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Biofilms and Their Consequences in the Oral Cavity

by

Tyler Olson

A paper submitted to the graduate faculty in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Sciences

Program of Study Committee
Steve Carlson
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Introduction:

Biofilms are a relatively recent discovery in the field of microbiology. Historically, bacteria and other microorganisms were thought of as planktonic, or freely suspended cells. It was Anton van Leeuwenhoek who was credited with the discovery of biofilms, he discovered and characterized microorganisms that grew universally on exposed surfaces, such as teeth; this laid the groundwork for future studies that displayed that these microorganisms had distinct phenotypes in regards to gene transcription and growth rate.

According to the author Donlan, the definition of a biofilm is “an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material”. Other non-cellular components may also be found in the matrix of the biofilm depending on the location and surface (Donlan, 2002). Biofilms can develop on a wide array of surfaces, that can range from living to non-living, above and below water, as well as hot and cold environments. Even though the electron microscope would prove to be an invaluable tool when evaluating and describing microbial biofilms, the pioneers of biofilm discovery would use transmission and light microscopes to try to explain this phenomenon. Using this earlier technology in the 1940's, Zobell discovered that in marine environments, microorganisms were more densely concentrated on exposed surfaces, then in the seawater that surrounded them. This proved to be the basis for scientists that would lead to many more discoveries that are still being sought after today (Zobell, 1943).
Just before the turn of a new decade in 1970, scanning and transmission electron microscopy was used to evaluate the filters at wastewater treatment plants. The electron microscope offered more complex images on biofilms (Figure 1). These images showed a multitude of organisms associated with the trickling filter and not a monoculture of a microorganism. This research that was led by Jones, also exposed that the matrix material that was surrounding the cells was primarily polysaccharides. This was achieved using a specific polysaccharide stain, Ruthenium Red, and combining it with an osmium tetroxide fixative (Jones, 1969). This new development primed Characklis in 1973 to test the perseverance of microbial slimes in an industrial water plant. He found that the microbial biofilms he was studying were very resilient to different chemicals and disinfectants such as chlorine. Since that time, research in the industrial and health care fields, in regards to biofilms, have mirrored each other. Both technological and genetic advances have aided this research. The development of the confocal laser scanning microscope has helped with the understanding of the ultrastructure of biofilms, and further research into genetics of organisms in biofilms have allowed for further disclosures within this field.
An interesting aspect of microbial biofilms is identifying how the organisms attach to the substratum, or underlying surface. The rougher or more disturbed a surface is, seems to lead to better attachment because of an increase in surface area (Characklis et al., 1990). Furthermore, researchers have revealed that hydrophobic surfaces, such as plastic or Teflon, are more easily attached to then hydrophilic surfaces (Bendinger et al. 1993).

Many more factors can exhibit a role in the formation and rate of biofilm development. For example, characteristics of the environment, conditioning films, hydrodynamics, properties of the cells that occupy the biofilm, gene regulation of the cells, biofilm structure and architecture, gene transfer, quorum sensing, predation and competition, pathogens, and dispersal all play a role in the unique ecology of this micro-environment (Donlan, 2002). These biofilms are complex structures that can range from different microbial hosts, to different mediums, and much more. Because of this diversity in biofilms, I am going to focus on biofilms that are formed in the oral cavity of the human species. This will allow me to spotlight the importance of proper oral hygiene and practices as well as new products that are being introduced to fight these brawny and enduring environments.

The oral cavity offers some different environments that allow different inhabitants to thrive. Dental plaque was a term most commonly used as a synonym for biofilms on the teeth. That definition has broadened to include the whole oral cavity. The soft tissues of the gums, cheek, and palate offer a much different environment for microbial biofilms compared to the hard surfaces of the teeth. The different subcategories of just non-shedding surfaces in the oral cavity include:
supragingival plaque, gingival margin plaque, subgingival plaque, and approximal plaque. The plethora of environments within the oral cavity provides a home for a vast amount of bacteria. There are more than 500 bacterial taxa that have been recorded with many of these diverse organisms being almost exclusively found in this particular environment (Paster et al. 2001). The original acquisition of microorganisms in the oral cavity was originally thought to have occurred in the womb of the mother. However, studies by Carlson and Gothefors found that even after birth and encounters with the mother’s resident microflora in the birth canal, the oral cavity of neonate is usually sterile. Instead, it is believed that micro flora of the oral cavity is developed by exposure to food, milk, water, and saliva from the mother (Li & Caufield, 1995). Under six months of age, during the immediate colonization phase, the plaque is dominated by *Streptococcus salivarius* and *S. mitis* (Pearce et al. 1995) (Figure 2). This decreased amount of diversity is due to a limit of non-shedding environments in the oral cavity. Diversity will start to increase once teeth start to erupt, usually between six and eighteen months.

