DENTAL PLAQUE BIOFILM

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DENTAL PLAQUE BIOFILM

Oral biofilms are functionally and structurally organized polymicrobial communities that are embedded in an extracellular matrix of exopolymers on mucosal and dental surfaces.

Dental plaque is defined clinically as a structured resilient yellow-grayish biofilm that adheres firmly to the intraoral hard surfaces, including removable and fixed restorations. The tough extracellular matrix makes it impossible to remove plaque by rinsing or the use of sprays. Plaque can thus be differentiated from other deposits that may be found on the tooth surface such as materia alba and calculus.

Materia alba refers to soft accumulations of bacteria, food deposits, and tissue cells that lack the organized structure of dental plaque and are easily displaced with a water spray.

Calculus is formed as a hard mineralized deposit of dental plaque and is generally covered by a layer of unmineralized plaque

Microorganisms represent the main component of dental plaque, with approximately $\mathbf{10^{11}}$ bacteria contained in one gram of plaque (wet weight). The number of bacteria in supragingival plaque on a single tooth surface can exceed $\mathbf{10^{9}}$. In a periodontal pocket, counts can range from $\mathbf{10^{3}}$ bacteria in a healthy crevice to $\mathbf{> 10^{8}}$ bacteria in a deep pocket.

Using highly sensitive molecular techniques for microbial identification, it has been estimated that more than 500 distinct microbial phenotypes can be present as natural inhabitants of dental plaque. Any individual may harbour 150 or more different species. Non-bacterial microorganisms that are found in plaque include archaea, yeasts, protozoa, and viruses.

Differences between dental plaque, materia alba and calculus

	Materia alba	Dental plaque	Claculus
1	White cheese-like accumulations	Resilient clear to yellow grayish	Hard deposits formed by mineralization of dental plaque
2	A soft accumulation of salivary proteins	Primarily composed of bacteria in a matrix of salivary proteins	
3	Lack of organized structure (not complex) as dental plaque)	Considered as a biofilm	Generally covered by a layer of un-mineralized dental plaque
4	Easily displaced by a water spray	Removed only by mechanical rinsing (tooth brushing)	Removed by scaling

Dental plaque is broadly classified as supragingival or subgingival based on its position on the tooth surface toward the gingival margin.

Supragingival plaque is found at or above the gingiva, when in direct contact with the gingival margin it is referred to as marginal plaque.

Supragingival plaque typically demonstrates a multilayered accumulation of bacterial morphotypes. Gram-positive cocci and short rods are predominant at the tooth surface, whereas gram-negative rods and filaments, as well as spirochetes, predominate on the outer surface of the mature plaque mass.

Subgingival plaque is found below the gingival margin between the tooth and the gingival pocket epithelium

In general, the subgingival microbiota differs in composition from the supragingival plaque, primarily because of the local availability of blood products and a low reduction-oxidation (redox) potential, which characterizes the anaerobic environment.

The environmental parameters of the subgingival region differ from those of the supragingival region. The gingival crevice or pocket is bathed by the flow of crevicular fluid, which contains many substances which bacteria may use as nutrients.

The tooth-associated cervical plaque, adhering to the root cementum, does not markedly differ from that observed in gingivitis. At this location, filamentous microorganisms dominate, but cocci and rods also occur. However, in the deeper parts of the pocket, the filamentous organisms become fewer in number, and in the apical portion, they seem to be virtually absent. Instead, the microbiota is dominated by smaller organisms without a particular orientation.

