Preventive Dentistry

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Microbiology of caries

Oral cavity is a unique ecological niche, which is warm, moist and relatively opens to the outer environment. Tooth surfaces as well as dental plaque constantly encounter different challenges from food intake, speech, and so on. Bacteria grow in two different ways: planktonic and biofilm forms.

Microbial ecology in the oral cavity

It has been estimated that the human body is made up of over 1×10^{14} cells, of which 90 % are the microorganisms that comprise the resident microflora of the host.

The resident microflora dynamically interacts with the human body, contributing directly and indirectly to the normal development of the physiology, nutrition, and defense systems of the host.

The composition of the resident microflora is distinct in different habitats/ niches such as the oral cavity. The resident microflora has a diverse composition, consisting of a wide range of Gram-positive and Gram-negative bacterial species, as well as yeasts and other types of microorganism. In addition, the composition of the oral microflora will change as the biology of the mouth alters over time, the oral cavity, for example, the tooth surfaces provide distinct binding factors for microorganisms. Moreover, the mouth is continuously bathed with saliva at a temperature of 35–36 °C and a pH of 6.75–7.25. The nutritional condition of the oral cavity is often described as “feast or famine”, further exerting far-reaching influence on the composition of microflora.

Acquisition of the resident oral microflora

The mouth of the newborn baby is usually sterile. Acquisition depends on the successive transmission of microbes to the site of potential colonization. In the mouth, although organisms can be derived from water, food and other nutritious fluids, the main route of transmission is via saliva. Molecular typing studies have shown that the acquisition of oral streptococci and Gram-negative species in children is predominantly from their mother (vertical transmission).

The diversity of the oral microflora increases during the first months of life. The earliest colonizers of a site are termed pioneer species, and these are streptococci, particularly *S. salivarius*, *S. mitis* and *S. oralis*. With time, Gram-negative anaerobes appear, including *Prevotella melaninogenica*, *Fusobacterium nucleatum* and *Veillonella* spp. The eruption of the dentition creates novel habitats for microbial colonization because teeth provide the only non-shedding surfaces within the body to which the resident microflora can normally attach. This results in the undisturbed accumulation of large communities of bacteria, especially at stagnant sites. Microbial deposits on teeth are an example of a microbial biofilm.

Mutans streptococci and *S. sanguinis* generally only appear in the normal mouth following tooth eruption, and the development and maturation of dental biofilms create conditions suitable for a greater range of more fastidious bacteria. In addition, the flow of gingival crevicular fluid (GCF) around the gingival margin provides a source of essential nutrients for many obligate anaerobes.

The oral microflora continues to increase in diversity until, eventually, a stable situation is reached, termed the climax community. The microbial populations that comprise such a climax community remain stable over time, despite regular minor disturbance to the local environment due to changes in diet, hormonal levels, oral hygiene, etc. The stability is termed 'microbial homeostasis'; this is not a passive response by the organisms, but reflects a highly dynamic equilibrium between the resident microflora and the local environmental conditions at that site in the host. A major change to the habitat, such as frequent sugar consumption, can lead to imbalances among the species comprising the resident microflora, a consequence of which can be an increased predisposition to disease.

Changes in the microflora can, however, occur as a direct or an indirect effect of aging. Direct effects, such as the waning of cell- mediated immunity, can lead to increases in the carriage of non-oral bacteria (e.g. staphylococci and enterobacteria). Indirect effects include the increased wearing of dentures among the elderly, which promotes colonization by yeasts. Older people are also more likely to be on long-term medication, a common side-effect of which is a reduced salivary flow rate promoting colonization by lactobacilli and yeasts.

Site distribution of oral bacteria Although the mouth is highly selective for the microorganisms that are able to colonize and become established, more than 700 different types have been detected in the mouth. The mouth is not a homogeneous environment for microbial colonization. Distinct micro- habitats exist such as mucosal surfaces (palate, cheek, tongue, etc.), the various surfaces of teeth (smooth, approximal, fissures) and the gingival crevice. For example, the tongue has a highly papillated surface providing protection in the crypts to fastidious bacteria including obligate anaerobes. Indeed, the tongue can act as a reservoir for many species that are commonly found in dental plaque. In this way, the biological and physical properties of each site result in only a subset of these organisms (often 20–30 distinct types) being able to predominate at an individual site.

