Periodontics

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 deposit, and tissue cells that lack the organized structure of dental plaque and are easily displaced with a water spray, whereas 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 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 10^9. In a periodontal pocket, counts can range from 10^3 bacteria in a healthy crevice to > 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 harbor 150 or more different species. Non- bacterial microorganisms that are found in plaque include archaea, yeasts, protozoa, and viruses.



Clinical picture of 10-day-old supragingival plaque. Balck rows indicate early signs of gingival inflammation.



Supragingival calculus is depicted on the buccal surface of maxillary molars adjacent to the orifice for the parotid duct.

Differences between bacterial deposits

	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)	

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 latter; when in direct contact with the gingival margin it is referred to as marginal plaque

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

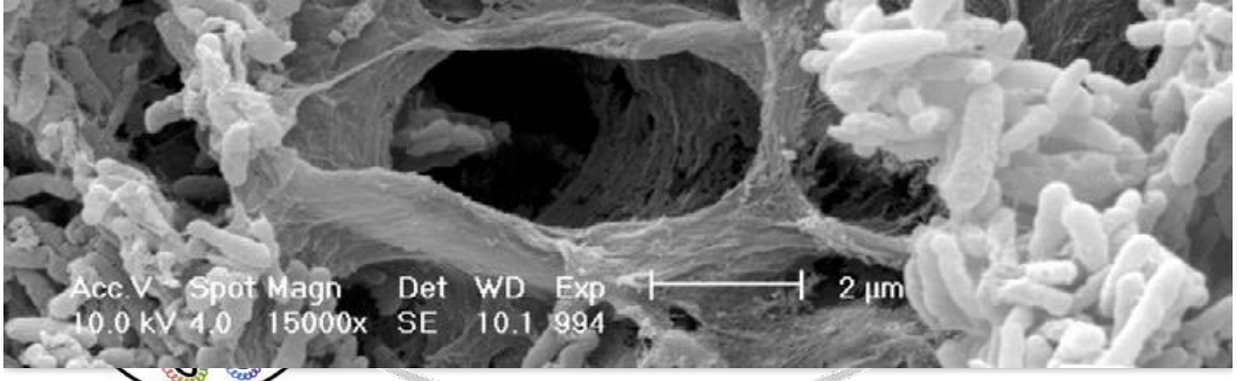
Supragingival plaque typically demonstrates a stratified organization of a multilayered accumulation of bacterial morphotypes. Gram-positive cocci and short rods predominat at the tooth surface, whereas gram-negative rods and filaments, as well as spirochetes, predominate in the outer surface of the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

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. Host inflammatory cells and mediators are likely to have considerable influence on the establishment and growth of bacteria in the subgingival region. Both morphologic and microbiologic studies of subgingival plaque reveal distinctions between the tooth-associated and soft tissue-associated regions of subgingival plaque.

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. This plaque is dominated by gram positive rods and cocci, including S. mitis, S. sanguinis, Actinomyces oris . However, in the deeper parts of the pocket, the filamentous organisms become fewer in numbers, 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 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 tooth surface consists of more than 180 peptides, proteins, and glycoproteins including keratins, mucins, proline-rich proteins, phosphoproteins e.g., statherin), histidine-rich proteins, and other molecules that can function as adhesion sites (receptors) for bacteria. Salivary pellicle can be detected on clean enamel surfaces within 1 minute after introduction into the mouths of volunteers. 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; however, the pellicle is not merely a passive adhesion matrix.

Many proteins retain enzymatic activity when incorporated into the pellicle, and some of these, such as peroxidases, lysozyme and a-amylase, may affect the physiology and metabolism of adhering bacterial cells.

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). The proteins and carbohydrates that are exposed on the bacterial cell surface become important once the bacteria are in loose contact with the acquired enamel pellicle. It is the specific interactions between microbial cell surface "adhesin" molecules and receptors in the salivary pellicle that determines 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 provide new binding sites for adhesion by other oral bacteria. 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 a new receptors for attachment by other bacteria in a process known as “coadhesion.” Together with growth of adherent microorganisms, coadhesion leads to the development of micro colonies and eventually to a mature biofilm.

