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# Microbial Biofilms

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**Abstract**

Biofilm formation constitutes an alternative lifestyle in which microorganisms adopt a multicellular behavior that facilitates and/or prolongs survival in diverse environmental niches. Biofilms form on biotic and abiotic surfaces both in the environment and in the healthcare setting. In hospital wards, the formation of biofilms on vents and medical equipment enables pathogens to persist as reservoirs that can readily spread to patients. Inside the host, biofilms allow pathogens to subvert innate immune defenses and are thus associated with long-term persistence. This review describes the process of biofilm formation its composition and

virulence and the role it plays in the pathogenisis of various infections mostly chronic.The review also makes an attempt to describe antimicrobial biofilm control.

## Introduction

A Biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS, which is also referred to as slime(although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms are usually found on solid substrates submerged in or exposed to an aqueous solution, although they can form as floating mats on liquid surfaces and also on the surface of leaves, particularly in high humidity climates. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings [1,2].The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism. Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics.[3,4.]When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated.[5] Because biofilms protect the bacteria, they are often more resistant to traditional antimicrobial treatments, making them a serious health risk.

### Composition and formation of biofilms

Biofilms consist of microorganisms and their self-produced extracellular polymeric substances (EPS). A fully developed biofilm contains many layers including a matrix of EPS with vertical structures, and a conditioning film. Vertical structures of microorganisms sometimes take the form of towers or mushrooms which are separated by interstitial spaces. Interstitial spaces allow the bulk of the biofilm to easily and rapidly take in nutrients from the surrounding liquid and move byproducts away from the biofilm.[3] Formation of biofilms are rather complex, but can

be generalized in four basic steps: deposition of the conditioning film, microbial (planktonic) attachment to the conditioning film, growth and bacterial colonization and finally biofilm formation.

1. Conditioning film: Conditioning films alter the surface properties of the substratum and allow microorganisms to adhere to the surface. For example, when sterile, medical implants are exposed to bodily fluids, proteins, polysaccharides, ions and various other components adhere to the surface and form a conditioning film which "invites" microorganisms that would otherwise be unable to attach to the original surface.[4] Rougher and more hydrophobic materials will develop biofilms faster.
2. Adsorption and attachment: While the exact mechanism of microorganism attachment is still unknown, DLVO theory and thermodynamic interaction mechanisms have been used to help explain the initial microbial attachment.[9,11] Cell properties: flagella, pili, fimbriae, or glycocalyx may impact rate of microbial attachment.
3. Growth and colonization: Production of polysaccharides that anchor the bacteria to the surface allow colonies to grow. The growth process is the most significant step in biofilm accumulation when accounting for biofilm mass.[5]
4. Biofilm formation: A fully developed biofilms will contain an EPS matrix and vertical structures separated by interstitial spaces. Biofilms have a heterogeneous structure and are capable of mass internal transport.[5,11] In addition to the polysaccharides, these matrices may also contain material from the surrounding environment, including but not limited to minerals, soil particles, and blood components.
5. Biofilm Dispersion:The final stage of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in shape and size.[7,11]

**Communication within and movement of biofilms**

Multiple studies have shown that during the time a biofilm is being created, the pathogens inside it can communicate with each other thanks to a phenomenon called quorum sensing. Although the mechanisms behind quorum sensing are not fully understood, the phenomenon allows a

single-celled bacterium to perceive how many other bacteria are in close proximity. If a bacterium can sense that it is surrounded by a dense population of other pathogens, it is more inclined to join them and contribute to the formation of a biofilm.[6]

Bacteria that engage in quorum sensing communicate their presence by emitting chemical messages that their fellow infectious agents are able to recognize. When the messages grow strong enough, the bacteria respond en masse, behaving as a group. Quorum sensing can occur within a single bacterial species as well as between diverse species, and can regulate a host of different processes, essentially serving as a simple communication network. A variety of different molecules can be used as signals.[3,6]

Biofilm bacteria can move in numerous ways: Collectively, by rippling or rolling across the surface, or by detaching in clumps. Individually, through a "swarming and seeding" dispersal whereby a biofilm colony differentiates to form an outer "wall" of stationary bacteria, while the inner region of the biofilm "liquefies", allowing planktonic cells to "swim" out of the biofilm and leave behind a hollow mound.[4]

**Pathogenic mechanisms**

Different pathogenic mechanisms of the biofilms have been proposed.

