Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 1)

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Dental caries is a complex disease process that afflicts a large proportion of the world's population, regardless of gender, age and ethnicity, although it does tend to affect more indivduals with a low socioeconomic status to a greater extent. The process of dental caries is dependent upon biological factors that are present within the saliva and dental plaque. There are many different agents within saliva and plaque that serve to protect the tooth surface against caries development. Salivary flow rate, buffering capacity, antimicrobial activity, microorganism aggregation and clearance from the oral cavity, immune surveillance, and calcium phosphate binding proteins all interact to inhibit or reverse demineralization of exposed tooth surfaces. Cariogenic bacteria levels within the saliva and plaque determine whether caries will occur or not, and the concentration in saliva and plaque are intimately related to the type of carbohydrate ingestion and the frequency of ingestion, as well as the oral hygiene practiced by the individual.

J Clin Pediatr Dent 28(1): 47-52, 2003

INTRODUCTION

Dental caries is one of the most prevalent infectious diseases to afflict mankind.[1-12] The proportion of the world's population affected by dental caries increased dramatically once refined carbohydrates became available to those within developed and developing countries. Although the United States and European countries have experienced a dramatic decline in caries, only a small percentage (approximately 10%) of late adolescents in the United States enters adulthood in a caries-free status. It is also important to remember that one-fifth of the population accounts for about two-thirds of the total caries experience. It is difficult, if not impossible, to identify this at risk population with currently available caries screening methods. With the aging process, the vast majority of individuals encounter periodontal disease with exposure of root surfaces and ensuing root surface caries.

The caries process is dependent upon: 1) the interaction of protective and deleterious factors in saliva and plaque, 2) the balance between the cariogenic and non-cariogenic microbial population within saliva and in particular plaque, and 3) the physicochemical characteristics of enamel, dentin and cementum that make the dental hydroxyapatite more or less vulnerable to an acidogenic challenge.[1-12] The structure of enamel is unique, in that it has no residual cellular components that can effect repair when the enamel is damaged by a cariogenic episode. In contrast, both cementum and dentin have cellular components that assist in maintenance and repair of these root structures.

Demineralization and remineralization (repair or healing) of enamel are continuous processes that are intimately related and occur episodically based upon the presence of cariogenic bacteria in dental plaque and the availability of refined carbohydrates for fermentation to organic acids. There are many and varied biological factors in saliva and plaque that protect enamel dentin and cementum from caries development and facilitate remineralization. The physicochemical process of demineralization and remineralization of dental hydroxyapatite (the mineral component of tooth

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structure) are influenced dramatically by the constituents comprising saliva and plaque.

Saliva: biological factors in dental caries

The oral cavity and exposed tooth surfaces are continuously bathed in oral fluid.[13-19] Oral fluid, also referred to as whole saliva, consists of the secretions from major (parotid, submandibular, sublingual) and minor (buccal, palatal, labial, lingual) salivary glands, and contributions from the oral cavity. Crevicular fluid, food particulate, lysed bacteria, degenerating inflammatory cells and sloughed epithelial cells are also present in oral fluid. Saliva serves many different functions in promoting oral health and in oral disease (Table 1).[13-19] Saliva provides a protective coating for both the mucosa and exposed tooth surfaces. It allows for lubrication and humidification of the oral cavity and associated structures due to its viscosity and elastic properties. Immunoglobulins are secreted into the saliva by plasma cells from lymphoid aggregates resident within the major and minor salivary glands and this provides for a certain degree of immune surveillance. The acinar epithelial cells of the salivary glands manufacture numerous proteins that protect the oral cavity against bacteria, fungi and even viruses. Certain elements induce aggregation of bacteria and other oral debris and result in improved clearance from the oral environs. Digestion of ingested foodstuff is initiated by secretion of certain enzymes into the oral cavity. Saliva also acts as a vehicle to carry certain chemicals from ingested material to the taste buds, and allows for the central nervous system to experience pleasurable sensations regarding certain foods. Noxious stimuli to harmful substances result in expectoration and rapid clearance of these undesirable ingested substances. Formation of a cohesive food bolus facilitates swallowing and unimpeded passage of the bolus from the oral cavity to the digestive tract. Phonation of utterances that are important for clear speech is aided by the lubricating ability of saliva. Of considerable importance to the dental profession is the ability of saliva to participate in the modulation of demineralization and remineralization of tooth structure exposed to the oral cavity.

