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Plaque Biofilm

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1. Introduction

Periodontal infections are caused by bacteria which colonize the tooth surface and the surrounding gingival tissue to form dental plaque. Dental plaque is a complex polymicrobial biofilm. The term biofilm has been used to describe a well-organized microbial community which adheres to an inanimate or living surface. Bacteria growing in biofilms adhere to a solid surface where they multiply and form microcolonies embedded in an extracellular polymeric matrix, which includes water and nutrient channels. (Costerton et al., 1999) Novel microscopic and molecular techniques have recently been used to investigate environmental biofilms and explore the properties of dental plaque. These studies have shown that dental plaque behaves as a classic biofilm (Socransky & Haffajje 2002, Marsh, 2004). The development of this microbial community is a process that involves cooperation and competition among an extremely diverse community of organisms. (Kolenbrander PE et al., 2002)

2. Definitions

The involvement of "very fine extracellular polymer fibrils" that anchored bacteria to surfaces were observed by Marshall (1976). Communities of attached bacteria in aquatic systems were found to be encased in a "glycocalyx" matrix that was polysaccharide in nature, and mediated adhesion (Costerton et al.,1978.) It was stated that biofilm consists of single cells and microcolonies, all embedded in a highly hydrated, predominantly anionic exopolymer matrix (Costerton et al., 1987.) Other defining aspects of biofilms, such as the characteristics of spatial and temporal heterogeneity and the involvement of inorganic or abiotic substances held together in the biofilm matrix have been described (Characklis and Marshall in 1990).

It was emphasized that biofilms could adhere to surfaces and interfaces and to each other, including in the definition microbial aggregates and floccules and adherent populations within spaces of porous media (Costerton et al., 1995). At the same time it was observed that adhesion triggered expression of genes controlling production of bacterial components necessary for adhesion and biofilm formation, emphasizing that the process of biofilm formation was regulated by specific genes transcribed during initial cell attachment (Costerton and Lappin-Scott, 1995).

More recently a biofilm was defined as a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each

other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. (Donla and Costerton 2002)

3. Significance of biofilms

Epidemiologic evidence indicates that biofilms are a source of several infectious diseases, although the exact mechanisms by which biofilm-associated bacteria induce disease are poorly understood. The pathogenicity of the biofilm in the oral cavity is increased by two biofilm characteristics: increased resistance to antibiotics and to phagocytosis by host inflammatory cells. Current intervention strategies are designed to prevent initial colonization by mechanical removal, minimizing microbial cell attachment to the oral tissues and increasing penetration of the biofilm matrix by antimicrobials. In the future, treatments may inhibit the genes involved in cell attachment and biofilm formation.

4. Formation of dental plaque biofilms

Distinct stages in plaque formation include:

4.1 Acquired pellicle formation

Within minutes of tooth eruption or after professional cleaning of the tooth, the surface rapidly becomes coated with a variety of salivary constituents including albumin, glycoproteins, acidic proline-rich proteins, mucins, cell debris, exoproducts (such as a-amylase and lysozyme), and sialic acid thus providing variety of receptors that are recognized by colonizing bacteria.

4.2 Transport of microorganisms to the pellicle

The primary colonizers of microorganisms attach to these receptors. They are mostly gram-positive cocci , followed by some gram-positive rods and filaments and a small number of gram-negative cocci. The gram-positive cocci species involved in this initial layer include, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus oralis*, *Rothia dentocariosa*, and *Staphylococcus epidermidis*. The gram-positive rod and filament species include *Actinomyces viscosus*, *Actinomyces israelis*. *Actinomyces gerencseriae* and *Corynebacterium* species. *Veillonella parvula* and *Neisseria* sp comprise the gram-negative cocci, which are aerobes or facultative aerobes and are able to adhere to the non-exfoliating hard tooth surfaces (Sbordone, L., Bortolaia.,2003). These early colonizers are able to withstand many of the frequent mechanisms of the oral cavity that contribute to bacterial removal such as swallowing, chewing, and the flow of saliva. The early colonizers are also able to survive in the aerobic conditions present in the oral cavity, without having much protection from other bacteria (Sbordone and Bortolaia 2003). This thin, biofilm is almost always present on the tooth surface as it forms immediately after cleaning.

4.3 Weak, long range physico-chemical interactions between microbes and tooth pellicle

As a consequence of bacterial attachment,a change in gene expression is likely to occur. Consequently, primary colonizers alter the surface not only by their physical presence but by developing a new surface-attached phenotype with distinct metabolic activity and

surface properties, thus altering their surroundings and creating new niches for other bacteria to colonize.(Davey & Costerton, 2006) Reversible adhesion involving weak long-range physicochemical interactions occur between the cell surface and the pellicle. It is reversible because the attraction is weak and the micro organisms can readily detach from the tooth surface.

