

ORAL MICROBES AND ITS ENVIRONMENT: A REVIEW ARTICLE

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ABSTRACT

The ecosystem within the oral cavity is unique as it is a result of interactions between components such as the oral fluids and oral microorganisms with structures of the oral soft and hard tissues. The soft structures are represented by the mucous membranes while the hard surface is represented by the teeth. Both the soft and hard tissue structures influence the distribution of microbes in the mouth. Some surfaces like those at the gingival junction protect their microbial inhabitants from any physical forces or/and chemical interactions, whereas other surfaces like the lingual incisal surface do not. Thus, the oral cavity represents a host environment that possessed features that support the establishment and growth of a great diversity of microbes. The cheek mucosa, the tongue, the gingival crevice and the tooth surface provide sites with different physicochemical and nutritional microenvironment that allows for the adherence and growth of selective microorganisms.

Keywords: oral cavity; oral microbes; soft tissues; hard tissues; oral microorganisms.

1. INTRODUCTION

Human mouth harbors a diverse, abundant and complex microbial community. The oral environment which is warm, rich in nutrients, continuous flow of saliva and has a pH towards neutrality makes it an ideal place for the growth of oral microorganisms. The microbe usually exists in the form of biofilm, which refer to the group of microorganisms embedded in a matrix of extracellular polymer. This community of microorganisms normally inhabits various surfaces in the human mouth.

2. COMPONENTS OF THE ORAL ENVIRONMENT

The tooth with its unique structure is a non-shedding surface. Thus, it is suitable for the colonization of oral microbes as it allows large masses of microbes to accumulate to form the oral biofilm. Each tooth is made up of the pulp, dentine, cementum and enamel (ten Cate, 1998; Bath-Balogh & Fehrenbach, 2006). The enamel being the outer layer of the tooth is the only part that is exposed to the oral environment under normal condition. In addition to the varying intrinsic biological properties, the tooth provides several distinct surfaces such as pits and fissures that influence the colonization and growth of different populations of microbes.

The tooth surface normally encourages the residence of aerobic, facultative and anaerobic microflora (Ross & Holbrook, 1984; Samaranayake, 1998; Samaranayake, 2002).

The oral mucosal surfaces provide specialized areas that contribute to the diversity of the oral microbes. The cheek mucosa supports the establishment of predominantly the facultative types, especially the streptococci (Ross & Holbrook, 1984). The tongue, with its papillary surface, provides sites of colonization that are protected from mechanical removal by the washing action of saliva (Samaranayake, 1998; Marcotte & Lavoie, 1998). The dorsum of the tongue was reported to be frequently colonized by *Candida albicans* (Ross & Holbrook, 1984).

Saliva that flows over all the internal surfaces of the oral cavity acts as moisturizer for the oral cavity. It is secreted by three paired major salivary glands; the parotid, submandibular and sublingual glands (Edgar & O'Mullane, 1996; Edgar, Dawes, & O'Mullane, 2004; Dodds, Johnson, & Yeh, 2005; Bath-Balogh & Fehrenbach, 2006). Additional to these are minor glands that include the labial, lingual, buccal and palatal glands (Marsh & Martin, 1999) which present on the soft palate and on the sub mucosal surfaces of the lips and cheeks (Cole & Eastoe, 1988; Bath-Balogh & Fehrenbach, 2006). The type of secretion and the chemical composition of the saliva in the mouth vary according to the secreting gland. The parotid secretion is serous, whereas the submandibular and sublingual are much more viscous as it has higher content of glycoprotein (Cole & Eastoe, 1988; Edgar & O'Mullane, 1996; Edgar et al., 2004). The types of secretion have influence on the adhesion of the oral microbes to the oral surfaces. It has been reported that parotid saliva supported whereas whole saliva inhibited the adherence of *Strep. mutans* to hydroxyapatite beads (Carlén, Olsson & Ramberg, 1996). According to Humphrey and Williamson (2001), whole saliva refers to the complex mixture of salivary contents that include stimulated and unstimulated saliva, gingival crevicular fluid, non-adherent oral bacteria and food debris, and traces of introduced chemicals or medicaments. The composition of whole saliva are controlled by parasympathetic and sympathetic stimuli and influenced by a number of physiological factors (Lingström & Moynihan, 2003).