The ability of saliva to affect dental caries development is dependent upon the quantity and composition of the secretions (Table 2).[13-19] Salivary flow rates[20-25] vary depending upon whether the salivary glands have been stimulated or are at rest. The resting flow rate is typically one-quarter to one-tenth that of the stimulated flow rate. When the salivary glands are stimulated, the composition changes from a more mucoid to a more serous character, allowing for increased clearance of ingested material from the oral cavity.[13-19] The difference among individuals with normal, low and very low flow rates is considerable; however, there is also a certain degree of overlap among groups.[20-25] The perception of low flow rate by individuals is quite variable with some people with low and very low flow rates lacking complaints of xerostomia (perception of oral dryness or dry mouth); whereas others with measured normal flow rates may express symptoms of xerostomia. In general, most patients diagnosed with dry mouth will have very low resting salivary flow rates compared to those without this oral condition. Advancing age and female gender are important determinants in lower salivary flow rates. In addition, hormone replacement therapy with menopause and oral contraceptives are associated with improving the salivary flow rate. Certain drugs contribute to suppression of the flow rate.[14-16,26] These medications include antipsychotics, antidepressants, diuretics, beta-adrenergics, sedatives, tranquilizers, narcotic analgesics, anticonvulsants, antiemetics, antimetabolites, antihistamines, anticholinergics, and vitamin D in large doses, anti-Parkinsonian agents, chemotherapeutic agents, antiarrhythmics, and antihypertensives. Particularly in the elderly, an already depressed salivary flow rate may be further exacerbated by multiple medication usage (polypharmacy). For specific medications and the effects, the dental professional should consult drug information inserts or a knowledgeable pharmacist. In addition, head and neck radiotherapy adversely affects salivary flow rates. Certain disease also may adversely affect salivary flow (Sjogren's syndrome, HIV infection, and systemic lupus erythematosus).[13-19,26]

The composition of whole saliva is complex and there are many components that interact with the oral environment to contribute to an individual's caries experience (Table 2).[13-19,27] Buffering capacity may be determined by simple testing methods and may give a measure of the ability of saliva to absorb an

Table 1. Functions of Saliva

Surface Coating of Mucosa and Teeth (pellicle)
Lubrication and Humidification via Visco-elastic Properties
Immune Surveillance (secretory IgA)
Antimicrobial Proteins Against Bacteria, Fungi and Viruses
Aggregation of Particle Material and Micro-Organisms for Oral Clearance
Digestive Enzymes for Starch Hydrolysis
Vehicle for Sensory Stimulus for Pleasurable and Noxious Taste Sensation
Cohesive Food Bolus Formation to Facilitate Swallowing
Phonation Improvement
Modulation of Demineralization and Remineralization of Tooth Structure

Compiled from references: 13-18

Table 2. Salivary Factors in Demineralization and Remineralization

Salivary Flow Rate (mL/min)				
	Normal	**Low**	**Very Low**	
Resting Saliva	0.25-0.35	0.10-0.25	<0.10	
Stimulated Saliva	1.0-3.0	0.7-1.0	<0.7	
	Age	**Men**	**Women**	
	35-44 years	0.54	0.34	
	45-54 years	0.48	0.35	
	55-64 years	0.49	0.28	
	65-69 years	0.42	0.27	
	70-74 years	0.40	0.28	
	>75 years	0.44	0.22	
	Resting Saliva Flow	**Dry Mouth Patients**	**Normal Patients**	
	< 0.1 mL/min	54%	4%	
	>0.1 mL/min	46%	96%	
Decreased Salivary Flow Rate (Clinical Xerostomia)				
	Diabetes Mellitus			
	Autoimmune Disease (Sjogren's Syndrome)			
	Medications			
	Advancing Age			
Buffering Capacity				
	Bicarbonate (primarily)	Urea	Arginine-Rich Proteins	Phosphates
	Estrogen/Progesterone (hormone replacement therapy, oral contraceptives)			
	Carbonic Anhydrase	Sialin	Basic (Alkaline) Proteins	
Antimicrobial Activity				
	Lactoferrin	Peroxidase	Histatins	Fluoride
	Lysozyme	Secretory IgA	Cystatins	Amylase
	Proline-Rich Proteins	Histatins		
Aggregation and Clearance of Micro-organisms Activity				
	Mucins	(MG2>MG1)	Proline-Rich Proteins	Statherin	Lysozyme
	Lactoferrin	Secretory IgA	Parotid Agglutinin	
Demineralization Inhibition and Remineralization Promotion (calcium and phosphate supersaturation)				
	Statherin	Histatins	Proline-Rich Proteins	Cystatins
	Mucins			
Cariogenic Bacteria				
	Streptococcus mutans	*Streptococcus sobrinus*		
	Lactobaccilus spp	*Actinomyces* spp		
	Streptoccoccus mitis	*Streptococcus oralis*		
	Streptococcus gordoni	*Streptococcus anginosus*		
Cariogenic Bacteria Properties				
	Rapid Transport of Fermentable Carbohydrates and Conversion to Organic Acid			
	Produce Extracellular and Intracellular Polysaccharide			
	Maintain Carbohydrate Metabolism under Adverse Conditions and Stress			