4.4 Strong, short-range interactions between adhesions of bacteria and receptors on pellicle

This reversible adhesion is followed by a much stronger, irreversible attachment, as short-range interactions between specific molecules (adhesins) on the bacterial cells and the complementary receptor proteins on the pellicle surface occur. Many oral microbial species have multiple adhesion types on their cell surface, and can, therefore, participate in a plethora of interactions both with other bacteria and host surface molecules (Marsh, 2004.)
Theoritically analogs could be synthesized to block adhesin-receptor attachment or co-adhesion thus making them less conducive to bacterial colonization. However, cells can express multiple types of adhesin (Hasty et al., 1992; Zhang et al., 2005) so that even if a major adhesin was blocked, other mechanisms of attachment may be invoked. Furthermore, although adhesion is necessary for colonization, the final proportions of a species within a mixed culture biofilm such as dental plaque will depend ultimately on the ability of an organism to grow and outcompete neighboring cells.

4.5 Co-aggregation

Socransky et al.(1998) examined over 13,000 subgingival plaque samples from 185 adult subjects and used cluster analysis and community ordination techniques to demonstrate the presence of specific microbial groups within dental plaque (Fig. 1).
Six closely associated groups of bacterial species were recognized. These included the *Actinomyces*, a yellow complex consisting of members of the genus *Streptococcus*, a green complex consisting of *Capnocytophaga* species, *Actinobacillus actinomycetemcomitans* serotype a, *Eikenella corrodens* and *Campylobacter concisus* and a purple complex consisting of *Veillonella parvula* and *Actinomyces odontolyticus*. These groups of species are early colonizers of the tooth surface, and their growth usually precedes the multiplication of the predominantly gram negative orange and red complexes. Certain complexes are observed together more frequently than others in subgingival plaque. For example, it is extremely unlikely to find red complex species in the absence of members of the orange complex. In contrast, members of the *Actinomyces*, yellow, green and purple complexes are often observed without members of the red complex or even the red and orange complexes. Most oral bacteria adhere to one another.This cell-to-cell adherence is known as **coaggregation**.

4.6 Multiplication of bacteria and confluent growth

Eventually, the bacterial cells continue to divide until a three-dimensional mixed-culture biofilm forms that is spatially and functionally organized. Polymer production causes the development of the extracellular matrix which is one of the key structural aspects of the plaque biofilm. The bacterial stratification is arranged according to metabolism and aerotolerance, with the number of gram-negative cocci, rods and filaments increasing as more anaerobic bacteria appear (Sbordone and Bortolaia,.2003). As the biofilm thickens and becomes more mature, anaerobic bacteria live deeper within the biofilm which protects them from the aerobic environment within the oral cavity.

