An Elsevier Indexed Journal ISSN-2230-7346

*Journal of Global Trends in Pharmaceutical Sciences*

# A REVIEW ON "BIOFILMS: MICROBIAL LIFE ON SURFACE"

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**ARTICLE INFO**

**Key Words**
Biofilm, Microorganism, Matrix, Cell

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

**ABSTRACT**

This review constitutes a detailed description about the term "BIOFILM" which can be defined as a "thin but robust layer of mucilage adhering to the solid surface and containing a community of bacteria and other microorganism. Biofilm have greater importance for the public health because of their role in certain infectious diseases and importance in a variety of device related infections. A greater understanding of biofilm processes should be lead to novel,effective control stratergies for biofilm control and resulting improvement in patient management. The biofilm has both its own advantages and disadvantages.

## INTRODUCTION

Microorganisms attach to surfaces and develop biofilms. Biofilm-associated cells can be differentiated from their suspended counterparts by generation of an extracellular polymeric substance (EPS) matrix, reduced growth rates, and the up- and down- regulation of specific genes. Attachment is a complex process regulated by diverse characteristics of the growth medium, substratum, and cell surface. An established biofilm structure comprises microbial cells and EPS, has a defined architecture, and provides an optimal environment for the exchange of genetic material between cells. Cells may also communicate via quorum sensing, which may in turn affect biofilm processes such as detachment. Biofilms have great importance for public health because of their role in certain infectious diseases and importance in a variety of device-related infections[1,3] Microbes form a biofilm in response to various different 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. A cell that switches to the biofilm mode of growth undergoes a phenotypic shift in behaviour in which large suites of genes are differentially regulated. Biofilm may also be considered a hydrogel, which is a complex polymer that contains many times its dry weight in water. Biofilms are not just bacterial slime layers but biological systems; the bacteria organize themselves into a coordinated functional community Biofilms are advantageous to bacteria because they provide a nutrient-rich environment that facilitates growth and because they confer resistance to antibiotics. Biofilms can cause severe infections in hospitalized patients; the formation of biofilms in these instances is typically associated with the introduction into the body of foreign substrates, such as artificial implants and urinary catheters.

Biofilms also form on the thin films of plaque found on teeth, where they ferment sugars and starches into acids, causing the destruction of tooth enamel. In the environment, biofilms fill an important role in the breakdown of organic wastes by filtering wastes from water and by removing or neutralizing contaminants in soil. As a result, biofilms are used to purify water in water treatment plants and to detoxify contaminated areas of the enviorment[2]

## BIOFILMS DEFINED

A thin but robust layer of mucilage adhering to a solid surface and containing a community of bacteria and other microorganisms. Non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix. Biofilm-associated organisms also differ from their planktonic (freely suspended) counterparts with respect to the genes that are transcribed. Biofilms may form on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems. The variable nature of biofilms can be illustrated from scanning electron micrographs of biofilms from an industrial water system and a medical device, respectively the figure. The water system biofilm is highly complex, containing corrosion products, clay material, fresh water diatoms, and filamentous bacteria. The biofilm on the medical device, on the other hand, appears to be composed of a single, coccoid organism and the associated extracellular polymeric substance (EPS)matrix[3,4,5]

## CONDITIONING FILMS

A material surface exposed in an aqueous medium will inevitably and almost immediately become conditioned or coated by polymers from that medium, and the resulting chemical modification will affect the rate and extent of microbial attachment. Loeb and Neihof were the first to report the formation of these conditioning films on surfaces exposed in seawater. These researchers found that films were organic in nature, formed within minutes of exposure, and continued to grow for several hours. The nature of conditioning films may be quite different for surfaces exposed in the human host. A prime example may be the proteinaceous conditioning film called "acquired pellicle," which develops on tooth enamel surfaces in the oral cavity. Pellicle comprises albumin, lysozyme, glycoproteins, phosphoproteins, lipids, and gingival crevice fluid bacteria from the oral cavity colonize pellicle-conditioned surfaces within hours of exposure to these surfaces. Mittelman noted that a number of host-produced conditioning films such as blood, tears, urine, saliva, inter vascular fluid, and respiratory secretions influence the attachment of bacteria to biomaterials. Ofek and Doyle also noted that the surface energy of the suspending medium may affect hydrodynamic interactions of microbial cells with surfaces by altering the substratum characteristics.[5,6]

