Mutans Streptococci and the Development of Dental Plaque

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Abstract: A study of dental plaque was first documented in the literature of the 17th century. It has been described as a biofilm composed of bacterial populations living in a plaque matrix. The plaque development starts short time after a tooth surface is cleaned and covered by salivary proteins and glycoproteins conditioning film. Mutans streptococci was described as the most important bacteria related to the etiology of dental caries. This bacteria has the basic properties of cariogenicity required in the dental caries process. Current studies have suggested that the behaviour of the oral microflora, with all its components, should be the aim of future understanding of the process of dental caries. The aim of this paper is to review literature about initiation and development of dental plaque and the influence of mutans streptococci in the dental caries.

Development of dental plaque

The first reference found about dental plaque was done by Anthony van Leeuwenhoek [1] in the 17th century. He described it as a microbe-containing deposit on the teeth. According to Dawes [2], dental plaque is "the soft tenacious material found on tooth surfaces which is not readily removed by rinsing with water". At present, dental plaque has been recognized as a microbial biofilm with properties similar to biofilms in other ecosystems [1].

Costerton [3] supplies a broader definition of the biofilm in 1995. He defined it as "matrix-enclosed bacterial populations' adherent to each other and/or to surfaces or interfaces". Several authors as Stickler [4], Watnick and Kolter [5] agree in the fact that attachment to the surfaces as well as close proximity of multiple species and presence of extra-cellular matrix result in a multicellular system with heterogeneous structure and functions, that is able to resist severe environmental shocks (i.e., pH fluctuations in dental plaque, antimicrobial treatments) and to maintain its homeostasis.

It was estimated that 1mm^3 of dental plaque – about 1mg of weight – contained more than 200 million of bacteria. These bacteria include streptococci, lactobacilli, as well as mycoplasma, "yeasts", and protozoa (in mature plaque); sticky polysaccharides and other products form the so-called *plaque matrix* and constitute 10% to 40% of the volume of supragingival plaques [1].

The development of the oral plaque was classified in four phases of formation:

Phase I, the development of dental plaque begins with a clean tooth surface covered by a conditioning film of salivary proteins and glycoproteins, called the *tooth pellicle*. Oral cavity contains more than 350 species of bacteria but only few can colonize a cleaned tooth surface. This first step depends on the interaction of the surface molecules on the bacteria and the

tooth pellicle. These molecules are easily vulnerable to alteration by chemical agents. Plaque adhesion is specially favoured by high free energy between microorganisms and tooth [1].

Gibbons and Van Houte [6] studied the process of colonization of oral bacteria on human teeth. They called *pioneer colonizers* to the first bacteria over the tooth surface. Pioneer colonizers have characteristic features to succeed when competing with other microorganisms in the oral flora for a place at the tooth surface. Principally, these bacteria are streptococci strains S oralis, S mitior and S sanguinis.

After initial deposition, pioneer-colonizing bacteria, especially *Streptococus sanguinis*, begin to expand away from the tooth surface forming columns that move outwards on long chains of pallisading bacteria. These parallel columns of bacteria are separated by uniform narrow spaces. Plaque growth continues with deposition of new species into these open spaces [7].

Di Renzo [8] in 1990 and Kolenbrander [9] in 1988 investigated the development of oral plaque. They concluded that after the deposition of pioneer bacteria new species start to attach to them in order to colonize the tooth surface. Plaque expansion is accomplished in a lateral direction, which causes the interbacterial spaces to merge. When these spaces are close enough, bacteria start to secrete substances, signalling the surrounding bacteria to undergo a growth spurt. Within a short time, intermeshed bacteria cover the tooth surface adjacent to the gingiva. New bacteria derived from saliva or surrounding mucous membranes attach by bonding to bacteria already attached to the plaque. These associations, *intergeneric coaggregations,* are mediated by specific attachment proteins and occur between two partner cells. At this time, plaque is composed mainly of cocci and few rods.

Phase II, there is an increasing of the levels of gram-positive rods, such as Actinomyces viscosus, and gram-negative cocci, including Neisseria and Veillonella species, occupy the remaining interstices. Tall rods cover the outer surface of the gingival plaque. At this time, there is an intensive increment of the plaque after 3 and 4 days compared to the first 2 days. This plaque is now mature and the so-called homeostasis is established among the different microorganisms.

Phase III, 5 to 7 days after initiation, plaque begins to migrate subgingivally and bacteria and their products permeate and circulate in the pocket.

Phase IV, 7 to 11 days after initiation, the adversity of the flora increases to comprise motile bacteria, including spirochetes and vibrios as well as fusiforms. Attached gingiva plaque fills the gingiva sulcus, while spirochetes and vibrios move around in the outer and more apical regions of the sulcus [1].