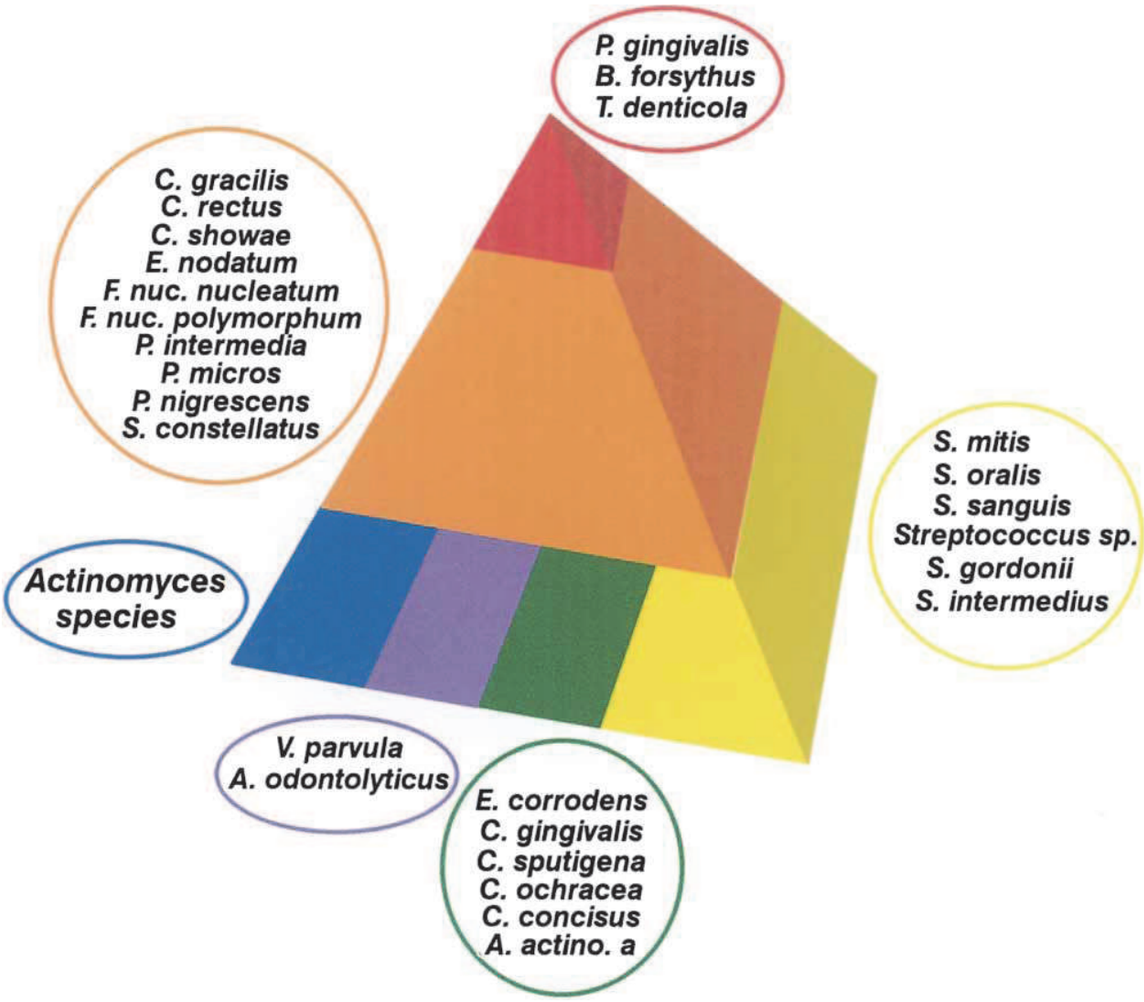


Fig. 1. Diagram of the association among subgingival species. The base of the pyramid is comprised of species thought to colonize the tooth surface and proliferate at an early stage. The orange complex becomes numerically more dominant later and is thought to bridge the early colonizers and the red complex species which become numerically more dominant at late stages in plaque development. (adapted from Socransky et al.,1998)

4.7 Active detachment of bacteria

The composition of the climax community of plaque is diverse, with many species being detected at individual sites. Molecular ecology approaches, in which 16S rRNA genes are amplified from plaque samples, have identified >600 bacterial and *Archae* taxa, of which approximately 50% are currently unculturable. (Wade 1999)

The detachment of bacteria from biofilms is essential to allow colonization of new habitats. It appears from *in vitro* studies that cells detach in different ways. Some of these involve the detachment of single cells in a continuous predictable fashion (erosion), the sporadic detachment of large groups of cells (sloughing) or an intermediate process whereby large pieces of biofilm are shed from the biofilm in a predictable manner. The more predictable intermediate process results in detached clusters consisting of about 10^4 cells.

5. Structure of biofilms

Plaque biofilms are complex three-dimensional structures composed of baterial microcolonies attached to a solid surface like the enamel of the teeth, the surface of the root or dental implants (Socransky and Haffajee 2002) embedded in an exo-polysaccharide matrix.

5.1 Microcolonies

Biofilms are composed of microcolonies of bacterial cells (15–20% by volume) that are non-randomly distributed in a matrix or glycocalyx (75–80%volume). Earlier studies of thick biofilms (.5 mm) that develop in sewage treatment plants indicated the presence of voids or water channels between the microcolonies. These permit the passage of nutrients and other agents throughout the biofilm acting as a primitive ''circulatory'' system. Nutrients make contact with the sessile (attached) microcolonies by diffusion from the water channel to the microcolony rather. (Socransky and Haffajee,2002) Microcolonies occur in different shapes which are governed by shear forces due to the passage of fluid over the biofilm. At low shear force, the colonies are shaped liked towers or mushrooms, while at high shear force, the colonies are elongated and capable of rapid oscillation (Stoodley et al., 1999).

5.2 Exopolysaccharides(EPS) – the backbone of the biofilm

The bulk of the biofilm consists of the matrix which composed predominantly of water and aqueous solutes. The ''dry'' material is a mixture of exopolysaccharides, proteins, salts and cell material.

Exopolysaccharides, which are produced by the bacteria in the biofilm, are the major components of the biofilm, making up 50–95% of the dry weight (Sutherland., 1999). The EPS are largely insoluble and have a complex structure. (Kopec et al., 1997) They play a major role in maintaining the integrity of the biofilm and confer other beneficial properties. Using sucrose primarily as a substrate, the EPS are synthesized mostly by bacterial glucosyltransferases and, to a lesser extent, by fructosyltransferases. (Hamada and Slade, 1980; Bowen, 2002).

Bacteria can produce several different polysaccharides depending on the physiological state of the bacteria and the presence of specific substrates. All biofilms contain exopolysaccharides, which can vary quite markedly in their composition. Some exopolysaccharides are neutral, such as the mutan from *Streptococcus mutans*, whereas others are highly charged polyanionic macromolecules. Different ionic charge and concentrations of exopolysaccharides alter the confirmation and cause rapid changes in the three-dimensional gel network of polysaccharides. Similar effects may also be produced by provision of sucrose or other sugars. The exopolysaccharides can be degraded and utilized by bacteria within the biofilm. One distinguishing feature of oral biofilms is that many of the microorganisms can synthesize and degrade the exopolysaccharides. Exopolysaccharides can exist in both ordered or disordered forms. At high temperatures and often at very low ionic concentrations, the disordered form predominates, although few biofilms exhibit total absence of an ordered structure (Sutherland, 1990). Biofilm matrices are complex structures that contain masses of fibers of varying size, structure, composition and rigidity that interact with each other, with cells and with surface matrices. A wide range of possible

conformations, flexibility and configurations can be expected among different classes of polysaccharides.