These include:

- Attachment to a solid surface;
- "Division of labor" thereby increasing metabolic efficiency of the community;
- Evading host defenses such as phagocytosis;
- A Repository of high density of microorganisms;
- Exchange genes that can result in more virulent strains of microorganisms;
- Production of large concentration of toxins;
- Protection from antimicrobial agents;
- Detachment of microbial aggregates thereby transmitiing microorganisms to other sites.[16,24,25]

**Biofilms and infectious diseases**

Biofilms have been found to be involved in a wide variety of microbial infections in the body. The biofilm formation has also been documented as survival strategy of pathogens.[9] Some microorganisms in biofilm can even modulate the pathogenic potential of bacteria as evident from cariogenic bacteria in plaque biofilms. According to a recent public statement from the National Institutes of Health, more than 65% of all microbial infections are caused by biofilms. This number might seem high, but if one recalls that such common infections as urinary tract infections (caused by *E. coli* and other pathogens), catheter infections (caused by *Staphylococcus aureus* and other gram-positive pathogens), child middle-ear infections (caused by Haemophilus influenzae, for example), common dental plaque formation, and gingivitis, all of which are caused by biofilms, are hard to treat or frequently relapsing, this figure appears realistic.[18]

Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear infections, formation of dental plaque,[13] gingivitis, legionellosis ,infections involving contact lenses, and less common but more lethal processes such as endocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves[]16],some of the biofilm associaed infections have been discussed.

**Dental plaque**

Perhaps the most well-studied biofilms are those that make up what is commonly referred to as dental plaque. Plaque is a biofilm on the surfaces of the teeth . The accumulation of microorganisms subject the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease.[27]

**Chronic sinusitis and osteomyelitis**

It has also recently been shown that biofilms are present on the removed tissue of 80% of patients undergoing surgery for chronic sinusitis. According to Parsek, biofilms may also cause osteomyelitis, a disease in which the bones and bone marrow become infected. This is supported

by the fact that microscopy studies have shown biofilm formation on infected bone surfaces from humans and experimental animal models.Parsek also implicates biofilms in chronic prostatitis since microscopy studies have also documented biofilms on the surface of the prostatic duct.[10] Microbes that colonize vaginal tissue and tampon fibers can also form into biofilms, causing inflammation and disease such as Toxic Shock Syndrome.[7,8]

**Kidney stones**

Biofilms may also cause the formation of kidney stones. The stones cause disease by obstructing urine flow and by producing inflammation and recurrent infection that can lead to kidney failure. Approximately 15%–20% of kidney stones occur in the setting of urinary tract infection. According to Parsek, these stones are produced by the interplay between infecting bacteria and mineral substrates derived from the urine. This interaction results in a complex biofilm composed of bacteria, bacterial exoproducts, and mineralized stone material.[7,8,10]

**Endocarditis and other device related infections**

Then there's endocarditis, a disease that involves inflammation of the inner layers of the heart. The primary infectious lesion in endocarditis is a complex biofilm composed of both bacterial and host components that is located on a cardiac valve. This biofilm, known as a vegetation, causes disease by three basic mechanisms. First, the vegetation physically disrupts valve function, causing leakage when the valve is closed and inducing turbulence and diminished flow when the valve is open. Second, the vegetation provides a source for near-continuous infection of the bloodstream that persists even during antibiotic treatment. This causes recurrent fever, chronic systemic inflammation, and other infections. Third, pieces of the infected vegetation can break off and be carried to a terminal point in the circulation where they block the flow of blood (a process known as embolization). The brain, kidney, and extremities are particularly vulnerable to the effects of embolization.[10,13]

A variety of pathogenic biofims are also commonly found on medical devices such as joint prostheses and heart valves. Electron microscopy of the surfaces of medical devices that have

been foci of device-related infections shows the presence of large numbers of slime-encased bacteria. Tissues taken from non-device-related chronic infections also show the presence of biofilm bacteria surrounded by an exopolysaccharide matrix.[15] These biofilm infections may be caused by a single species or by a mixture of species of bacteria or fungi.

It was observed that biofilms were responsible for most infections associated with contact lens use. In 2006, Bausch & Lomb withdrew its ReNu with Moisture Loc contact lens solution because a high proportion of corneal infections were associated with it.[21,19]

**Leptospirosis**

Leptospirosis is a major public health problem in southeast Asia and South America, with over 500,000 severe cases every year. Between 5% and 20% of these cases are fatal. Rats and other mammals carry the disease-causing pathogen *Leptospira interrogans* in their kidneys. When they urinate, they contaminate surface water with the bacteria, which can survive in the environment for long periods.Previously, scientists believed the bacteria were planktonic. But Professor Picardeau and his team have shown that *L. interrogans* can make biofilms, which could be one of the main factors controlling survival and disease transmission.[14]

**Cystic fibrosis**

As mentioned previously, infection by the bacterium *Pseudomonas aeruginosa* (*P. aeruginosa*) is the main cause of death among patients with cystic fibrosis. Pseudomonas is able to set up permanent residence in the lungs of patients with cystic fibrosis where, if you ask most mainstream researchers, it is impossible to kill. Eventually, chronic inflammation produced by the immune system in response to Pseudomonas destroys the lung and causes respiratory failure. In the permanent infection phase, *P. aeruginosa* biofilms are thought to be present in the airway, although much about the infection pathogenesis remains unclear.[13,14]Cystic fibrosis is caused by mutations in the proteins of channels that regulates chloride. How abnormal chloride channel protein leads to biofilm infection remains hotly debated. It is clear, however, that cystic fibrosis patients manifest some kind of host-defense defect localized to the airway surface. Somehow this leads to a debilitating biofilm infection.[20]

**Ear infections**

It wasn't until July of 2006 that researchers realized that the majority of ear infections are caused by biofilm bacteria. These infections, which can be either acute or chronic, are referred to collectively as otitis media (OM). They are the most common illness for which children visit a physician, receive antibiotics, or undergo surgery in the United States.