Plaque matrix

Plaque matrix is composed of almost 80% water, proteins and carbohydrates. The type of carbohydrates and the bulk of polysaccharide in plaque are determined by the diet. The synthesis of polysaccharide probably represents an attempt of microorganisms to store sources of energy. Zaura and ten Cate evaluated the effects of nutrients on plaque pH and dentin demineralization; they concluded that food retention is also a determining factor for the formation of caries-like lesions [1].

Most of the polysaccharides in plaque are extracellular and form the named extracellular matrix material. The two most common and important extra-cellular polysaccharides are glucans and fructans. Glucan is a polysaccharide made of by glucose units and it probably mediates the initial adherence to the tooth surface, enables bacterial accumulation on smooth surfaces and acts as reservoir for metabolized polysaccharides outside of the cell; fructans are believed to function as extracellular storage compounds [10].

Sucrose is the most common sugar in the diet. Several investigations are focused in the ability that sucrose has inducting the demineralisation of others sugars as maltose, glucose and fructose [11]. Lactose has less acidogenic potential than the other sugars and, as a constituent of milk is not considered to be cariogenic mainly due to the protective factors in milk [12]. When sucrose is compared to other sugars (glucose, fructose and lactose) it seems to be more cariogenic. However, this effect appears to be related to specific strains, type of animal model and associated with smooth surfaces [13, 14]. The fact that MS can enhance markedly demineralization from sucrose compared to others sugars [15] has been attributed to an alteration of the diffusion properties of plaque due to the presence of water-insoluble glucan synthesized from sucrose [12].

Cariogenicity of mutans streptococci

In order to be cariogenic, bacteria should fill three conditions:

1. To be able to colonize a tooth surface.
2. To produce acids faster than the local neutralization of the plaque.
3. To be able to carry out the two items described above in a pH lower than the critical pH for enamel dissolution [16].

Mutans streptococci (MS) are acidogenic as well as aciduric and can adhere to tooth surfaces [17]. These bacteria can produce intracellular and extracellular polysaccharides from sucrose. In periods of low nutrient supply, MS can degrade intracellular polysaccharides, indicating that these polysaccharides increase the virulence of some MS species (*S mutans and S Sobrinus*) [18].

Studies in vitro have demonstrated that *S Sobrinus* is more acidogenic and aciduric than MS [19, 20]. Polymerase chain reaction (PCR) detection methods indicate that *S Sobrinus* may be more prevalent than indicated by cultural studies, however, it is rarely present at the same level in plaque as MS [21]. The reason for the inability of *S Sobrinus* to proliferate appears to be due to its inability to catabolize transported N-acetylglucosamine, an energy-requiring process, which depletes intracellular levels of phosphoenolpyruvate to the detriment of the organism [22]. Only when external sources of fermentable carbohydrates are high, or the environment is very acidic as in bulimic subjects, this inhibitory effect becomes insignificant and *S Sobrinus* proliferates.

The species most commonly isolated from dental plaque in humans are S. mutans (serotypes c, e and f) and S sobrinus (serotypes d, g). They have been isolated from populations all over the world and have been related to human caries. The bacteria prevalence differs among populations and test values also differ, depending on the method of detection [23]. About 10% to 30% of a population may have little or no MS, to 100, 000 CFUs/mL of saliva. The percentage of individuals with very high levels of MS (mayor 1 million CFUs/mL of saliva) in a population may vary considerably, depending on age, caries prevalence, dietary habits and so on [24].

Although several studies have shown the relationship among MS presence, plaque accumulation and dental caries lesions [25] they have failed in the prediction of caries based on MS levels in dental plaque or saliva [26]. Only the presence of MS in dental plaque is not enough for dental caries development. The fact that dental caries is a multifactorial disease makes us to consider other alternatives as the mechanism of MS colonization, the presence of different strains of MS, the host defence against MS infection, etc, in order to understand better and to prevent this disease.

One of these factors is the virulence of MS. MS cells possess a glucan-binding lectin and at least three types of glucosyltransferases (GTF), one of them GTF-S catalyzes the formation of a relatively water-soluble glucan composed entirely of α [1, 6] linkage and the others GTF-I and GTF-SI enzymes synthesize mainly water-insoluble glucans rich in α [1, 3] linkage but also with α ([1, 6] linkage. MS synthesizes a single FTF, which catalyses the production of fructan, composed predominantly of β [2, 1] linkages. Plaque fructans is rapidly accumulated in vivo after the ingestion of sucrose and then hydrolyzed to fructose by fructan hydrolyses produced mainly by MS [10].