The density of the fibrillar masses will affect accessibility of both cells and surfaces to nutrients and other solutes. The chemical composition and tertiary structure of the exopolysaccharides will determine whether it forms an effective adhesive. It will also affect the hydrophilic or hydrophobic nature of the surface. Exopolysaccharides aid in protecting microbial cells within the biofilm by preventing desiccation and attack by harmful agents. They may also bind essential nutrients such as cations to create a local nutritionally rich environment favoring specific microorganisms.The exopolysaccharide matrix could also act as a buffer and assist in retaining extracellular enzymes (and their substrates), enhancing substrate utilization by bacterial cells. They are effective in maintaining biofilm structure through the formation of networked, cross-linked linear macromolecules. In most mixed biofilms, numerous types of polysaccharide are found, complicating the network structure. The quantity of exopolysaccharides in a biofilm does not necessarily reflect the proportion of the bacterial species that produce it. Loss or removal of one type of exopolysaccharide may have a more drastic effect on the biofilm matrix than another even if the removed polymer is not dominant. (Socransky and Haffajee 2002)

6. Cell to cell communication (quorum sensing)

Bacteria are now known to lead highly social lives. (West, et al., 2006) They communicate and respond to local cell density through a process known as *quorum sensing*.

Quorum sensing is widely employed by a variety of gram-positive and gram-negative bacterial species to coordinate communal behavior. Quorum sensingwas originally discovered in the luminescent bacterium *Vibrio fischeri*.

Each individual bacterium is capable of producing a signaling molecule (inducer) and each also has a receptor for the inducer. When the inducer binds to the receptor, it activates the transcription of certain genes, including those responsible for the synthesis of the inducer itself. Imagine that only a few bacteria of the same kind are nearby. Diffusion reduces the concentration of the inducer in the surrounding medium to a negligible amount.

However, as the bacterial population grows, the concentration of the inducer in the surroundings increases, causing more inducer molecules to be synthesized. This forms a positive feedback loop and the concentration of the molecule keeps increasing. Once a threshold concentration is attained, activation of the receptor leads to a signal transduction cascade to switch on specific genes in the bacterial cells, leading to a coordinated population response. As a group, bacteria behave differently if there are few or many bacteria around them. Quorum sensing thus enables bacteria to co-ordinate and respond quickly to environmental changes, such as the availability of nutrients, other microbes or toxins. (Figure II)

6.1 Key players in a quorum-sensing network (table 1)

6.1.1 Autoinducers

Autoinducers are usually small molecules that either diffuse freely across the cell membranes or are actively transported out of the cell.