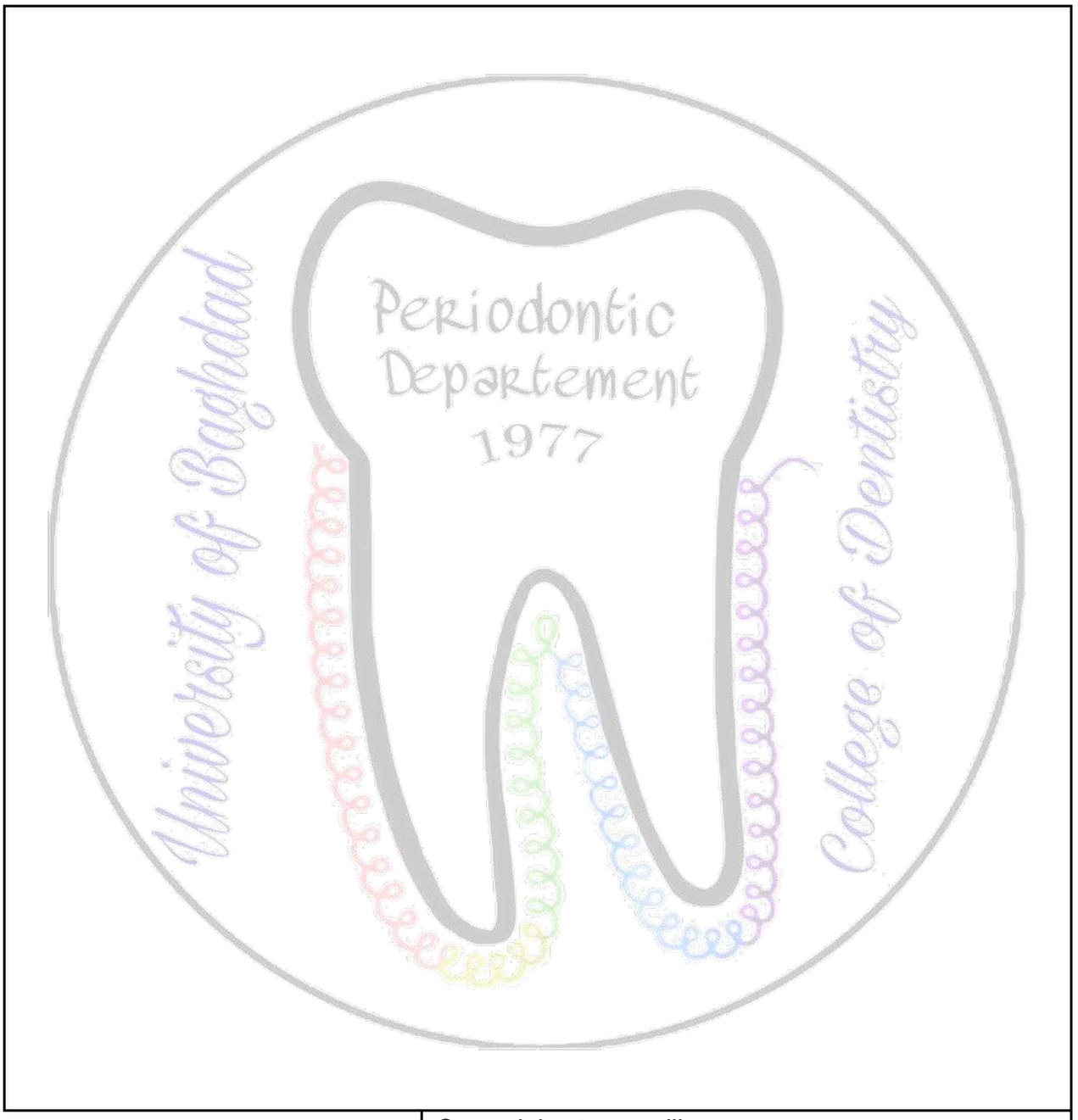
Different species or even different strains of a single species have distinct sets of coaggregation partners. Fusobacteria coaggregate with all other human oral bacteria while Veillonella spp., Capnocytophaga spp. and Prevotella spp. bind to streptococci and/or actinomyces. Each newly cell becomes itself a new surface and therefore, may act as a coaggregation bridge to the next potentially accreting cell type that passes by.