Saliva contains several ions including sodium, potassium, calcium, chloride, bicarbonate and phosphate that contribute to the buffering property of the saliva (Cole & Eastoe, 1988; Marsh & Martin, 1999; Humphrey & Williamson, 2001). This property is important to reduce the cariogenic effect of acids produced by the bacteria. The major organic components of saliva are proteins and glycoproteins, such as mucin which serve to cleanse, aggregate and attach oral microorganism and contribute to dental plaque metabolism (Humphrey & Williamson, 2001). The proteins and glycoproteins influence the oral ecosystem in many ways. These proteins form components of the acquired pellicle and serves as host receptors that can interact with the adhesins on the bacterial surface. Thus, the acquired pellicle plays an important role to enhance the attachment and colonization of the pioneer bacteria on the tooth surface. Besides providing indigenous nutrient for the resident microflora, the organic constituents also include antimicrobial factors such as lysozyme, lactoferrin and the sialoperoxidase system (Samaranayake, 1998). The presence of these antimicrobial factors is important to monitor and control the population of microbes and fungi in the mouth.

The gingival crevice which is located between the junctional epithelium of the gingiva and teeth also provides a unique site for bacterial colonization as it includes both the hard and soft

tissues (Marcotte & Lavoie, 1998). It is relatively well protected from the forces that dislodge bacteria. The crevicular fluid provides a rich nutrient environment that is suitable for both the anaerobic and facultative microbial communities (Ross & Holbrook, 1984). Gingival Crevicular Fluid (GCF) is the exudate originating from the plasma that passes through the gingiva. Thus, the composition of the gingival fluid is similar to that of plasma; it contains proteins, albumin, leukocytes, antibodies and complements (Marcotte & Lavoie, 1998). The host proteins and glycoproteins are broken down by enzymes of the subgingival bacteria into peptides, amino acids and carbohydrates which contribute to the richness of the GCF (Marsh & Martin, 1999). The antibodies in the GCF are directed specifically against a variety of periodontally important organisms. These components may help to keep the flora of subgingiva in check by inhibiting colonization, acting as opsonins, or by activating the complement system (Schonfeld, 1992).

The coexistence of microorganisms with a wide spectrum of requirements in the oral biofilm is accommodated by various physico-chemical interactions within the microbial community. The streptococci forms the largest group of the resident oral microflora (White, 1991). According to Facklam (2002), hemolysis is one of the most useful characteristics for the identification of streptococci. Basically, the streptococci bacteria are divided into four groups; *mutans*-group, *salivarius*-group, *anginosus*-group and *mitis*-group (Marsh & Martin, 1999; Samaranyake, 2002). The members in *mutans*-group have been associated with the human dental caries (Samaranyake, 2002). The *salivarius*-group usually colonizes mucosal surfaces (tongue) and vestibular mucosa of the human mouth (Maiden, Lai & Tanner, 1992). The members in *anginosus*-group belong to carbon dioxide-dependent group (Maiden et al, 1992; Samaranyake, 2002) and are commonly found in abscesses of internal organs. They have been reported to play an important role in causing purulent disease in humans (Marsh & Martin, 1999). The members of *mitis*-group are opportunistic pathogens and are often associated with infective endocarditis (Samaranyake, 2002). Although this taxonomy has been widely used, Facklam (2002) has proposed five major groups of oral streptococci which include *mutans*-group, *salivarius*-group, *anginosus*-group, *mitis*-group and *sanguinis*-group based on phenotypic characteristics.

Streptococcus sanguinis (*Strep. sanguinis*) or previously known as *Streptococcus sanguis* (Handley, McNab & Jenkinson, 1999) belongs to the *mitis*-group (Samaranyake, 2002). *Strep. sanguinis* is capable of hydrolyzing arginine and esculin. It also has the ability to produce water-insoluble glucan α -1,3- + α -1,6- and water-soluble dextran α -1,6 from sucrose (Marsh & Martin, 1999).

Strep. sanguinis is one of the early colonizers of dental plaque (Maiden et al., 1992; Fathilah & Rahim, 2003) and also the most frequently identified streptococci from patients with subacute bacterial endocarditis (Facklam, 2002). Once a tooth erupted into the oral cavity, *Strep. sanguinis* will colonize its surfaces and the colonization is prior to the colonization of *Strep. mutans* (Caufield et al., 2000). The colonization of *Strep. sanguinis* to the tooth surfaces begins at the age of nine months in infants and the numbers was shown to increase with the age of the infants (Caufield et al., 2000; Lucas, Beighton, & Roberts, 2000) before the colonization of mutans streptococci. Lucas et al. (2000) reported that *Strep. sanguinis* forms about 39.4% of the oral residents of children.