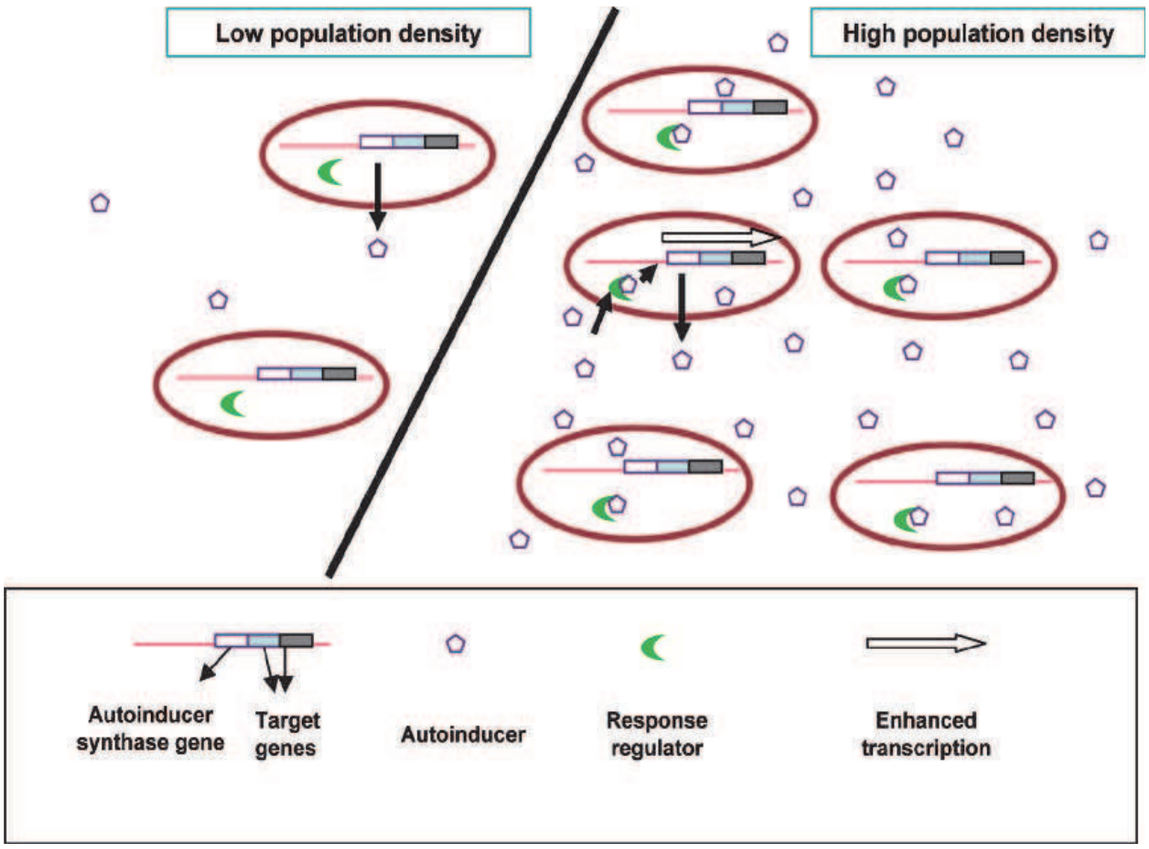


Fig. 2. Schematic representation of bacterial quorum sensing. (Adapted from Gonza´lez and Keshavan, 2006.)

Acyl homoserine lactones(AHL). Acyl homoserine lactones are the major group of autoinducer signals in gram-negative bacteria. They have a conserved homoserine lactone (HSL) ring with a variable acyl side chain. Based on the length of the acyl groups, AHLs can be broadly classified as short- or longchain molecules.

Autoinducer 2. AI-2 was first recognized as a quorum-sensing signal in *Vibrio harveyi* by Bassler et al. (1993). Since then, this type of signaling has been discovered in many gram-negative bacteria. AI-2 is described as a global signal molecule for interspecies communication. It is produced by gram-positive and gram-negative bacteria.

Cyclic dipeptides. A new class of autoinducers was recently identified in strains of *Pseudomonas.*

Bradyoxetin

Other types of autoinducers. In addition to the above-mentioned autoinducers, additional signals have been identified in gram-negative bacteria, including autoinducer (AI-3) in *E. coli* and diffusible signal factor (DSF) in *Xanthomonas campestris*

6.1.2 Autoinducer synthases

AHL synthases.

AI-2 synthase.

Synthases for other types of autoinducers

6.1.3 Quorum-sensing regulators

Quorum-sensing-dependent gene regulation is mediated by transcriptional regulator proteins that are activated upon binding autoinducer molecules.

LuxR-type regulators.

LuxP/Q-type regulators.

6.2 Negative regulation of quorum sensing

Negative regulation in general is the phenomenon of interfering with the bacterial quorum sensing. (Table 2) Of particular interest are the bacterial components used to manipulate quorum sensing called **Quorum Quenchers.**

Several AHL-degrading enzymes identified in various bacteria have the potential to be used as quorum quenchers. Dong et al. initially identified AiiA was isolated from *Bacillus* species and inactivates the AHL signal and attenuates virulence when expressed in *Erwinia carotovora* (Dong et al., 2000)

More than 20 bacteria belonging to the *Bacillus cereus* group are capable of enzymatic inactivation of AHLs. Further genetic analyses revealed that the enzymes responsible for AHL inactivation were homologs of AiiA from *Bacillus* species strain 240B1. This enzyme is an AHL lactonase, known to act by hydrolyzing the lactone bond in the AHL (Dong et al., 2001).

AUTOINDUCERS
Acyl homoserine lactones
Autoinducer 2
Cyclic dipeptides
Bradyoxetin
Other types of autoinducers
AUTOINDUCER SYNTHASES
AHL synthases
AI-2 synthase
Synthases for other types of autoinducers
QUORUM SENSING REGULATORS
LuxR-type regulators
LuxP/Q-type regulators

Table 1. Key Players In A Quorum-Sensing Network

7. Antibiotic resistance

Periodontitis is an infection induced by multiple species of bacteria and the host's response to the bacterial insult. The disease is usually successfully controlled by mechanical debridement, but some cases benefit from adjunctive antibiotic therapy. Antibiotics have been used to treat periodontal infections in the past and they still hold their use today.

The indiscriminate use of antimicrobial agents has the potential of leading to the development of resistant bacteria.(Levy, 1998; Pallasch, 2000).