<table>
<thead>
<tr>
<th>Time during a lifetime</th>
<th>MAJOR COMPONENTS &amp; CHANGES IN ORAL FLORA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>Oral cavity sterile. Soon colonised by facultative and aerobic organisms; esp S. salivarius</td>
</tr>
<tr>
<td>6 months</td>
<td>Flora becomes more complex &amp; includes anaerobic orgs eg. Veillonella sp. &amp; Fusobacteria</td>
</tr>
<tr>
<td>Tooth eruption</td>
<td>Increase in complexity. S sanguis, S mutans and A viscosus appear. New habitats include hard surfaces and gingival crevice.</td>
</tr>
<tr>
<td>Child to adult</td>
<td>Various anaerobes frequently found inc. Members of the Bacteroidaceae. Spirochaetes isolated more frequently</td>
</tr>
<tr>
<td>Loss of teeth</td>
<td>Disappearance of S mutan, S sanguis, spirochaetes and many anaerobes</td>
</tr>
<tr>
<td>Dentures etc</td>
<td>Reappearance of bacteria able to grow on hard surfaces</td>
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(Figure 2) [https://slideplayer.com/slide/3936510/]
months of age. More organisms start to inhabit the dental plaques of the new environment created. Even more diversity occurs when sex hormones become present in the gingival crevicular fluid, usually between ages twelve to sixteen years. As the age of the host increases, there are not many notable changes except for the inverse population changes between *Actionobacillus actinmycetemcomitans* and *Porphyromonas gingivalis*. *Actionobacillus actinmycetemcomitans* has an evident decrease in concentration with increasing age while the latter, *Porphyromonas gingivalis*, has an increase in concentration later in life (Darby et al. 2000).

As more teeth erupt and hormone levels change, the availability of shedding and non-shedding environments increases. Adherence of bacteria to the shedding and non-shedding substratum can generally be categorized into two events. Initially there is the absorption of bacteria into the salivary pedicle, and will conclude with the adherence of bacterial cells to the existing cells. Saliva is an important component in the initiation of dental plaques. A mL of saliva includes up to $10^8$ bacteria along with glycoproteins that are the primary source of nutrition for the microorganisms. This adhesion can loosely be compared to primary and secondary succession of plants in desolate landscapes. Some organisms are better suited to colonize empty substratum while others are better at utilizing an already colonized substratum (Spratt & Pratten, 2004). Additionally, co-aggregation, or the process of genetically distinct cells attaching to each other via separate molecules (Figure 3), can be vital in the formation and further growth of dental plaques. The process of co-aggregation can be categorized into two different routes. The first is lone cells in
suspension that can adhere to other cells in the initial dental plaque. The second is prior co-aggregation in a medium that is followed by adhesion of this co-aggregate to the dental plaque. Especially in the oral cavity, these two routes are important succession events in bacterial adhesion to substratum, while also proving mutually beneficial to each other (Rickard et al. 2003). Generally, relationships between dental plaque microorganisms are very specific and primary colonizers and secondary colonizers prefer to co-aggregate with their own instead of crossing over. Of course, there are always exceptions. *Streptococcus gordonii* a secondary colonizer that can co-aggregate in a synergistic event with the primary colonizer, *Porphyromonas gingivalis*. This is possible through *Fusobacterium nucleatum*; this microorganism forms a physical bridge to help co-aggregate the two differing species. Furthermore, this bridge allows for anaerobic microbes because this aggregation event promotes an anaerobic microenvironment in an otherwise aerobic environment. The further co-aggregation events are called succession and they modify the population diversity, properties, and distribution until a mature plaque is formed (Spratt & Pratten, 2004).

