging, by a multitude of prescription medications and is from a practical standpoint nonexistent during sleep. Radiation therapy and conditions like Sjögren's syndrome or other autoimmune connective tissue diseases, diabetes, hepatitis C, and HIV infection all can reduce the amount of saliva flow. Inadequate saliva flow is a known risk factor for dental caries, less than 0.5 ml of stimulated saliva per minute places a patient at high risk or extreme risk. Recently, reduced saliva flow has been tied to childhood obesity and increased risk for dental caries in children.

Saliva flow rate should be assessed in both stimulated and resting saliva. Commonly, clinicians only assess the stimulated flow rate because stimulated saliva flow is easy to measure. Simply have the patient chew on paraffin wax and spit into a graduated cup, measuring mls/minute. While this test is very accurate to measure stimulated saliva flow, the test itself may not be highly predictive for dental caries experience. If the stimulated flow rate is below 0.5 ml/minute, then the patient can now be

diagnosed as having “salivary gland hypofunction,” the preferred term when flow is measured quantitatively rather than using the subjective term “xerostomic.”

Measuring resting saliva flow is less often done perhaps because it requires the patient to “drool” into a collection vessel measuring the mls/minute of flow. When performing the test, patients were instructed to initially swallow and then tilt their head forward with the chin near the chest and instructed to avoid any lip or tongue movements, talking, or swallowing. The saliva was allowed to pool in the front of the mouth for exactly two minutes without swallowing. It was then gently drooled into a graduated cylinder.

The two-minute collection process was repeated twice and then patients were asked to gently empty their mouths of any remaining saliva into the collection vessel. Patients should be informed that there may be little or no saliva to expectorate and not be concerned if that should prove to be the case. The total quantity was divided by four minutes to obtain a flow rate per minute. The lower end of normal flow rate may be as low as .5 ml/min.

Admittedly the drool test described above is not very popular in clinical practice and a surrogate test to help identify the presence of abnormal resting saliva flow, such as the physical appearance of saliva (thick and bubbly) and the inability of minor salivary glands of the lips to produce visible “beads” of saliva within one minute after drying with gauze, have been reported in clinical use. Although using the appearance of resting saliva has the support of at least one study that correlated surface tension of saliva to dental caries using technology like droplet surface tensiometry, these surrogate tests should be considered anecdotal at best and should be lightly weighted until a larger body of evidence is presented.

Salivary pH (Resting and Stimulated)

Although resting and stimulated salivary pH is easily measured with a high degree of accuracy with the use of pH sensitive test strips, they must be interpreted carefully. While the data accurately reflect the pH of the saliva, the real challenge for pH measurements is how to use these data to make useful clinical decisions. It is important to keep in mind that the value in using any salivary test will depend not only on the type of saliva measured (resting or

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stimulated), time and location sampled, but most importantly what is going on with the local biofilm, chemistry, and salivary composition; together they will determine remineralization or demineralization of dental hard tissues.

Minor salivary glands normally produce saliva that has a lower pH than the major salivary glands and, considering the amount of time saliva is actually being stimulated, resting (unstimulated) saliva may perhaps be more important to evaluate clinically than stimulated saliva. Although one study by Subramaniam in 1998 demonstrated a significant correlation between resting salivary pH and dental caries in the primary dentition of children with cerebral palsy, in the end, the real value of pH testing may well be as a teaching tool rather

than attempting to relate pH to predicting caries risk. It may help in the selection of products to neutralize acid and restore the mouth to a healthy state. Part of this is because the salivary pH may or may not be reflective of the actual biofilm pH on the teeth or it may reflect affects of diet if the patient has eaten.

Resting saliva will also have different pH measurements, depending on the areas of the mouth in which they are sampled, thus pH (like biofilm and caries lesions) is site-specific. In fact, an interesting exercise is to sample saliva at different areas of the mouth and measure pH using pH-sensitive test strips. Although not validated, one technique is to collect saliva from a patient (in a resting state) by having them expectorate only once into a cup and measuring the pH of that single collection. A clinician may use this as an example to educate patients about the effects of resting saliva pH on remineralization/demineralization dynamics as well as the role it plays on the selection process of biofilm. Although measuring pH may help patients learn how their behaviors affect this dynamic balance, there is little evidence it predicts future caries risk and should not be used for that purpose.

In summary, while salivary pH (resting and stimulated) can accurately and easily be measured chairside in real time, one should use constraint in using any test by itself as being predictive of caries experience; one also must consider the local biofilm, chemistry, and salivary composition. It may help in modifying behavior and choosing products that will help neutralize acid. Using pH in conjunction with these other factors will, however, produce a rubric to provide an opportunity to modify the local environment using chemical interventions. Future research is needed to determine if this approach will actually result in better outcomes.

Bu ering Capacity

The quantitative measure of resistance to pH changes is called bu er capacity. In 1959, Ericsson introduced a test to measure the bu ering capacity of an individual's saliva. The expression of this test described the ability of a patient's saliva to bu er or neutralize the salivary pH during acidic challenges. The Ericsson test proved to be highly predictive for dental caries. The challenge is that this is not a chairside test but rather a laboratory procedure that requires several hours to complete. Chairside tests are currently available for measuring bu ering capacity of stimulated saliva; however, some studies question their reliability. A recent study of these tests demonstrated one technique used on resting saliva that was consistent with the Ericsson data, but the other tests on stimulated saliva were not. Other analyses on bu ering capacity recommended a need for additional research. The salivary-bu ering capacity appears to have caries predictive value in the Ericsson test, but available chairside tests may not be as accurate.