## PROPERTIES

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. Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic (visible to the naked eye). Biofilms can contain many different types of microorganism, e.g. bacteria, archaea, However, some organisms will form single-species films under certain conditions. The social structure (cooperation/competition) within a biofilm depends highly on the different species present[7]

## DISPERSAL

Dispersal of cells from the biofilm colony is an essential stage of the biofilm life cycle. Dispersal enables biofilms to spread and colonize new surfaces. Enzymes that degrade the biofilm extracellular matrix, such as dispersin B and deoxyribonuclease, may contribute to biofilm dispersal. Enzymes that degrade the biofilm matrix may be useful as anti-biofilm agents. Recent evidence has shown that a fatty acid messenger, cis-2-decenoic acid, is capable of inducing dispersion and inhibiting

growth of biofilm colonies. Secreted by Pseudomonas aeruginosa, this compound induces cycloheteromorphic cells in several species of bacteria and the yeast Candida albicans. Nitric oxide has also been shown to trigger the dispersal of biofilms of several bacteria species at sub-toxic concentrations. Nitric oxide has the potential for the treatment of patients that suffer from chronic infections caused by biofilms.[8] It is generally assumed that cells dispersed from biofilms immediately go into the planktonic growth phase. However, recent studies have shown that the physiology of dispersed cells from *Pseudomonas aeruginosa* biofilms is highly different from those of planktonic and biofilm cells. Hence, the dispersal process is a unique stage during the transition from biofilm to planktonic lifestyle in bacteria. Dispersed cells are found to be highly virulent against macrophages and Caenorhabditis elegans, but highly sensitive towards iron stress, as compared with planktonic.

## BIOFILM STRUCTURE

### Extracellular polymeric substance

Biofilms are composed primarily of microbial cells and EPS. EPS may account for 50% to 90% of the total organic carbon of biofilms and can be considered the primary matrix material of the biofilm. EPS may vary in chemical and physical properties, but it is primarily composed of polysaccharides. Some of these polysaccharides are neutral or polyanionic, as is the case for the EPS of gram-negative bacteria. The presence of uronic acids (such as D-glucouronic, D-galacturonic, and mannuronic acids) or ketal-linked pyruvates confers the anionic property. This property is important because it allows association of divalent cations such as calcium and magnesium, which have been shown to cross-link with the polymer strands and provide greater binding force in a developed biofilm. In the case of some gram-positive bacteria, such as the staphylococci, the chemical composition of EPS may be quite different and may be primarily cationic. Hussain et al. found that the slime of coagulase-negative bacteria consists of a teichoic acid mixed with small quantities of proteins.[11,12,13] EPS is also highly hydrated because it can incorporate large amounts of water into its structure by hydrogen bonding. EPS may be hydrophobic, although most types of EPS are both hydrophilic and hydrophobic. EPS may also vary in its solubility. Sutherland noted two important properties of EPS that may have a marked effect on the biofilm. First, the composition and structure of the polysaccharides determine their primary conformation. For example, many bacterial EPS possess backbone structures that contain 1,3- or 1,4-β-linked hexose residues and tend to be more rigid, less deformable, and in certain cases poorly soluble or insoluble. Other EPS molecules may be readily soluble in water. Second, the EPS of biofilms is not generally uniform but may vary spatially and temporally. Leriche et al. used the binding specificity of lectinsto simple sugars to evaluate bacterial biofilm development by different organisms. These researchers' results showed that different organisms produce differing amounts of EPS and that the amount of EPS increases with age of the biofilm. EPS may associate with metal ions, divalent cations, other macromolecules (such as proteins, DNA, lipids, and even humic substances). EPS production is known to be affected by nutrient status of the growth medium; excess available carbon and limitation of nitrogen, potassium, or phosphate promote EPS synthesis. Slow bacterial growth will also enhance EPS production. Because EPS is highly hydrated, it prevents desiccation in some natural biofilms. EPS may also contribute to the antimicrobial resistance properties of biofilms by impeding the mass transport of antibiotics through the biofilm, probably by binding directly to these agents.[14]