Within the oral cavity, teeth are continuously coated with saliva, and this thin layer of deposited salivary components forms the acquired pellicle (Samaranayake, 2002). The *Strep. sanguinis* being the early colonizer-group, possesses the ability to bind to the acquired pellicle (Bleiweis & Oyston, 1993). The acquired pellicle contains receptors that facilitate the binding of the *Strep. sanguinis*. The binding involves specific interactions between cells and the components of acquired pellicle (Ke Gong, Mailloux & Herzberg, 2000).

The surface of *Strep. sanguinis* possesses hydrophobic properties (Nesbitt, Doyle, & Taylor, 1982; Fives-Taylor & Thompson, 1985; Morris & McBride, 1984; Black, Allan, Ford, Wilson & McNab, 2004) which have been associated with the presence of several amino acids with hydrophobic side chains (Nesbitt et al., 1982). This causes the cells to demonstrate a high affinity for hydrocarbon solvents (Nesbitt et al., 1982). However, it has been reported that the hydrophobic properties of oral streptococci diminished after repeated *in vitro* subculture (Westergren & Olsson, 1983). Conversely, a different observation was made when *Strep. sanguinis* was grown as biofilm. In biofilm, *Strep. sanguinis* appeared to be more hydrophobic compared to those in the planktonic form (Black et al., 2004) and this was accounted for by the difference in the protein expressions.

Streptococcus mitis (*Strep. mitis*) is a common species in the mouth and frequently predominates with *Strep. sanguinis* during the initial colonization of the tooth surface (Maiden et al., 1992). These bacteria also may produce dextran from sucrose (Melville & Russell, 1975). *Strep. mitis* is commonly found on the soft tissues of the cheeks, lips and the ventral surface of the tongue as they tend to adhere to non-keratinized mucosa in the mouth (Samaranayake, 2002). *Strep. mitis* is distinguished from other oral streptococci by being negative for esculin hydrolysis, inulin fermentation and extracellular polysaccharide formation (Maiden et al., 1992). *Strep. mitis* also has been associated with endocarditis (Samaranayake, 2002). Following dental treatment, lack of prophylactic antibiotics may lead to the development of endocarditis. It has been shown that endocarditis is caused by penicillin-resistant *Strep. mitis* (Huang, Chiou, Liu, & Hsieh, 2002). In addition, it has been shown that some strains of *Strep. mitis* appeared to be associated with high-level resistance to beta-lactams antibiotics which have been used in dental therapeutics (Nakayama et al., 2006).

Apart from streptococcal group, most of the oral *Actinomyces* species have been isolated at the approximal sites and within the gingival crevice (Marsh & Martin, 1999). *Actinomyces* are gram-positive and their cells usually appear as short rods or long filaments (Maiden et al., 1992). All *Actinomyces* ferment glucose and produce succinic, acetic, formic and lactic acids (Melville & Russell, 1975; Marsh & Martin, 1999; Samaranayake, 2002).

Actinomyces viscosus (*A. viscosus*) strains are catalase-positive and facultative aerobes which grow well in the presence of oxygen. In plate cultures they form long hyphal elements in the early stages before dividing into irregular rod-shaped forms (Melville & Russell, 1975). *A. viscosus* has been associated with the development of extensive subgingival dental plaque, periodontal diseases (Melville & Russell, 1975) and supragingival plaque (Bleiweis & Oyston, 1993).

Like other oral bacteria, *A. viscosus* have fibrils on their cell surfaces that may play a role in its adhesion to surfaces. A study by Wheeler and Clark (1980) had reported that the adhesion of *A. viscosus* to saliva-coated substratum was mediated by surface fibrils. In a later study by

Rozen, Bachrach, Bronshteyn, Gedalia, and Steinberg (2001), the adhesion was found to be fructan-dependent. *A. viscosus* produces fructosyltransferase (FTF) which is involved in the synthesis of fructans with sucrose as the substrate (Rozen et al., 2001). The affinity of *A. viscosus* was shown to be higher towards fructan than glucans. Inhibition of fructan synthesis resulted in a reduction in the adhesion of *A. viscosus* to the substratum (Rozen et al., 2001).