Compiled from references: 13-26

acidogenic challenge. The primary buffer in saliva is bicarbonate. Arginine-rich protein degradation by bacterial-derived urease to urea and ammonia contributes to maintaining a neutral pH. The influence of urea on salivary pH is noted by the akaline nature of saliva in individuals suffering from chronic kidney failure. In children with chronic kidney disease, caries is markedly decreased. In addition, both phosphates and the enzyme carbonic anhydrase also modulate acidogenic assaults. Buffer capacity has also been shown to be improved when supplemental estrogen and/or progesterone are being taken. A protein called pH rise factor, sialin, and other basic (alkaline) proteins allow a more rapid return to a neutral pH following an acidic challenge. The resting pH of saliva tends to predict the caries experience of the individual and also is an indicator of salivary buffering capacity. Individuals with a resting salivary pH of approximately 7.0 tend to have low caries activity or no caries; while those with a resting pH of 5.5 have very high caries experience. Those with pH values between 5.5 and 7.0 have less severe caries activity. Lower resting salivary pH value is also predictive of a markedly low pH when exposed to a refined carbohydrate challenge and maintenance of this low pH

for a longer period of time before returning to the baseline resting pH level.

Certain proteins and enzymes secreted by the acinar epithelial cells of the salivary glands provide protection against microorganisms (Table 2).[13-19] The mechanism of antimicrobial activity is quite different. Lactoferrin is an iron-binding protein that has the ability to sequester iron from the oral environment. Iron is essential for bacterial metabolism, with aerobic and anaerobic facultative organisms being most affected. In addition, this protein inhibits mutans streptococci growth by an iron-independent mechanism. Endogenous peroxidase produced by acinar cells interacts via a heme moiety with thiocyanate and hydrogen peroxide to form hypothiocyanate and cyanosulfurous acid. These agents created by salivary peroxidase oxidize bacterial sulfhydryl groups and inhibit glucose metabolism. Of importance is that mutans streptococci are particularly susceptible to the actions of the resulting hypothiocyanate. In addition, peroxidase protects other glycoproteins in saliva from bacterial degradation. Lysozyme is a well-known antimicrobial enzyme that is found in many secretory fluids in the body, and by disruption of bacterial cell walls leads to their destruction. Several other protease inhibitors (cystatins), proteins (histatins, proline-rich proteins) and enzymes (amylase) work through various antimicrobial mechanisms to affect cariogenic bacteria. Although IgG and IgM may be detected in saliva, secretory IgA is the dominant salivary factor in immune surveillance and neutralization of bacteria and virus and their products by an antibody-antigen mechanism. Relatively small quantities of fluoride in oral fluids are capable of inhibiting enzymes in the metabolic pathway of bacteria, and reducing bacterial growth and proliferation. In particular, fluoride disrupts enolase enzyme activity, glucosyltransferase, proton-extruding ATPase, intracellular polysaccharide synthesis and storage pathway, and sugar transport via the phosphotransferase system within bacteria.

Bacteria are also removed from the oral cavity by aggregation (agglutination) and oral clearance by saliva and several proteins and enzymes (Table 2).[13-19] The mucins (MG1 and MG2) are high molecular weight glycoproteins that are rich in carbohydrates, and provide much of saliva's visco-elastic properties. These glycoproteins promote clearance of bacteria from the oral cavity by masking the bacterial surface adhesion molecules and inhibiting bacterial colonization of the mucosa and exposed tooth structures. MG1 tends to coat the tooth surface and forms a barrier against acid attack. MG2 has greater affinity for bacteria and binds to a larger number of bacteria.