Well-characterized interactions of secondary colonizers with early colonizers include the coaggregation of F. nucleatum with S. sanguinis, Prevotella loescheii with A. oris, and Capnocytophaga ochracea with A. oris. Streptococci show intrageneric coaggregation, allowing them to bind to the nascent monolayer of already bound streptococci.

Secondary colonizers ,such as Prevotella intermedia, Prevotella loescheii, Capnocytophaga spp., F. nucleatum, and P. gingivalis do not initially colonize clean tooth surfaces but adhere to bacteria already in the plaque mass. 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 co aggregation of F. nucleatum with P. gingivalis or T. denticola.

Overview of primary and secondary colonizers in dental plaque

Primary colonizers	Streptococcus gordonii
	Streptococcus intermedius
	Streptococcus mitis
	Streptococcus oralis
	Streptococcus sanguinis
	Actinomyces gerencseria
	Actinomyces israelii
	Actinomyces naeslundii
	Actinomyces oris
	Aggregatibacter actinomycetemcomitans serotype a
	Capnocytophaga gingivalis
	Capnocytophaga ochracea
	Capnocytophaga sputigena
	Eikenella corrodens
	Actinomyces odontolyticus
	Veillonella parvula



Campylobacter gracilis
Campylobacter rectus
Campylobacter showae
Eubacterium nodatum
Aggregatibacter actinomycetemcomitans serotype b
Fusobacterium nucleatum ssp nucleatum
Fusobacterium nucleatum ssp vincentii
Fusobacterium nucleatum ssp polymorphum

Secondary colonizers	Fusobacterium periodonticum
	Parvimonas micra
	Prevotella intermedia
	Prevotella loescheii
	Prevotella nigrescens
	Streptococcus constellatus
	Tannerella forsythia
	Porphyromonas gingivalis
	Treponema denticola

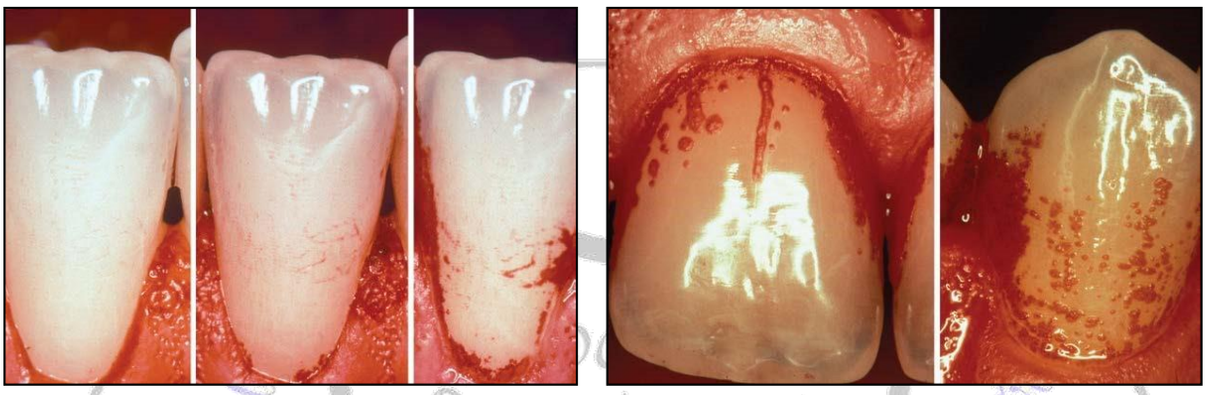
Factors Affecting Supragingival Dental dental plaque formation

Clinically, early undisturbed plaque formation on teeth follows an exponential growth curve when measured planimetrically. During the first 24 hours starting from a clean tooth surface, plaque growth is negligible from a clinical viewpoint (<3% coverage of the vestibular tooth surface, which is an amount nearly undetectable clinically). This “lag time” is due to the fact that the microbial population must reach a certain size before it can be easily detected by the clinician. During the following 3 days, coverage progresses rapidly to the point where, after 4 days, on average 30% of the total coronal tooth area will be covered with plaque.