Figure 3
[https://www.sciencedirect.com/science/article/pii/S0966842X02000343]
The adhesion and growth of dental plaques creates an environment that can offer a wide array of microenvironments with differing factors such as oxygen availability, pH, and nutrient availability. Additionally, nutrients that are consumed and byproducts of metabolism may create different ideal environment for other types of microorganisms to colonize. These environments can also be affected by synergistic or antagonistic actions of other organisms in the dental plaque. Co-aggregation and the degradation of complex polymers are considered synergistic while antagonistic actions include the production of enzymes and organic acids as well as a low pH.

With an overabundance of microorganisms present in the oral cavity, naturally some can lead to negative effects on the host. They can cause a multitude of problems including caries (on the coronal or root surface), endodontic infections, periodontal disease, gingivitis, periodontitis, oral malodor, and other diseases associated with the epithelial surface.

Caries, or cavities, can occur in two locations; on any surface of the crown of the tooth or on the root surface of the tooth. Both of these caries are a result of demineralization of the tooth because of acid production due to microorganism’s metabolism in dental plaques. The most common site of coronal caries seems to be in crevasses on the crown surface and between teeth, both locations that also seem the hardest to clean thoroughly. Root surface caries are a secondary cause due to the recession of the gums; because a healthy root would not be exposed to bacteria in the mouth if the gums were in place. Gram-positive bacteria are the most
common cause of these two diseases. Although a few gram-negative species can be a cause of caries also (Spratt & Pratten, 2004).

Endodontic infections are a result of chronic caries that demineralize enamel, cementum, and dentine to gain access to the pulp and periapical tissues that are usually sterile. Once exposed, the pulp tissue can be colonized as fast as three days after its introduction to the micro flora of the oral cavity (Watts & Paterson, 1990). Prolonged colonization of bacteria will lead to necrosis of the pulp tissue and reabsorption of bone tissue and eventual tooth loss. The types of dental plaques that can occupy this pulp are specific due to the significant changes in local environment from the coronal end of the root to the apex, or bottom of the root. At the coronal end of the root, bacteria in the biofilms are more aerobic and rely on the hosts diet for more of their nutrient supply, but in the apex of the root, the environment is anaerobic and nutrients not as prevalent or are the byproducts of other bacteria in the biofilms. It is not uncommon for more than one bacterial species to be causing an endodontic infection. Typically, four to twelve species are common throughout a root canal infection. Streptococci are the initial colonizers of pulp and use enough oxygen to make the environment anaerobic contributing to secondary colonization of other organisms. A unique characteristic of periodontal infections is the incorporation of yeast into the dental plaque. Although this is not a constant, it has been reported in approximately ten percent of cases studied (Egan et al. 2002).

Gingivitis, at least in a minor fashion, affects nearly everyone in the population. The gingiva becomes damaged after biofilm microflora cause non-
specific inflammation. (Figure 4) The cause of this inflammation is commonly poor oral hygiene but in most cases can be reverted to healthy tissue with proper cleaning. The inhabitants of the dental plaques at the gum margin that cause this disease can change depending on the level of oral hygiene. Streptococci usually dictate dental plaques restrained by moderate to good oral hygiene but it shifts to a biofilm controlled by *Actinomyces* species when less then adequate oral hygiene prevails (Spratt & Pratten, 2004).

Periodontitis is the result of dental plaque formation on the tooth’s supragingival, and subgingival surfaces. (Figure 4) Anaerobic gram-negative bacteria are the main colonists in regards to this disease. These biofilm plaques are responsible for the destruction of bone that supports the tooth and if not corrected could result in the loosening or loss of teeth affected (Bathla, 2011). There are also predisposing factors that alter formation and severity of periodontitis disease. A
distinct phenotype of the polymorphic interleukin-1 could increase the harshness of the disease. Smoking and diabetes mellitus also can increase the risk of developing severe chronic periodontitis.

Oral malodor, or bad breath, reportedly affects up to about forty percent of the population today. This ailment is caused by the microflora that occupy the oral cavity. The bacterial species responsible for these odors have been identified as gram-negative, proteolytic and anaerobic. These problematic organisms colonize the dental plaques in the gingival crevice, interdental spaces, and the dorsal region of the tongue. There seems to be more diversity of causative microflora species that cause bad breath in the tooth biofilms compared to the biofilms that occupy the tongue (Loesche & Kazer, 2002). The elements that comprise the gaseous components of this disease are largely due to volatile sulphur compounds such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide. These gaseous compounds are generated due to the microbial metabolism of amino acids, namely cysteine and methionine. Additionally, some microbes emit non-sulphur compounds that contribute to this gaseous mix. These are produced by the metabolism of the amino acid tryptophan (Loesche & Kazer, 2002).