Saliva is supersaturated with calcium and phosphate with respect to hydroxyapatite.[13-19] The presence of calcium, phosphate and fluoride within saliva enhances the resistance of exposed tooth surfaces to a cariogenic attack, therefore decreasing the likelihood of demineralization and favoring reprecipitation of organized mineral components (remineralization) into previously demineralized enamel and root surfaces. Several salivary proteins bind hydroxyapatite and aid in the maintenance of the supersaturated state of saliva. This allows calcium and phosphate containing mineral components to remain in solution at the resting pH of saliva (approximately 7.0) and avoid removal by precipitation out of solution. These salivary proteins release calcium and phosphate ions when levels drop within the saliva. With increased calcium and phosphate secretion upon salivary gland secretion, the level of these proteins is also increased to maintain the supersaturated state. Not only do these salivary components maintain high calcium and phosphate suspended within the saliva, but they also increase the stability of the mineral phases within exposed tooth surfaces.

A measure of caries activity and caries risk is the concentration of cariogenic bacteria within saliva (Table 2).[1,13-19] Although mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus* in humans) and lactobacilli are most commonly associated with dental caries development, several organisms have the ability to produce organic acids at levels that induce demineralization of tooth structure and lead to clinically detectable caries. Salivary levels of mutan streptococci at $\geq 10^6$ colony-forming units/mL of saliva and/or lactobacilli at $\geq 10^5$ colony-forming units/mL of saliva place an individual at high risk for caries development.

Dental plaque: biologic factors in dental caries

The foundation of dental plaque begins with the formation of an acellular organic pellicle on the exposed tooth surface.[28-37] This organic coating (salivary pellicle) is derived from adsorption of mucinous proteins from the saliva. This tenacious membrane is considered to be insoluble in oral fluids and is between 0.1 to 1.0 micrometers in thickness. Pellicle forms rapidly whenever a clean tooth surface is exposed to saliva. The function of this acquired pellicle is to protect the tooth surface from abrasion and grinding forces during mastication. In addition, friction is reduced between the oral mucosa and teeth. Other salivary constituents found within the pellicle include proline-rich proteins, statherin, cystatins, histatins, lysozyme, amylase, secretory IgA, and bacterial-derived glucosyltransferase. Many of these are proteins that bind and protect hydroxyapatite in the tooth surface from demineralization and promote supersaturation of calcium and phosphate ions within fluid phases.

Once the pellicle foundation is established, the development of dental plaque goes through several stages.[30] There is passive colonization of the pellicle by salivary-derived bacteria. The bacteria become firmly attached to the pellicle by electrostatic, hydrophobic ion and van der Wahl forces. Irreversible adhesion to the pellicle occurs via adhesion factors on the micro-

organisms' surfaces and complimentary receptors on the surface of the pellicle. Coaggregation or coadhesion occurs between various microorganisms and already adherent bacteria, allowing for increased diversity of the plaque microflora. These results in late colonizing bacteria binding to already attached early colonizers. The bacteria then proceed to growth to confluence and produce a biofilm. During this phase, extracellular polymer synthesis occurs with the plaque matrix becoming composed of glucans and fructans from sucrose metabolism. The plaque matrix appears to have channels within the biofilm that traverse the entire thickness of the biofilm. Plaque acts as a biofilm and allows for restricted penetration of antimicrobials, concentration of extracellular enzymes that inactive antimicrobials, and reduced bacterial growth. In addition, there is spatial organization with metabolic interaction among the various bacterial strains and both synergism and antagonism occurring. With maturation, bacteria become detached from the plaque biofilm to colonize other tooth surface sites.

Cariogenic bacteria possess several properties (Table 2): 1) rapid transport of fermentable carbohydrates and conversion to organic acid; 2) production of extracellular and intracellular polysaccharides; and 3) maintenance of carbohydrate metabolism under adverse conditions and stress.[28-30,35-37] Mutan streptococci and lactobacilli have these features. These cariogenic bacteria also prefer an acidic environment (aciduric). Plaque overlying areas of demineralization without cavitation are heavily colonized by mutans streptococci (11 to 18% of total streptococcal count). Typically, such heavy colonization occurs 12 to 18 months prior to clinical detection of the white spot lesion. With white spot lesions undergoing remineralization, mutan streptococci are reduced substantially (2 to 5% of total streptococcal count).