Several reports have shown that the microbial composition of the dental plaque will change with a shift toward a more anaerobic and a more gram-negative flora including an influx of fusobacteria, filaments, spiral forms, and spirochetes. This was in this beautifully illustrated in experimental gingivitis studies ecologic shift within the biofilm, there is a transition from the early aerobic environment characterized by gram-positive facultative species to a highly oxygen-deprived environment in which gram negative anaerobic microorganisms predominate. Bacterial growth in older plaque is much slower than in newly formed dental plaque presumably because nutrients become limiting for much of the plaque biomass.

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 contains irregularities that offer a favorable growth path. Plaque formation can also start from grooves, cracks, or pits. By multiplication, the bacteria subsequently spread out from these starting up areas as a relatively even monolayer. 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.



Irregular plaque growth patterns follow tooth surface irregularities

Surface Micro roughness.

Rough intraoral surfaces (e.g. crown margins, implant abutments, and denture bases) accumulate and retain more plaque and calculus in terms of thickness, area, and colonyforming units. Ample plaque also reveals an increased maturity/pathogenicity of its bacterial components, characterized by an increased proportion of motile organisms and spirochetes and/or a denser packing of them. Smoothing an intraoral surface decreases the rate of plaque

formation.

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. Moreover, the saliva from light plaque formers reduced the colloidal stability of bacterial suspensions of, for example, S. sanguinis.

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 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.

Impact of Gingival Inflammation and Saliva .

Several studies clearly indicate that early in vivo plaque formation is more rapid on tooth surfaces facing inflamed gingival margins than on those adjacent to healthy gingival. These studies suggest that the increase in crevicular fluid production enhances plaque formation. Probably, some substance(s) from this exudate (e.g. minerals, proteins, or carbohydrates) favor both the initial adhesion and/or the growth of the early colonizing bacteria. Additionally, it is known that during the night, plaque growth rate is reduced by some 50%. This seems surprising, since one would expect that reduced plaque removal and the decreased salivary flow at night would enhance plaque growth. The fact that the supragingival plaque obtains its nutrients mainly from the saliva appears to be of greater significance than the antibacterial activity of saliva.

The Impact of Patient's Age.

Although older studies were contradictory, more recent papers clearly indicate that a subject's age does not influence de novo plaque formation. The developed plaque in the older patient group resulted however, in a more severe gingival inflammation, which seems to increased susceptibility to gingivitis with aging.

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.

Metabolism of Dental Plaque Bacteria

The majority of nutrients for dental plaque bacteria originate from saliva or GCF, although the host diet provides an occasional but nevertheless 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 protoheme from 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 inter-dependencies 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 signaling 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. Two types of signaling molecules have been detected from dental plaque bacteria: peptides released by gram-positive organisms during growth and a “universal” signal molecule autoinducer 2(AI-2). Peptide signals are produced by oral streptococci and are

recognized by cells of the same strain that produced them. Responses are induced only when a threshold concentration of the peptide is attained, and thus the peptides act as cell density, or quorum, sensors.

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 the 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.

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

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.

Antibiotic resistance may be spread through a biofilm by intercellular exchange of DNA. The high density of bacterial cells in biofilm facilitates the exchange of genetic information among cells of the same species and across species and even genera. Conjugation (the exchange of genes through a direct interbacteria connection formed by a sex pilus), transformation (movement of small pieces of DNA from the environment into the bacterial chromosome), plasmid transfer, and transposon transfer have all been shown to occur in biofilms.