It took over ten years for researchers to realize that otitis media is caused by biofilms. In a subsequent study, Ehrlich and Post obtained middle ear mucosa – or membrane tissue – biopsies from children undergoing a procedure for otitis. The team gathered uninfected mucosal biopsies from children and adults undergoing cochlear implantation as a control. The team obtained three dimensional images of the biopsies and evaluated them for biofilm morphology using generic stains and species-specific probes for *Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis*. Effusions, when present, were also evaluated for evidence of pathogen specific nucleic acid sequences (indicating presence of live bacteria).[14]

The study found mucosal biofilms in the middle ears of 46/50 children (92%) with both forms of otitis. Biofilms were not observed in eight control middle ear mucosa specimens obtained.

**Chronic infections**

it is now increasingly understood that chronic inflammatory diseases result from infection with a large microbiota of chronic biofilm and L-form bacteria (collectively called the Th1 pathogens).[17,19]

**Treating biofilm infections**

Biofilms have been reported to be less susceptible to antimicrobial agents and have reduced sensitivity to inhibitors, thereby adding to their survival.[10] The findings have shown delayed penetration of ciprofloxacin into *Pseudomonas aeruginosa* biofilms.[11] *E.coli* biofilms exhibited decreased susceptibility to cetrimide.[12] Similar reports are available in *Staphylococcus aureus* exposed to tobramycin.[26,13] The resistance shown by these biofilms, in general, has been attributed to factors such as poor penetration of antimicrobials, nutrient limitation, accumulation of toxic metabolites and decreased oxygen tension.[23]

Although the mainstream medical community is rapidly acknowledging the large number of diseases and infections caused by biofilms, most researchers are convinced that biofilms are difficult or impossible to destroy, particularly those cells that form the deeper layers of a thick biofilm. Most papers on biofilms state that they are resistant to antibiotics administered in a standard manner. Mainstream researchers have repeatedly tried to kill biofilms by giving patients high, constant doses of antibiotics.[22] Unfortunately, when administered in high doses, the antibiotic may temporarily weaken the biofilm but is incapable of destroying it, as certain cells inevitably persist and allow the biofilm to regenerate. . The catch is that antibiotics are only effective against biofilms if administered in a very specific manner. Furthermore, only certain antibiotics appear to effectively target biofilms. For eg studies have shown that the penetration of oxacillin, cefotaxime (β-lactams) and vancomycin (a glycopeptide) is significantly reduced through *S. aureus* and *S. epidermidis* biofilms whereas that of amikacin (an aminoglycoside) and ciprofloxacin (a fluoroquinolone) remains unaffected.After decades of research, much of which was derived from molecular modeling data, Marshall was the first to create an antibiotic regimen that appears to effectively target and destroy biofilms.[15,17] Central to the treatment, which is called the Marshall Protocol, is the fact that biofilms and other Th1 pathogens succumb to specific bacteriostatic antibiotics taken in very low, pulsed doses. It is only when antibiotics are administered in this manner that they appear capable of fully eradicating biofilms.[19][20] Thus, a dose of antibiotics – particularly in immunocompromised patients – eradicates most of the biofilm population but leaves a small fraction of surviving persisters behind. Unfortunately, in the same sense that the beta-lactam antibiotics promote the formation of L-form bacteria, persister cells are actually preserved by the presence of an antibiotic that inhibits their growth. Thus, paradoxically, dosing an antibiotic in a constant, high-dose manner (in which the antibiotic is always present) helps persisters persevere.[15,17]

But in the case of low, pulsed dosing, where an antibiotic is administered, withdrawn, then administered again, the first application of antibiotic will eradicate the bulk of biofilm cells, leaving persister cells behind. Withdrawl of the antibiotic allows the persister population to start growing. Since administration of the antibiotic is temporarily stopped, the survival of persisters

is not enhanced. This causes the persister cells to lose their phenotype (their shape and biochemical properties), meaning that they are unable to switch back into biofilm mode. A second application of the antibiotic should then completely eliminate the persister cells, which are still in planktonic mode.[19]

**Conclusion**

Infectious disease processes such as otitis media, periodontitis, cystic fibrosis and chronic prostatitis all appear to be caused by biofilm-associated microorganisms. In addition, indwelling medical devices have been shown to harbour biofilms, which have been implicated in infections. Apart from acting as a repository biofilms are highly resistant to most antimicrobial agents and disinfectants; sessile bacteria within a biofilm are able to acquire resistance through the transfer of resistance plasmids. This acquisition of resistance is particularly important in the healthcare environment for patients with colonised urinary catheters,artifical heart valves and chronically ill patients. Hence further studies on Biofilms are warrented which include effective control strategies ,effective treatment strategies and further understanding of the mechanisms which make bacteria within biofilms so different from their planktonic counterparts.

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