## PROPERTIESOF THE CELL

Cell surface hydrophobicity, presence of fimbriae and flagella, and production of EPS all influence the rate and extent of attachment of microbial cells. The hydrophobicity of the cell surface is important in adhesion because hydrophobic interactions tend to increase with an increasing nonpolar nature of one or both surfaces involved (i.e., the microbial cell surface and the substratum surface). Fimbriae, i.e., non-flagellar appendages other than those involved in transfer of viral or bacterial nucleic acids (called pili), contribute to cell surface

hydrophobicity. Fimbriae play a role in cell surface hydrophobicity and attachment, probably by overcoming the initial electrostatic repulsion barrier that exists between the cell and substratum. Cell surface polymers with nonpolar sites such as fimbriae, other proteins, and components of certain gram-positive bacteria (mycolic acids) appear to dominate attachment to hydrophobic substrata, while EPS and lipopolysaccharides are more important in attachment to hydrophilic materials. Flagella are important in attachment also, although their role may be to overcome repulsive forces rather than to act as adsorbents or adhesives. In general, attachment will occur most readily on surfaces that are rougher, more hydrophobic, and coated by surface "conditioning" films. An increase in flow velocity, water temperature, or nutrient concentration may also equate to increased attachment, if these factors do not exceed critical levels. Properties of the cell surface, specifically the presence of fimbriae, flagella, and surface-associated polysaccharides or proteins, also are important and may possibly provide a competitive advantage for one organism where a mixed community is involved.[15]

## BIOFILM FORMATION

The formation of a biofilm begins with the attachment of free-floating microorganisms to a surface The first colonist bacteria of a biofilm may adhere to the surface initially by the weak van der Waals forces and hydrophobic effects If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.[16,17] Hydrophobicity can also affect the ability of bacteria to form biofilms. Bacteria with increased hydrophobicity have reduced repulsion between the substratum and the bacterium. Some bacteria species are not able to attach to a surface on their own successfully due to their limited motility but are instead able to anchor themselves to the matrix or directly to other, earlier bacteria colonists. Non-motile bacteria cannot recognize surfaces or aggregate together as easily as motile bacteria. [16]

During surface colonization bacteria cells are able to communicate using quorum sensing (QS) products such as N-acyl homo serine lactone (AHL). Once colonization has begun, the biofilm grows by a combination of cell division and recruitment. Polysaccharide matrices typically enclose bacterial biofilms. 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, such as erythrocytes and fibrin. 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.[18] The development of a biofilm may allow for an aggregate cell colony (or colonies) to be increasingly resistant to antibiotics. Cell-cell communication or quorum sensing has been shown to be involved in the formation of biofilm in several bacterial species.[19,20]

## DEVELOPMENT

1. Conditioning
2. Contact
3. Adsorption
4. Growth
5. Production of extracellular products
6. Attachment
7. Re-entrainment

### 1.Conditioning

• Acleansurface is immediately covered with a conditioning film of organic molecules and macromolecules macro molecules

• Transport of molecules and small particles is rapid and as a result adsorption of conditioning rapid and as a result adsorption of conditioning film occurs instantaneously

• The presence of the conditioning film alters the characteristics of the substratum.[21,22]

Effect

• Substrate hydrophobicity decreases

• Substrate obtains a negative charge

• Substrate potentials increase or decrease

• Critical surface tensions increase or decrease

**2. Contact**

• Bacteria in fluid contact the substrate via mass transport mechanisms

• Strongly influenced by mixing in the bulk fluid –Related to flow regime

• Laminar flow transport

– Sedimentation
– Motility
– Molecular diffusion
• Turbulent flow transport (larger particles)
– Convection
– Diffusive transport
• Bacteria penetrate the viscous sublayer (~1 cm) via eddy diffusion

• Bacteria actively migrate through the diffusive sublayer using pili (~1 mm)

**3. Adsorption**

• At the substrate the cells absorb reversibly or irreversibly

– Primary(early)colonization Primary (early) colonization
– mediated through specific or non-specific physiochemical interactions with components of conditioning film
– Adsorption
– accumulation of cells directly on surface substrate
– Desorption
– re-entrainment of cells into the bulk fluid

• Complex process

• Initial adsorption occurs through long-range(100s), weak interactions with low specificity

– Electrostatic or van der Waals forces

• Irreversible adsorption is short-range (5nm), highly specific interaction highly specific interaction

– Dipole, ionic, hydrogen bonding, hydrophobic interactions, etc.