Antiactivator Proteins Homologs of Transcriptional Regulators AHL-Degrading Enzymes mRNA-Dependent Regulation
EUKARYOTIC INTERFERENCE IN BACTERIAL QUORUM SENSING Quorum-Sensing Cross Talk between *A. tumefaciens* and Its Host Plant Furanones: Structural Mimics L-Canavanine as a Quorum-Sensing Inhibitor Human Hormones Interfere with Bacterial Quorum Sensing Other QSI Compounds
USING BACTERIAL COMPONENTS TO MANIPULATE QUORUM SENSING Quorum Quenchers Transgenic Plants Synthetic Analogs for Quorum-Sensing Autoinducers

Table 2. Negative Regulation Of Quorum Sensing

The phenomenon of increased antimicrobial resistances and reduced susceptibilities in biofilms is well recognized. (Walker and Karpinia . 2002; Walker et al., 2004)
Almost without exception, bacteria grown in biofilms are more resistant to antibiotics than are the same cells grown in a planktonic state. Estimates of 1000 to 1500 times greater resistance for biofilm-grown cells than planktonically grown cells have been suggested (Costerton JW. 1999)
One important mechanism of resistance appears to be the slower rate of growth of bacterial species in biofilms,which makes them less susceptible to many, but not all, antibiotics (Ashby MJ et al., 1994; Brooun A et al., 2000; Costerton et al., 1999).
It has been shown in many studies that the resistance of bacteria to antibiotics, biocides or preservatives is affected by their nutritional status, growth rate, temperature, pH and prior exposure to ineffective concentrations of antimicrobial agents (Brown and Williams 1985; Brown et al., 1990; Williams P.1988). Variations in any of these parameters can lead to a varied response to antibiotics within a biofilm.
The matrix performs a ‘‘homeostatic function”. Cells deep in the biofilm experience different conditions, such as hydrogen ion concentration or redox potentials, than cells at the periphery or cells growing planktonically. The growth rates of these deeper cells will be decreased allowing them to survive better than faster-growing cells at the periphery when exposed to antimicrobial agents. In addition, the slower- growing bacteria often overexpress ‘‘nonspecific defense mechanisms’’ including shock proteins and multi-drug efflux pumps (arcAB) and demonstrate increased exopolymer synthesis. (Gilbert and Allison 1999)
The exopolymer matrix of a biofilm, although not a significant barrier in itself to the diffusion of antibiotics, does have certain properties that can retard diffusion. For example, strongly charged or chemically highly reactive agents can fail to reach the deeper zones of the biofilm because the biofilm acts as an ion-exchange resin removing such molecules from solution . (Gilbert and Allison 1999)

In addition, extracellular enzymes such as b-lactamases, formaldehyde lyase and formaldehyde dehydrogenase may become trapped and concentrated in the extracellular matrix, thus inactivating susceptible, typically positively charged, hydrophilic antibiotics. Some antibiotics such as the macrolides, which are positively charged but hydrophobic, are unaffected by this process. Thus, the ability of the matrix to act as a physical barrier depends on the type of antibiotic, the binding of the matrix to that agent and the levels of the agent employed. (Nichols WW 1993) Since reaction between the agent and the matrix will reduce the levels of the agent, a biofilm with greater bulk will deplete the agent more readily. Further, hydrodynamics (de Beer et al., 1994) and the turnover rate of the microcolonies will also affect antibiotic effectiveness. (Kumon et al 1994)

Alteration of genotype and/or phenotype of the cells growing within a biofilm matrix is receiving increased attention. Such cells express genes that are not observed in the same cells grown in a planktonic state, and they can retain this resistance for some time after being released from the biofilm.

Recently, the notion of a subpopulation of cells within a biofilm that are ''super-resistant'' was proposed. Such cells might explain remarkably elevated levels of resistance to certain antibiotics that have been suggested in the literature. The contribution of multi-drug resistance pumps to antibiotic resistance of organisms grown in biofilms was examined by Brooun et al.(2000). These ''pumps'' can extrude chemically unrelated antimicrobial agents from the cell. Since extrusion places the antibiotics outside the outer membrane, the process offers protection against antibiotics that target cell wall synthesis. They postulated the presence of a ''super-resistant'' subpopulation of cells when grown as biofilms. No ''super-resistant'' subpopulation was detected when the same strains were grown in a planktonic state.

8. Methods of analyzing the biofilm

The Leeds *in situ* device:

Plaque biofilms can be generated using "Leeds *in situ* device" (Robinson et al 1997; Watson et al 2004): Devices are bonded to teeth and worn for seven days, during which time volunteers carried out their normal oral hygiene regime. Devices are then debonded and recovered, with undisturbed plaque in situ.