With all the diseases that can be generated by bacteria, why are more compounds not being developed to eliminate these biofilms from the oral cavity? Generally, biofilms are comprised of five to twenty five percent bacterial cells with the remainder comprised of a glycocalyx matrix. The majority of bacteria that inhabit the oral cavity are actually beneficial to human health. There are only a slight minority of species that are pathogenic. Recent research has found that the
type of bacteria that inhabit these biofilms ultimately influence the health of the human host. These pathogenic and synergistic bacteria constantly are battling each other to gain the upper hand in the environments they occupy in the oral cavity; utilizing methods such as bacteriocin synthesis, quorum sensing, and hydrogen peroxide excretion (Mingyun & Gregory 2011).

Furthermore, bacteria that populate biofilms are one thousand to fifteen hundred times more resistant to antibiotics when compared to planktonic cells. Biofilms that contain a greater diversity of microflora have experienced greater resistance compared to monoculture biofilms. Researchers describe this via the insurance hypothesis that states that a single species is more vulnerable to the environment then multiple species. Dental plaques with multiple species are able to form a more complex and mature structure that increases resistance to antibiotics (Costertan, 1999). Also, plasmids that carry genes responsible for the antibiotic properties are commonly transmitted via conjugation, because of the decreased distance between cells in plaques; it is more common for these cells to share these genes (Mingyun & Gregory 2011).
Future Research:

Although many breakthroughs and findings have rewarded this field of study, our knowledge and understanding of biofilms in the oral cavity are in their infancy and are far from being completely understood. It is beneficial that it is generally accepted that the oral cavity is seen as an ecosystem that has beneficial and harmful organisms that call it home. Future research will almost certainly be focused on trying to find compounds that are controlling the harmful organisms instead of trying to sterilize the cavity. These compounds are likely to come from organisms that are already occupying this environment and isolating antagonistic compounds that are effective against harmful neighboring cells. Improvements in technology, especially electron microscopy, have allowed a better understanding of the structure of these complex biofilms. This, in turn has allowed researchers to replicate those structures in their studies instead of doing tests on planktonic cells that may not transfer to biofilm functionality. There is; however, still important work in this sub-field that can help researchers understand the interactions that take place in dental plaques.
**Summary:**

The oral cavity offers a wide variety of microenvironments that can support a wide array of microflora. These different environments create a diversity of microflora. In the oral cavity, this microflora produces biofilms that are distinct to the mouth and that can be both beneficial and pathogenic. Practically all bacteria prefer to live in biofilms compared to planktonic solutions. Oxygen availability, nutrient availability, shedding and non-shedding substratum, along with other factors determines which organisms inhabit specific regions of the oral cavity.

These organisms attach to surfaces through a succession; primary colonizers initially use the host’s glycoproteins to attach to the substratum, otherwise known as attachment to the acquired pellicle. Streptococcus species are notorious primary colonizers. After the attachment of the bacteria to the pellicle (Figure 5), the bacteria start to secrete chemicals that allow them to stay attached to each other. Microorganisms that come in to further mature the biofilm recognize receptors on primary colonizers and co-aggregate on these bacteria.

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**Figure 5**

https://www.tandfonline.com/doi/full/10.4161/viru.2.5.16140?scroll=top&needAccess=true
These biofilms usually contain pores and channels that allow organisms to obtain water and nutrients. These bacteria use synergistic and antagonistic actions to better the situations for themselves and mature the biofilms. Antagonistic actions include the secretion of hydrogen peroxide, quorum sensing, and bacteriocin synthesis. Contrarily, bacteria also use synergistic actions to grow or protect the biofilm, for example bacteria utilize conjugation to transfer plasmids that contain genes beneficial for antibiotic resistance. Bacteria in biofilms are one thousand to fifteen hundred times more resistant to antibiotics compared to planktonic bacteria. This makes it difficult to remove bacteria in the oral cavity, which can prove to become problematic to host health with colonization of pathogenic bacteria. These bacteria can cause ailments such as caries, endodontic disease, periodontitis, gingivitis, and oral malodor. Future work will be beneficial if it can mimic the natural competition between the beneficial bacteria and the pathogenic bacteria, which will allow treatments that can control the pathogenic organisms without a reduction in the favorable microbiome.
References