Ecological factors affecting the growth and metabolism of oral bacteria

The mouth provides both a friendly and a hostile environment for microbial growth. Resident oral microorganisms are adapted to use endogenous (host-derived) nutrients for growth (e.g. salivary proteins and glycoproteins), but superimposed on this can be sudden and irregular intakes of dietary carbohydrates in excess (e.g. readily fermentable sugars such as glucose, fructose and sucrose). The mouth is overtly aerobic, and yet obligate anaerobes and facultative anaerobic bacteria are able to persist within biofilms on oral surfaces (tongue, teeth) and comprise the most numerous group of bacteria at these sites. Organisms have to attach firmly to a surface to avoid being washed away by the flow of saliva and swallowed. Thus, the majority of organisms (and the most disease) are found at protected and stagnant sites around the dentition.

Saliva plays other roles in regulating the growth and metabolic activity of the oral microflora.

Saliva:

- Contains glycoproteins and proteins that act as the primary source of carbohydrates, peptides and amino acids for microbial growth. Bacteria cooperate to degrade the oligosaccharide side-chains of glycoproteins such as mucins. Acid is produced relatively slowly from the metabolism of these compounds, and so there is little risk of enamel demineralization.
- Considers a sufficient source of nutrients to sustain the growth of a natural and diverse oral microflora in the absence of other nutrients.
- Delivers a spectrum of innate and specific immune host defense factors which are essential to the maintenance of a healthy mouth.

A carbohydrate-rich diet increases the acid production and growth rate of many oral bacteria. Thus, it has been shown that the accumulation of dental plaque after 4 days, as regards extension, weight and actual numbers of bacteria, is higher when individuals consume a diet supplemented with sucrose candies compared with a control diet.

Excess dietary carbohydrate is stored by some species as intracellular glycogen-like storage compounds, which can be metabolized to acid at a later time in the absence of fermentable exogenous substrates. All of these factors can contribute towards increasing the likelihood of developing caries.

Dental biofilms: development, structure, composition and properties

In order to persist, oral micro-organisms have to attach to a surface and grow; otherwise they will be lost from the habitat. The term 'biofilm' has been used to signify the common features among biofilms forming on teeth and biofilms forming in other environments. Biofilm is defined as aggregates of bacterial cells attached to a surface and embedded in a polymeric matrix that is self-produced and helps the community to gain tolerance against antimicrobials and host defenses.

Development of dental biofilms

The development of dental biofilms can be divided into several stages:
- Pellicle formation
- Attachment of single bacterial cells (0–24 h) is a reversible attachment.
- Growth of attached bacteria by specific molecules on their cells interact with the complementary receptor proteins on the pellicle surface leading to the formation of distinct micro-colonies (4–24 h).
- Microbial succession (and co-adhesion) leading to increased species diversity concomitant with continued growth of micro-colonies (1–7 days)
- Climax community/mature biofilm (1 week or older) including synthesis of extra polysaccharides.

Biofilm formation is a highly dynamic process, and that attachment, growth, removal and reattachment of bacteria may occur at the same time. There are direct- and indirect-mediated changes in bacterial gene expression during biofilm development.

Pellicle formation

- The teeth are always covered by an acellular proteinaceous film; this is the pellicle that forms on the 'naked' tooth surface within minutes to hours before microbial colonization.
- The major constituents of the pellicle are salivary glycoproteins, phosphoproteins, lipids and, to a lesser extent, components from the GCF.
- In uncolonized areas the pellicle reaches a thickness of 0.01–1 μm within 24 h.

- Remnants of cell walls from dead bacteria, and other microbial products (e.g. glucosyltransferases and glucans), have also been identified in the pellicle.
- Some salivary molecules undergo conformational changes when they bind to the tooth surface; this can lead to exposure of new receptors for bacterial attachment.
- The pellicle plays an important modifying role in caries and erosion because of its permeable-selective nature restricting transport of ions in and out of the dental hard tissues.
- The presence of a pellicle inhibits subsurface demineralization of enamel *in vitro*.
- The composition of the pellicle may aid in determining the composition of the initial microflora. It has been speculated that the surface characteristics of different dental hard tissues and dental materials may influence the profile of amino acids in the pellicle and thereby modify the number of potential adsorption sites for different bacterial species.