Direct light and electron microscopic observation:

Direct light and electron microscopic observation clearly showed that biofilm bacteria were enveloped in very large amounts of a fibrous, highly hydrated, exopolysaccharide matrix whose chemical composition was species specific (Sutherland, 1977)

Microelectrodes:

Christiane von Ohle, et al (2010) demonstrated the utility of using microelectrodes to measure the influence of nutrients and antimicrobial agents on the physiology of human dental biofilms nondestructively and in real time. The microelectrode data can be corroborated with microscopy and culture techniques. Microelectrodes with tip diameters of < 10 ,um are useful in the study of microbial biofilms because they allow the in situ measurement of pH, dissolved oxygen (DO), sulfide, and other chemical species with minimal disturbance of the biofilm structure (Lewandowskiet al., 1991; Revsbechn and Ward 1983.)

Chemical probes:

During the examination of eukaryotic tissues by CSL microscopy, a large number of fluorescent chemical probes have been developed. (Haugland, 1992.) These probes can be

introduced into fully hydrated living bacterial biofilms and their fluorescent emissions can be monitored for location and intensity to yield very valuable direct data concerning chemical and physical conditions in virtually all parts of these complex matrix-enclosed adherent populations.(Costerton et al., 1994)

The introduction and application of **"metagenomics"** approach has greatly enhanced and will continue to increase our ability to study microbial community, including dental plaque, in greater detail. The term "Metagenomics" was first invented by Handelsman J,et al (1998), and is defined as "the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and laboratory cultivation of individual species". The advances in refinements of DNA amplification, bioinformatics, and enhanced computational power for analyzing DNA sequences have enabled the adaptation of shotgun sequencing, such as chip-based pyrosequencing, to metagenomic samples. The approach randomly shears DNA, sequences many short sequences, and reconstructs them into a consensus sequence (Breitbart M et al 2002). By performing metabolic function analyses on genes identified via metagenomic approach, researchers are able to retrieve information both on which organisms are present and more importantly, what functions or metabolic processes are possible in that particular community (Gill SR et al 2006). Using comparative genetic studies coupled with expression experiments such as microarray and proteomics, microbiologist will be able to piece together a metabolic network that goes beyond species boundary, and gain valuable insight into the metabolism within the community.

9. Biofilm formation around implant surfaces

Biofilm formation on oral implants can cause inflammation of peri-implant tissues, which endangers the long-term success of osseointegrated implants.

Heuer et al. (2007) examined the crevicular fluid around 14 dental implants/healing abutments over a period of 14 days. Despite massive supragingival biofilm formation, no periodontal pathogens were isolated from the sulcus fluid around the implants/healing abutments during initial bacterial colonization. They concluded that the attachment of peri-implant tissue by means of hemidesmosomal, actin filaments and microvilli, reduced the risk of formation of anaerobic subgingival pockets.

In some studies, *H. actinomycetemcomitans* and *P. gingivalis* were found in greater amounts in peri-implant lesions (George et al., 1994 and Shibli et al., 2003)

No *P. gingivalis* or *H. actinomycetemcomitans* were isolated from stable osseointegrated implant surfaces, in contrast to peri-implant lesions, in which high levels of periodontal pathogens were present. (Botero et al., 2005)

A study of implants in the partially edentulous patient, Quirynen et al. (2006) reported that initial colonization of peri-implant pockets with bacteria associated with periodontitis occured within two weeks. Four subgingival plaque samples were taken from shallow and medium pockets around implants (test sites), and control teeth within the same quadrant one, two, four, 13, 26 and 78 weeks after abutment connection. Checkerboard DNA-DNA hybridization and real-time PCR revealed a complex microbiota (including several pathogenic species) in the peri-implant pockets within two weeks after abutment connection. After seven days, the detection frequency for most species, including the red complex microbiota, was almost identical to samples from the fresh peri-implant pockets (5 per cent and 20 per cent of the microbiota belonging to red and orange complex,

respectively) when compared with samples from the reference teeth. Between weeks 2 and 13, the number of bacteria in peri-implant pockets only slightly increased, with minor changes in the relative proportions of bacteria associated with periodontitis (8 per cent and 33 per cent of the microbiota belonging to red and orange complex, respectively). Although small differences were seen between teeth and implants at week two, a striking similarity in subgingival microbiota was found after three months.

10. Treatment and control of biofilm formation

Due to the structure of biofilms, their physical removal by a professional and the individual remains the most effective means of control. Subgingival debridement of root surfaces is an essential component in the treatment of periodontitis.

The use of antimicrobials can be grouped into two broad categories; those that attempt to kill or affect the metabolism of the organism such as antiseptics and antibiotics and those that affect the environment of the organisms. Other types of therapy are on the horizon, such as possible vaccines against oral pathogens or replacement therapy in which a species is introduced to the biofilm in order to control potentially pathogenic microorganisms. (Socransky and Haffajee 2002)

The main impetus behind the desire to control the bacterial composition of dental plaque is to prevent or reduce the incidence of periodontal diseases. Some potential strategies to achieve these aims were elaborated by Marsh and Bradshaw in 1997.(Table 3)

In a clinical trial, a seven-day treatment regime involving methylene blue led to a decrease in the proportions of Gramnegative anaerobes (including spirochetes) and motile bacteria and a reduction in the flow of GCF, while bacteria associated with gingival health increased (Wilson et al. 1992), suggesting that this approach has genuine potential. Further work on the influence of surface growth on the behavior of plaque communities will also be needed before the full potential of physiological approaches to biofilm control will be realized.