With a cariogenic diet high in refined carbohydrate (sucrose) and with frequent carbohydrate ingestion, the scenario is established for caries development with progression to clinically detectable white spot lesions.[11,16,28-30,35-38] Cariogenic bacteria, such as mutans streptococci and lactobaccilli, have a terminal pH of 3.9 to 4.1, much below the critical pH of 5.5 when dental hydroxyapatite undergoes dissolution. The buffering capacity of plaque and its degree of supersaturation with respect to calcium and phosphate will determine if the tooth surface will undergo demineralization or not. Dental plaque sequesters buffering agents (bicarbonate, phosphate, urea) and calcium and phosphate ions from saliva.[13-19] In general, the buffering capacity of plaque is substantially greater than that for saliva (10-fold) and it also has a greater concentration of calcium, phosphate and fluoride than saliva (3 to 4-fold). Supersaturation of dental plaque and saliva implies that the mineral phases in saliva and plaque will undergo dissolution prior to the hydroxyapatite forming the tooth structure.[14-17,30-34] In addition, fluoride sequestered by plaque increases the ability of hydroxyapatite to resist organic acid dissolution, effectively allowing for a lower critical pH in the presence of fluoride. Certain plaque bacteria may modulate the effect of mutans streptococci.[13-19,28-30,35-40] *Veillonella* metabolizes lactic acid produced by acidogenic organisms and partially ameliorates acid production. In acidic conditions, both *Streptococcus salivarius* and *Streptococcus sanguis* have urease and arginine deaminase activity resulting in urea and ammonia production that raises the plaque pH.

The physical character of plaque must also be considered. Plaque may be considered a gel through which diffusion occurs.[13-19,28-30] This is important for access to the plaque-tooth interface by protective proteins, enzymes, buffers, calcium, phosphate and fluoride derived from saliva. Likewise, diffusion of acidic byproducts and caries-promoting elements away from the tooth surface are critical in avoiding caries development. Increased water content in plaque promotes rapid diffusion, and lessens the effects of acidogenic episodes. High glucan and colloid content, as well as increased thickness of the plaque, decrease transit time and prolong exposure to organic acids.

The process of caries development occurs over a considerable length of time (many months to years), and is dependent upon repeated episodes of demineralization for prolonged periods of time.[2,11,13-19,24,25,30-34] With repeated and prolonged exposure to a low pH, plaque buffering capacity and supersaturation, with respect to calcium and phosphate, will be compromised leading to eventual demineralization of tooth structure.

Those individuals most prone to caries development typically have high mutan streptococci and lactobacilli salivary levels, low salivary buffering capacity, decreased saturation of plaque and saliva with respect to calcium and phosphate, low fluoride levels in the plaque and saliva, and a high sucrose diet with frequent carbohydrate exposure. The most effective means of caries control in these individuals is removal of dental plaque using mechanical means (toothbrushing and flossing) with the addition of topical fluoride agents to enhance remineralization of demineralized enamel, and antimicrobials (chlorhexidine) to limit cariogenic bacterial growth.[1-3,13-19,23-25,27-37]

It should also be understood that caries is an infectious disease that may be transmitted from one person to another. This is exemplified by the fact that mothers with a high caries risk or experience harbor substantial numbers of mutan streptococci and lactobacilli in oral cavities and saliva.[11,41] Within a short time after eruption of the primary teeth in their infants, these caries-prone mothers transmit cariogenic bacteria to their young children, leading to increased caries susceptibility.

SUMMARY

Dental caries is a complex disease process that afflicts a large proportion of the population of the world, regardless of gender, age and ethnicity, although it does tend to affect more people with a low socioeconomic status to a greater extent. The process of dental caries is dependent upon biological factors that are present within the saliva and dental plaque. There are many different agents within saliva and plaque that serve to protect the tooth surface against caries development. Salivary flow rate, buffering capacity, antimicrobial activity, microorganism aggregation and clearance from the oral cavity, immune surveillance, and calcium phosphate binding proteins all interact to inhibit or reverse demineralization of exposed tooth surfaces. Cariogenic bacteria levels within the saliva and plaque determine whether caries will occur or not, and the concentration in saliva and plaque are intimately related to the type of carbohydrate ingestion and the frequency of ingestion, as well as the oral hygiene practiced by the individual.

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