– Can take place by secretion of EPS structures[23]

**4. Growth**

• The number of irreversibly adsorbed cells increase due to replication

– Limited by physiological processes

–Concentration of rate-limiting nutrient important

**5. Production of extra cellular products**

• Affixed cells transition from planktonic form to attached form

– Processes controlled by gene encoding for the production of products

**6. Attachment**

• Secondary (late) colonizing cells from bulk fluid attach to the existing biofilm

– Can result in species displacement

Attachment and co-aggregation

• Co-aggregation–is the attachment of distinct bacteria via specific molecules

– Single cells in bulk fluid specifically recognize and adhere to genetically distinct cells in developing biofilm
– Prior co-aggregation in suspension followed by subsequent adhesion to existing biofilm
  • Multi-species biofilms are a functional consortium that often possess a combined metabolic activity that is greater than the individual component species

**7. Re-entrainment**

• Cells detach from the surface and return to the bulk fluid and planktonic form of growth

• Detachment can occur

- Erosion
- Sloughing
- Human intervention
- Predatory grazing
- Abrasion
- Starvation

• Detachment can be an active or passive process leading to further survival or colonization.[24,25]

## USES AND IMPACT

In medicine

Infections associated with the biofilm growth usually are challenging to eradicate. [81] This is mostly due to the fact that mature biofilms display tolerance towards antibiotics and the immune response. Biofilms often form on the inert surfaces of implanted devices such as catheters, prosthetic cardiac valves and intrauterine devices. [83].The rapidly expanding worldwide industry for biomedical devices and tissue engineering related products is already at $180 billion per year, yet this industry continues to suffer from microbial colonization. No matter the sophistication, microbial infections can develop on all medical devices and tissue engineering constructs. 60-70% of nosocomial or hospital acquired infections are associated with the implantation of a biomedical device. This leads to 2 million cases annually in the U.S., costing the healthcare system over $5 billion in additional healthcare expenses. [28]

### In industry

Biofilms can also be harnessed for constructive purposes. For example, many sewage treatment plants include a secondary treatment stage in which waste water passes over biofilms grown on filters, which extract and digest organic compounds. In such biofilms, bacteria are mainly responsible for removal of organic matter (BOD), while protozoa and rotifers are mainly responsible for removal of suspended solids (SS), including pathogens and other microorganisms. Slow sand filters rely on biofilm development in the same way to filter surface water from lake, spring or river sources for drinking purposes. What we regard as clean water is effectively a waste material to these microcellular organisms. Biofilms can help eliminate petroleum oil from contaminated oceans or marine systems. The oil is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called *hydrocarbonoclastic bacteria* (HCB). Biofilms are used in microbial fuel cells (MFCs) to generate electricity from a variety of starting materials, including complex organic waste and renewable biomass. [7] [85] [86] Biofilms are also relevant for the improvement of metal dissolution in bioleaching industry.[26]

### Food industry

Biofilms have become problematic in several food industries due to the ability to form on plants and during industrial processes. Bacteria can survive long periods of time in water, animal manure, and soil, causing biofilm formation on plants or in the processing equipment. The build-up of biofilms can affect the heat flow across a surface and increase surface corrosion and frictional resistance of fluids. These can lead to a loss of energy in a system and overall loss of products. Along with economic problems, biofilm formation on food poses a health risk to consumers due to the ability to make the food more resistant to disinfectants. As a result, from 1996 to 2010 the Center for Disease Control and Prevention estimated 48 million foodborne illnesses per year. Biofilms have been connected to about 80% of bacterial infections in the United States.[27] In produce, microorganisms attach to the surfaces and biofilms develop internally. During the washing process, biofilms resist sanitization and allow bacteria to spread across the produce. This problem is also found in ready-to-eat foods, because the foods go through limited cleaning procedures before consumption Due to the perishability of dairy products and limitations in cleaning procedures, resulting in the build-up of bacteria, dairy is susceptible to biofilm formation and contamination.[89][91] The bacteria can spoil the products more readily and contaminated products pose a health risk to consumers. One bacteria that can be found in various industries and is a major cause of foodborne disease is Salmonella. Large

amounts of salmonella contamination can befound in the poultry processing industry as about 50% of salmonella strains can produce biofilms on poultry farms. Salmonella increases the risk of foodborne illnesses when the poultry products are not cleaned and cooked correctly. Salmonella is also found in the seafood industry where biofilms form from seafood borne pathogens on the seafood itself as well as in water. Shrimp products are commonly affected by salmonella because of unhygienic processing and handling techniques. The preparation practices of shrimp and other seafood products can allow for bacteria build up on the products. [29,31] New forms of cleaning procedures are being tested in order to reduce biofilm formation in these processes which will lead to safer and more productive food processing industries. These new forms of cleaning procedures also have a profound effect on the environment, often releasing toxic gases into the groundwater reservoirs. [30]

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