The **bioelectric effect**, in which electric fields are used to enhance the efficacy of biocides and antibiotics in killing biofilm bacteria, has been shown to reduce the very high concentrations of these antibacterial agents needed to kill biofilm bacteria to levels very close to those needed to kill planktonic (floating) bacteria of the same species. Biofilm bacteria are readily killed by an antibiotic on all areas of the active electrodes and on the surfaces of conductive elements that lie within the electric field but do not themselves function as electrodes (Costerton et al. 1994). Considerations of electrode geometry indicate that very low (< 100 μA/cm2) current densities may be effective in this electrical enhancement of antibiotic efficacy against biofilm bacteria, and flow experiments indicate that this bioelectric eflect does not appear to depend entirely on the possible local electrochemical generation of antibacterial molecules or ions. These data are expected to facilitate the use of the bioelectric effect in the prevention and treatment of device-related bacterial infections that are caused by bacteria that grow in biofilms and thereby frustrate antibiotic chemotherapy.

Photodynamic therapy (PDT) has been suggested as an alternative to chemical antimicrobial agents to eliminate subgingival species and treat periodontitis (Wilson. 1993). PDT is based on the concept that non-toxic photosensitizers can be preferentially localized in certain tissues and activated by light of the appropriate wavelength to generate singlet oxygen and free radicals that are cytotoxic to cells of the target tissue (Dougherty et al. 1998). Several studies have shown that oral bacteria are susceptible to PDT in planktonic cultures

(Wilson . 1993 and Wilson et al. 1993) and plaque scrapings (Williams et al. 2003 and Sarkar , Wilson 1993). Recent studies have reported that PDT-induced bacterial cell killing reduced bacterial numbers by more than 10-fold in *Streptococcus mutans, Streptococcus sobrinus* and *Streptococcus sanguinis* (Metcalf et al., 2006; Zanin et al., 2005) biofilms using toluidine blue O or erythrosine as the photosensitizer.

Control of plaque pH • inhibition of acid production - fluoride - sugar substitutes - antimicrobial agents • stimulation of base production - arginine - urea - peptides Control of redox potential • • redox agents • oxygenating agents Control of nutrients • addition of base-generating nutrients - arginine • reduction of GCF flow • anti-inflammatory agents • inhibition of key microbial enzymes

Table 3. (Marsh And Bradshaw 1997) Physiological Strategies For The Control Of Oral Biofilms

Efflux pump inhibitors: Bacteria rely on efflux pumps to get rid of toxic substances. It was discovered that efflux pumps are highly active in bacterial biofilms, makingthem attractive targets for antibiofilm measures. A number of efflux pump inhibitors (EPIs) are known. EPIs were shown to reduce biofilm formation, and in combination they could abolish biofilm formation completely. Also, EPIs were able to block the antibiotic tolerance of biofilms. The results of this feasibility study might pave the way for new treatments for biofilm-related infections and may be exploited for prevention of biofilms in general.(Kvist et al., 2008)
The use of **probiotics** (introduction of beneficial bacteria) or **prebiotics** (nutrients that favour the growth of beneficial bacteria)
The role of **nanoscience** in microbiology needs to be assessed. Nanoparticles could be a new delivery mechanism for antimicrobial agents or vaccines that could disrupt biofilms; however, consideration needs to be given to the behavior of nanoparticles in ecosystems and their long-term effects.

11. Conclusion

This chapter attempts to throw light on the nature of plaque biofilms and the strategies towards their control. Biofilms are very complex structures and pose great challenges for

clinicians on a daily basis. Nevertheless, advances in science have made it possible to dissect their complex microbiology and guide the control of plaque biofilm related periodontal and peri implant infections.

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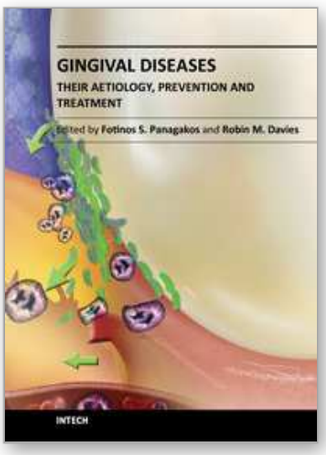
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Gingival diseases are a family of distinct pathological entities that involve the gingival tissues. These signs and symptoms of these diseases are so prevalent in populations around the world that they are often considered to be â€œnormalâ€ features. The diseases are now classified into two main groups namely: Plaque-Induced and Non-Plaque Induced Gingival Diseases. This book provides dentists, dental hygienists, dental therapists and students with a comprehensive review of gingival diseases, their aetiology and treatment.

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