2.1 Environmental Factors Affecting The Optimal Growth Of Microbes In The Oral Cavity

Temperature is a crucial factor that can affect key parameters associated with the oral habitat, such as pH, ion activity, aggregation of molecules and gas solubility. Oral bacteria grow optimally at the human body temperature of 37°C (Schonfeld, 1992; Marcotte & Lavoie, 1998). The temperature in the mouth varies at different sites and this variation may influence the proportions of the bacterial species (Marsh & Bradshaw, 1999).

In general, the oral microorganisms can be divided into the aerobes and anaerobes based on their ability to grow in the presence or absence of oxygen. Majority of the oral microbes are however, either facultative anaerobic or obligate anaerobic. The former refers to microbes that can grow in the presence or absence of oxygen while the latter refers to microbes that cannot grow in the presence of oxygen (Schonfeld, 1992). Only a few is truly aerobic species that requires oxygen for their optimal growth. The dorsum of the tongue and the buccal and palatal mucosa provide aerobic environments and thus supports the growth of facultative anaerobic bacteria (Marcotte & Lavoie, 1998). Anaerobic organisms such as bacteroides, fusobacteria, spirochaetes and some actinomycetes will only multiply in a reduced surrounding and can be found mostly in the gingival crevice and in the deeper layer of dental plaque (Ross & Holbrook, 1984).

pH or hydrogen ion concentration of an environment affects the survival of microorganisms while some oral microbes require a pH around neutrality for optimal growth whereas the cariogenic species prefer acidic condition to grow (Marsh & Bradshaw, 1999). For example, *Strep. sanguinis* and *A. viscosus* prefer neutral environment, *Lactobacillus acidophilus* prefers an acidic condition and *Porphyromonas gingivalis* requires alkaline pH for optimal growth (Marsh & Martin, 1992). These microorganisms are usually sensitive to the extremes of acid or alkali. The optimum pH in the mouth is within the range of 6.7 to 7.3 and is continuously regulated by the saliva (Marcotte & Lavoie, 1998). Although there are different sites in the mouth, a study by Aframian, Davidowitz and Benoliel (2006) has shown that the mean pH of all sites was 6.78, which is around neutrality.

The fluctuating pH value in the mouth is influenced by the food consumed. Following sugar intake, pH in the plaque usually falls rapidly to below pH 5.0 (Marsh & Martin, 1992; Marcotte & Lavoie, 1998). On the other hand, bicarbonate has been shown to increase the pH value to 8.06 (Anderson & Orchardson, 2003). Since bacteria in the plaque is exposed to fluctuating pH depending on the sugar intake frequency per day, most of the predominant bacteria in the plaque can tolerate a slight change in the pH. However, their growth are inhibited or suppressed when the condition becomes extreme.

Members of the microbial community depend on the habitat for nutrients supply. The nutrients required for bacterial growth can be divided into endogenous and exogenous nutrients. The host provides the endogenous nutrients whereas diet is the source of the exogenous nutrients (Marcotte & Lavoie, 1998).

The main source of endogenous nutrients is the saliva, which contains amino acids, proteins and glycoproteins (Samaranayake, 1998). In addition, the gingival crevice fluid components around the teeth also provide albumin and other host proteins (Schonfeld, 1992). The varieties in the source of endogenous nutrients contribute to the variation of oral microbes in the mouth.

Fermentable carbohydrate influences the ecology of the mouth (Bowden & Li, 1997; Ye Jin, L. P. Samaranayake, L. Samaranayake, & Yip, 2004). Carbohydrates can be broken down for energy metabolism (Marsh & Bradshaw, 1999). The acids which are produced in the process may lead to low pH in the oral cavity (Marcotte & Lavoie, 1998). The levels of bacteria that are aciduric for example, *Streptococcus mutans* and lactobacilli increase, while *Strep. sanguinis* decreases when a person frequently consumes carbohydrates (Marsh & Martin, 1999).

3. CONCLUSION

The oral cavity is comprised of many surfaces, with each of the surface is coated with specific bacteria. The flow rate of saliva, the endogenous and exogenous nutrients that can be found in the mouth and the oral pH contribute to the variation of the bacteria distribution. Some of the bacteria are species-specific to certain surfaces but some do not.

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