As with testing salivary pH, care must be exercised in the interpretation of a bu ering test, even if the result is "normal." For example, the test does not measure the presence of salivary hypofunction (xerostomia), so one could conceivably get a "normal" bu ering capacity on a patient who has severe salivary hypofunction. As mentioned above, the reliability of these chairside tests to assess bu ering capacity is in question and should be considered along with other factors to modify the local environment using chemical interventions.

Measuring Bacterial Load or Activity

Research data have long established a strong predictive relationship between levels of salivary mutans

streptococci, lactobacillus, and, more recently, *S. sobrinis* and bifidobacteria and dental caries. Blood agar plating and polymerase chain reaction (PCR)-based bacterial identification provide accurate measurements of these known pathogens in the saliva. The challenge for this parameter again is one of an accurate and predictive chairside test.

While several chairside cultures are available, recent independent research indicated that none of them accurately identified the level of cariogenic bac-

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teria or *S. mutans* present. A sample of the patient's saliva is collected and then cultured on selective agar media for 24 hours. It is a management challenge to schedule patients to collect the sample then recall them for reading and discussion of the test after 24 hours.

Monoclonal antibody testing has been established for measuring individual pathogens and a test for mutans streptococcus is currently available. Measuring specific pathogens in light of the multipathogen biofilm model for this disease is questionable. In other words, such specific pathogen targeting may not be able to provide adequate predictive value. In addition, in light of the extended ecological plaque hypothesis where low pH nonmutans bacteria and actinomyces are acid-producing and

thought to be precursors to a mature acidogenic biofilm dominated by MS and LB, the diagnosis would be missed by both specific monoclonal antibody test and selective culturing methods. In summary, bacterial identification offers some promise of predictability; however, there is a need for additional evidence correlating the chairside test currently available to actual caries disease risk.

The cariogenic potential of a plaque/biofilm sample is another chairside test that is currently available. It measures the ability of the patient's plaque/biofilm to metabolize sugar. A small sample of the patient's plaque is collected, a sugar solution is added, and then a pH-sensitive dye is added. The resulting color change is read and indicates the patient's "cariogenic potential." While one study indicated the cariogenic potential correlated well with the bacterial levels, additional research and validation correlating the test to the patient's caries risk are needed. In the meantime, the test has strong educational value for patients to understand the role of diet and pH in dental caries. This chairside test takes about 10 minutes to perform.

ATP Bioluminescence is a technology that has been around for a long time. It is used in a multitude of environments where precise measurement of bacterial activity is necessary, e.g., food manufacturing, wastewater treatment. The concept behind ATP bioluminescence in dental caries is based on the known adaptive mechanism of aciduric bacteria. These bacteria survive and thrive in acidic pH environments because they have the ability to pump the hydrogen (protons) ions out of their cell. In addition to other adaptive mechanisms, they maintain a more neutral intracellular pH in this harsh environment. This requires a

tremendous expenditure of ATP. By measuring ATP levels in the biofilm, a determination of overall bacterial load and biofilm activity can be assessed. Recent scientific studies further indicate a significant positive correlation to the patient's overall *Streptococcus* count, mutans streptococcus counts, and directly correlates to the patient's caries risk status. ATP bioluminescence then becomes a risk tool and a potential biometric to identify and assess the level of cariogenic bacteria, and also act as a surrogate endpoint to measure effectiveness of anti-caries therapy. ATP bioluminescence is a simple chairside test that involves swabbing a specific site on the teeth and then a 1-second measurement with a meter. It is efficient, effective, and provides reasonable predictability without recalling the patient as in the culturing technique.

Conclusion

The complex nature of a multifactorial, pH-driven biofilm disease such as dental caries whose onset and progression is influenced by so many diverse bacterial, dietary, environmental, socioeconomic, physiological, and genetics risk factors exemplifies the need for the dental profession to look beyond tooth restoration. It requires a careful assessment of each of these factors, not in isolation but taking all unique and dynamic factors into account in the assessment of each individual patient. Science has made significant advances in bacteria and saliva assessment including new methods of measuring bacterial load, pH, buffering capacity, and flow of both stimulated and unstimulated saliva. The challenge in a complex biofilm disease like dental caries is identifying a risk assessment/screening/biometric

tool that is time-efficient at chairside and provides real-time results, and is predictive for the disease activity.

Salivary diagnostics offers the opportunity to develop such instruments, without which the biometric becomes tooth cavitation (the endpoint of the disease process). It is preferable to objectively treat and prevent caries disease rather than wait to see if cavitations continue to develop. However, the lack of evidence, minimal evidence, and conflicting evidence for many of these new methodologies will inevitably result in variations on how concepts and products are used in clinical practice. Differing points of view are to be expected in the presence of imperfect evidence.

In addition to dental caries disease, the future for salivary diagnostics for other diseases and conditions as an emerging science is very promising. New tests for saliva include multiple factors that relate to oral and systemic diseases. Currently, saliva is being analyzed for periodontal disease indicators including genetic testing for IL-1A and B genes, along with measurements of periodontal pathogens present. The saliva has been a reliable source for markers of myocardial infarction, and stroke. Future tests also include examining for irregular exosomes released by epithelial minor salivary glands that indicate the presence of oral cancer. Breast cancer markers have also been identified in saliva and provide better information about prognosis at point of diagnosis, and act as biometrics during cancer therapy. The database for this emerging field is being collected at the Saliva Ontology Project at UCLA. Saliva is a complex fluid that provides many important roles in the body and it promises to provide many opportunities for diagnosis in the near future. ■■■■

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