Microbial colonization

Microbial colonization of teeth requires that bacteria adhere to the surface. As the microbial cell approaches the pellicle-coated surface, long-range but relatively weak physicochemical forces between the two surfaces are generated. Initially, bacteria are non-specifically associated with the tooth surface under the net influence of van der Waal's attractive forces as well as repulsive electro- static forces. Within a short time, these weak physicochemical interactions may become stronger owing to adhesins on the microbial cell surface becoming involved in specific, short-range interactions with complementary receptors in the acquired pellicle.

Initial microbial colonization

Cocci are probably the first to adhere because they are small and round. The first or primary colonizers tend to be aerobic (oxygen-tolerant) bacteria including Neisseria and Rothia. The streptococci, the *Gram-positive facultative rods*, and the *actinomycetes* are the main organisms in both early fissure and approximal plaque. As plaque oxygen levels fall, the proportions of Gram-negative rods, for example *fusobacteria,* and *Gram-negative cocci* such as *Veillonella* tend to increase and they are predominating in the subgingival plaque during the later phases of plaque development.
Irrespective of the type of tooth surface (enamel or root), the initial colonizers constitute a highly selected part of the oral microflora, mainly *S. sanguinis, S. oralis* and *S. mitis*. Together, these three-streptococcal species account for 95% of the streptococci and 56% of the total initial microflora.

The modification of pellicle constituents is probably an important factor in the regulation of colonization.

Microbial succession

The initial establishment of a streptococcal flora appears to be a necessary forward for the subsequent proliferation of other organisms. Such population shifts are known as microbial succession. As the microbiota ages the most striking change is a shift from a *Streptococcus*-dominated plaque to a plaque dominated by *Actinomyces.*

The principle of microbial succession is, briefly, that pioneer bacteria create an environment that is either more attractive to secondary invaders or increasingly unfavorable to themselves because of a lack of nutrients, accumulation of inhibitory metabolic products, and/or increase in anaerobiosis, etc. In this way, the resident microbial community is gradually replaced by other species more suited to the modified habitat. The secondary colonizers also attach to the established pioneer species via adhesin–receptor interactions (termed coaggregation or coadhesion). As dental biofilms develop, some of the bacteria produce polysaccharides, especially from the metabolism of sucrose, and these contribute to the biofilm matrix. the matrix is biologically active and is involved in retaining nutrients, water and key enzymes within the biofilm. As the composition of the developing biofilm becomes more diverse, the bacteria can interact both in a conventional biochemical manner and via specific signaling molecules.

As the bacterial deposits become thicker, a lowering of the oxygen concentration (increased anaerobiosis) is one of the factors that help to drive microbial succession. Thus, in developing coronal plaque, a progressive shift is observed from mainly aerobic and facultatively anaerobic species in the early stages to a situation in which facultatively and obligately anaerobic organisms predominate after 9 days.

Microbial composition of the climax community (mature biofilm)

Environmental conditions on a tooth are not uniform. Differences exist in the degree of protection from oral removal forces and in the gradients of many biological and chemical factors that influence the growth of the resident oral microflora on particular surfaces. These differences will be reflected in the composition of dental biofilms, particularly at sites so obviously distinct as approximal surfaces, occlusal fissures and gingival crevices.

The composition of dental plaque is diverse, and includes a range of Gram-positive and Gram-negative bacteria, most of which are facultatively or obligately anaerobic. Of relevance to dental caries is the presence in dental biofilms of high numbers of acid-producing Gram-positive cocci, such as the mutans streptococci, other streptococci (non-mutans streptococci) and Gram-positive rods (lactobacilli and some *Actinomyces* spp.). However, the acidogenic potential of these bacteria can be reduced by other organisms in plaque, such as the anaerobic Gram-negative coccus, *Veillonella* spp., which converts lactic acid to weaker acids as part of a food chain, or by bacteria generating alkali from arginine (*S. sanguinis*) or urea (*S. salivarius*, *A. naeslundii*). This demonstrates the complexity of the challenge when attempting to find correlations between the microbial composition of dental plaque and the development of caries.

From studies, there is specific type of bacteria to develop dental caries following the type of tooth surfaces:

- Smooth surfaces
S. mutans, S. salivaris, Actinomyces.

- Occlusal fissures
S. mutans, S. sanguinis, *lactobacilli*, *Actinomyces* spp.

- Approximal surfaces
Actinomyces spp., Gram negative bacteria., Fewer streptococci.

- Cervical surfaces
Actinomyces spp., Anaerobic bacteria.