The virulence of glucans is more in relation with the change of the plaque ecology than the accumulation of specific bacteria. For that, the synthesis of glucans (mainly mediated by sucrose) increases the porosity of plaque, allowing the deeper penetration of dietary sugar into the biofilm and increases the acid production close to the tooth surface [27, 28]. The extracellular matrix material synthesized from sucrose by MS in plaque alters the diffusion properties of

plaque, and thus prolongs the depression of plaque pH [15]. In recent studies related to synthesis of water insoluble glucans by MS and caries incidence in young children, it has been suggested that the capacity of synthesis of these glucans may be more important than their levels in plaque [18, 29].

Sucrose is not the only source of these polysaccharides, isomers as palatinose, trehalulose, turanose, maltulose and leucrose have been studied and, in human dental plaque, where significant numbers of bacteria are able to ferment these sucrose isomers [11]. The most important question is if there is another bacteria able to produce acids and survive in such a low pH environment. Current investigations concluded that the increase of the number of bacteria and their acidogenic and aciduric ability is also related with the quantity of fermentable carbohydrate consumption, which increase the proportion of polysaccharide-storing bacteria [30, 31]. The investigators suggest a succession in the microflora resulting in dental caries caused by increased fermentable carbohydrate fermentation, in which many different bacterial taxa respond to the changing environment and the increased numbers exhibit a greater ability to produce and withstand exposure to acid. They found the emergence of MS in plaque was often preceded by an increase in the number of other types of acidogenic bacteria, which included not only the non-MS but also other members of the plaque flora which were not necessarily streptococci [32].

Mutans streptococci and dental caries

Many investigators have shown the relationship between high caries incidence and high salivary counts of MS. In deciduous dentition, Fujiwara [33] evaluated the prevalence of caries and the number and species distribution of salivary mutans streptococci in 356 children (aged 0–2 years old) during an interval of 1 year. They found that the detection rate of MS and prevalence of caries increased with age and the concentration of MS is correlated with the number of erupted teeth. They concluded that the establishment of MS is associated with caries initiation of early childhood.

Kowash [34] studied the association of salivary MS with caries in young children; they wanted to determine the effect of a long-term dental health education for mothers with young children during three years. Even when the difference in the level of salivary MS between groups given various programs of dental health education was not statistically significant, there was a statistically significant relationship between salivary MS and caries children.

Seibert [35] evaluated the MS levels and caries prevalence in low-income schoolchildren in US. 242 schoolchildren were tested and they concluded that high levels of MS ware related to higher number of decayed teeth and

conversely, low MS levels ware related to less frequent dental caries. In US adolescents, Kingman [36] also showed that subjects with high salivary MS levels developed more new carious surfaces than did subjects with lower MS levels.

Jalil [37] studied the correlation MS counts in saliva with plaque amount, gingival inflammation and caries experience. Samples of stimulated whole saliva were obtained from 94 schoolchildren aged 12 to14years, living in inner London. He found no association between counts' of MS in saliva with plaque amount and gingival inflammation. However, there was a significant trend of increased decayed, missing, filled surfaces (DMFS) with increasing MS counts.

The presence of high levels of MS in active lesions of dental caries is widely demonstrated by the literature. However, current studies evaluate not only MS but all the bacteria in the biofilm. This biofilm is metabolically active, causing fluctuations in pH and these fluctuations may cause a loss of mineral from tooth when the pH is dropping or gaining mineral when pH is increasing. The cumulative result of de- and re-mineralization processes may be a net of loss of mineral, leading to dissolution of the dental hard tissues and the formation of a caries lesion [38].

Although MS are the bacteria which show the best characteristics of cariogenicity in the process of dental caries, new investigations are focused on the evaluation of the oral microflora, and new species are observed and compare with it [39].

Studies published by van Houte underlined and revealed the intricate relationships between diet, the microflora and caries. They clearly demonstrated the heterogeneity of oral bacteria and their capacity to respond to the impact of dietary carbohydrates that consequently increased the acid production by in turn exhibited increased acidogenicity [40, 41]. Once the acid produced by bacteria was able to alter the biochemical balance of the tooth, the process of dental caries could start.

Conclusions

Dental plaque is a microbial film composed of more than 300 species of bacteria. Plaque matrix is part of dental plaque and contains mainly polysaccharides, proteins and water. Sucrose appears to be the most cariogenic sugar. Mutans streptococci are the most important caries-inducing bacteria because of their cariogenic characteristics. However, this feature seems to be related to the specific strains, type of animal models and smooth surfaces. Glucans are polysaccharides produced by MS enzymes (glucosyltransferases and fructosyltransferases). These enzymes are responsible for the initial adherence of the bacteria at the tooth surface, enabling bacterial accumulation, reservoir and extracellular storage.

Although MS have been recognized as the bacteria with highest cariogenicity in the process of dental caries, the study of this disease as multifactorial process-must consider the whole microfilm and the interrelationship among all the bacteria inside it.

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