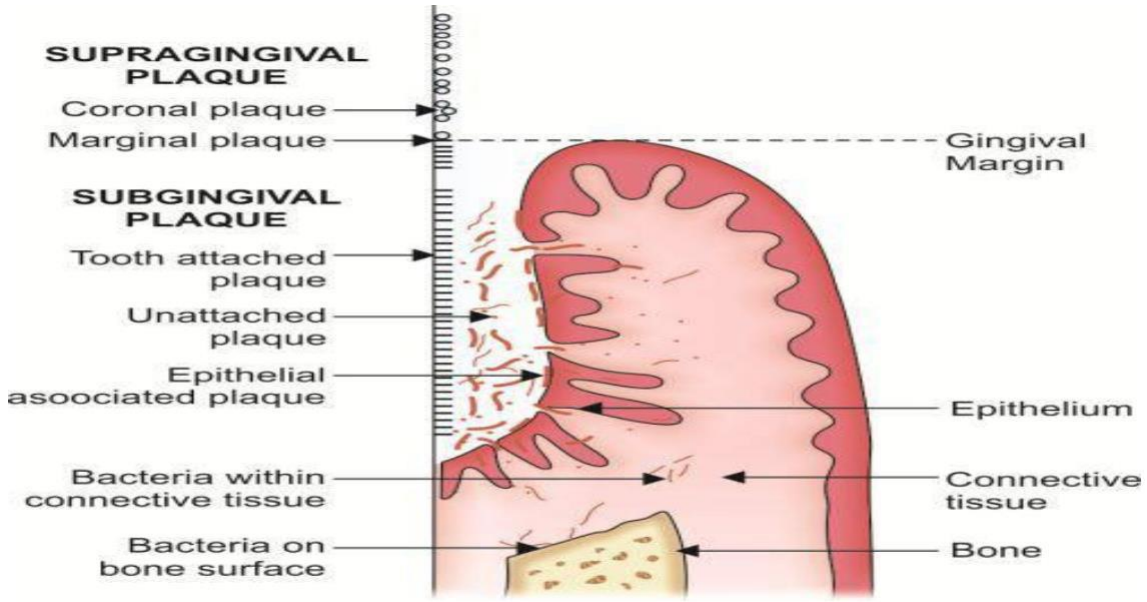
The apical border of the plaque mass is separated from the junctional epithelium by a layer of host leukocytes, and the bacterial population of this apical tooth-associated region shows an increased concentration of gram-negative rods.

The layers of microorganisms facing the soft tissue lack a definite inter-microbial matrix and contain primarily gram-negative rods and cocci as well as large numbers of filaments, flagellated rods, and spirochetes. Host tissue cells (e.g., white blood cells and epithelial cells) may also be found in this region. Bacteria are also found within the host tissues, such as in the soft tissues and within epithelial cells, as well as in the dentinal tubules.

The composition of the subgingival plaque depends on pocket depth; the apical part is more dominated by spirochetes, cocci and rods, whereas in the coronal part, more filaments are observed.

The site specificity of plaque is significantly associated with diseases of the periodontium. Marginal plaque, for example, is of prime importance in the initiation and development of gingivitis. Supragingival plaque and tooth-associated subgingival plaque are critical in calculus formation and root caries; whereas tissue-associated subgingival plaque is important in the tissue

destruction that characterizes different forms of periodontitis. Biofilms also form on artificial surfaces exposed to the oral environment such as prostheses and implants.



Accumulation of a Dental Plaque Biofilm

The process of plaque formation can be divided into several phases:

1- The formation of the pellicle on the tooth surface.

2- Initial adhesion/attachment of bacteria.

3- Colonization/plaque maturation.

Formation of the Pellicle

All surfaces in the oral cavity including hard and soft tissues, are coated with a layer of organic material known as the acquired pellicle. The pellicle on the tooth surface consists of more than 180 peptides, proteins, and glycoproteins including keratins, mucins, proline-rich proteins, phosphoproteins, histidine-rich proteins, and other molecules that can function as adhesion sites (receptors) for bacteria. Salivary pellicles can be detected on clean enamel surfaces within 1 minute after tooth cleaning. By two hours, the pellicle is essentially in equilibrium between adsorption and detachment, although further pellicle maturation can be observed for several hours. Consequently, bacteria that adhere to tooth surfaces do not contact the enamel directly but interact with the acquired enamel pellicle.

Initial Adhesion/Attachment of Bacteria

Tooth brushing removes most but not all bacteria from the exposed surfaces of teeth. However, recolonization begins immediately, and bacteria can be detected within 3 minutes of introducing sterile enamel into the mouth.

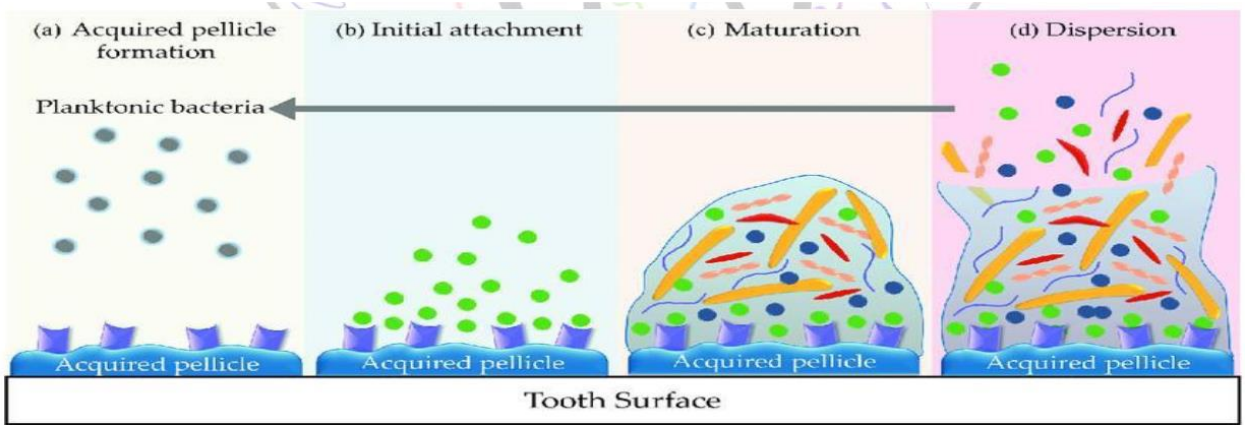
The initial steps of transport and interaction with the surface are essentially nonspecific (i.e., they are the same for all bacteria). Initial adhesion is performed by specific interactions between microbial cell surface "adhesin" molecules and receptors in the salivary pellicle that determine whether a bacterial cell will remain associated with the surface. Only a relatively small proportion of oral bacteria possess adhesins that interact with receptors in the host pellicle, and these organisms are generally the most abundant bacteria in biofilms on tooth enamel shortly after cleaning.

Over the first 4 to 8 hours, 60% to 80% of bacteria present are members of the genus Streptococcus. Other bacteria commonly present at this time include species that cannot survive without oxygen (obligate aerobes), such as Haemophilus spp. And Neisseria spp., as well as organisms that can grow in the presence or absence of oxygen (facultative anaerobes) including Actinomyces spp. and Veillonella spp. These species are considered the "primary colonizers" of tooth surfaces.

The primary colonizers' function

1. provide new binding sites for adhesion by other oral bacteria.

2. The metabolic activity of the primary colonizers modifies the local microenvironment in ways that can influence the ability of other bacteria to survive in the dental plaque biofilm. For example, by removing oxygen, the primary colonizers provide conditions of low oxygen tension that permit the survival and growth of obligate anaerobes.



Colonization and Plaque Maturation

The primary colonizing bacteria adhered to the tooth surface provide new receptors for attachment by other bacteria in a process known as "co-adhesion." Together with the growth of adherent microorganisms, coadhesion leads to the development of microcolonies and eventually to a mature biofilm. Different species or even different strains of a single species have distinct sets of coaggregation partners.

Fusobacteria coaggregate with all other human oral bacteria while Veillonella spp., Capnocytophaga spp. and Prevotella spp. bind to streptococci and/or actinomyces. Each new cell

becomes itself a new surface and therefore, may act as a coaggregation bridge to the next potentially accreting cell type that passes by.

The transition from early supragingival dental plaque to mature plaque growing below the gingival margin involves a shift in the microbial population from primarily gram-positive organisms to high numbers of gram-negative bacteria. Therefore, in the later stages of plaque formation coaggregation between different gram-negative species is likely to predominate. Examples of these types of interactions are the coaggregation of F. nucleatum with P. gingivalis or T. denticola.

Factors Affecting Supragingival dental plaque formation

1. Topography of Supragingival Plaque

Early plaque formation on teeth follows a typical topographic pattern with initial growth along the gingival margin and from the interdental space (areas protected against shear forces). Later, a further extension in the coronal direction can be observed. This pattern may fundamentally change when the tooth surface is rough or contains irregularities that offer a favourable growth path. Plaque formation can also start from grooves, cracks, or pits.

Surface irregularities are also responsible for the so called “individualized plaque growth pattern, which is reproduced in the absence of optimal oral hygiene. This phenomenon illustrates the importance of surface roughness in plaque growth, which should lead to proper clinical treatment options.

2. Individual Variables Influencing Plaque Formation

The rate of plaque formation differs significantly between subjects, differences that might overrule surface characteristics. A distinction is often made between “heavy” (fast) and “light” (slow) plaque formers. It has shown that the clinical wettability of the tooth surfaces, the saliva-induced aggregation of oral bacteria, and the relative salivary flow conditions around the sampled teeth explained 90% of the variation.

3. Variation within the Dentition

Within dental arch large differences in plaque growth rate can be detected. In general early plaque formation occurs faster: in the lower jaw (when compared to the upper jaw); in the molar areas; on the buccal tooth surfaces when compared to palatal sites (especially in the upper jaw); and in the interdental regions when compared to the buccal or lingual surfaces.

4. Impact of Gingival Inflammation and Saliva

Plaque formation is more rapid on tooth surfaces facing inflamed gingival margins than on those adjacent to healthy gingival. Due to increase in crevicular fluid production enhances plaque formation.

Probably, some substance(s) from this exudate (e.g. minerals, proteins, or carbohydrates) favour both the initial adhesion and/or the growth of the early colonizing bacteria.

5. The Impact of Patient's Age

The subject's age does not influence denovo plaque formation. The developed plaque in the older patient group resulted, however, in a more severe gingival inflammation, which seems to increase susceptibility to gingivitis with ageing.

6. Spontaneous Tooth Cleaning

Many clinicians still believe that plaque is removed spontaneously from the teeth, such as during eating. However, based on the firm attachment between bacteria and surface, this seems unlikely. Even in the occlusal surfaces of the molars, plaque remains, even after chewing fibrous food carrots, apples, or chips.

7. Metabolism of Dental Plaque Bacteria

Most nutrients for dental plaque bacteria originate from saliva or GCF, although the host diet provides an occasional important food supply. The transition from gram-positive to gram-negative microorganisms observed in the structural development of dental plaque is paralleled by a physiologic transition in the developing plaque.

The growth of P. gingivalis is enhanced by metabolic byproducts produced by other microorganisms, such as succinate from Capnocytophaga ochrecea and protohemefrom Campylobacter rectus. Overall, the total plaque population is more efficient than any one constituent organism at releasing energy from the available substrates.

Metabolic interactions occur also between the host and plaque microorganisms. Increases in steroid hormones are associated with significant increases in the proportions of P. intermedia found in subgingival plaque. These nutritional interdependencies are probably critical to the growth and survival of microorganisms in dental plaque and may partly explain the evolution of highly specific structural interactions observed among bacteria in plaque.

Communication between Biofilm Bacteria

Bacterial cells do not exist in isolation. In a biofilm, bacteria have the capacity to communicate with each other. One example of this is quorum sensing, in which bacteria secrete a signalling molecule that accumulates in the local environment and triggers a response such as a change in the expression of specific genes once they reach a critical threshold concentration. The threshold concentration is reached only at a high-cell density, and therefore bacteria sense that the population has reached a critical mass or quorum.

There is some evidence that intercellular communication can occur after cell-cell contact and, in this case, may not involve secreted signaling molecules.

Biofilms and Antimicrobial Resistance

Bacteria growing in microbial communities' adherent to a surface do not "behave" the same way as bacteria growing suspended in a liquid environment (in a planktonic or unattached state).

For example, the resistance of bacteria to antimicrobial agents is dramatically increased in biofilm. Almost without exception, organisms in a biofilm are ***1000 to 1500 times*** more resistant to antibiotics than in their planktonic state.

The mechanisms of this increased resistance differ from species to species, from antibiotic to antibiotic, and for biofilm growing in different habitats.

1. the resistance of bacteria to antibiotics is affected by their nutritional status, growth rate, temperature, pH, and prior exposure to sub-effective concentrations of antimicrobial agents. Variations in any of these parameters will thus lead to a varied response to antibiotics within a biofilm.

2. An important mechanism of resistance appears to be the slower rate of growth of bacterial species in a biofilm, which makes them less susceptible to many but not all antibiotics.

3. The biofilm matrix, although not a significant physical barrier to the diffusion of antibiotics, does have certain properties that can retard antibiotic penetration.

4. In addition, extracellular enzymes such as β-lactamases, formaldehyde lyase, and formaldehyde dehydrogenase may become trapped and concentrated in the extracellular matrix, thus inactivating some antibiotics (especially positively charged hydrophilic antibiotics).

Recently, "super-resistant" bacteria were identified within a biofilm. These cells have multidrug resistance pumps that can extrude antimicrobial agents from the cell. Since these pumps place the antibiotics outside the outer membrane, the process offers protection against antibiotics that target, for example, cell wall synthesis. The penetration and efficacy of antimicrobials against biofilm bacteria are critical issues for the treatment of periodontal infections.