



Review Article

## Bacterial biofilm and associated infections

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### Abstract

Microscopic entities, microorganisms that drastically affect human health need to be thoroughly investigated. A biofilm is an architectural colony of microorganisms, within a matrix of extracellular polymeric substance that they produce. Biofilm contains microbial cells adherent to one-another and to a static surface (living or non-living). Bacterial biofilms are usually pathogenic in nature and can cause nosocomial infections. The National Institutes of Health (NIH) revealed that among all microbial and chronic infections, 65% and 80%, respectively, are associated with biofilm formation. The process of biofilm formation consists of many steps, starting with attachment to a living or non-living surface that will lead to formation of micro-colony, giving rise to three-dimensional structures and ending up, after maturation, with detachment. During formation of biofilm several species of bacteria communicate with one another, employing quorum sensing. In general, bacterial biofilms show resistance against human immune system, as well as against antibiotics. Health related concerns speak loud due to the biofilm potential to cause diseases, utilizing both device-related and non-device-related infections. In summary, the understanding of bacterial biofilm is important to manage and/or to eradicate biofilm-related diseases. The current review is, therefore, an effort to encompass the current concepts in biofilm formation and its implications in human health and disease.

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### 1. Introduction

In 17th century, Antoine Von Leeuwenhoek, for the first time observed a type of creature on his own teeth, a discovery considered to be a biofilm.<sup>1</sup> Zobell in 1943 stated that “the

surrounding sea water have less number of bacteria than on the surface”.<sup>2</sup> Even at the end of 1960 and the start of 1970, physical and chemical properties of biofilms were not investigated.<sup>3</sup> Heukelekian and Heller observed “*Bottle Effect*” of marine microorganisms – the growth and activity enhances when they are attached to a surface.<sup>4</sup> However, the curious observation of microbial biofilm awaited the invention of electron microscopy to examine in detail the biofilm with high-resolution, as compared to light microscopy. The employment of scanning electron and transmission electron microscopy allowed to identify biofilm on trickling filters in a wastewater treatment plant. It was then concluded that the

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biofilm cell morphology is evident of the clustering of a variety of microorganisms.<sup>5</sup>

## 2. Microbial biofilm composition

Biofilm is an organized aggregate of microorganisms living within an extracellular polymeric matrix that they produce and irreversibly attached to fetish or living surface which will not remove unless rinse quickly.<sup>6,7</sup> Formation of extracellular polymeric substances (EPS) occurs in the attachment stage of a biofilm to the surface. Whether a microbial biofilm will form on an inanimate or solid surface or not is a consequence of the formation of an exopolysaccharide matrix, which provides strength to the interaction of the microorganisms in the biofilm.<sup>9–11</sup> Usually thickness of EPS matrix is 0.2–1.0  $\mu\text{m}$ , however the size of the biofilm does not exceed 10–30 nm.<sup>12</sup> Typically 5–35% of the biofilm volume is constituted by the microorganisms while the remaining volume is extracellular matrix. This extracellular matrix is partially or mostly composed of proteins.<sup>13</sup> Some important nutrients and minerals are trapped from the surrounding environment through the scavenging system, created by the extracellular matrix.<sup>7</sup> Different types of components are present in extracellular polymeric substances: protein in majority (>2%); other constituents, such as polysaccharides (1–2%); DNA molecules (<1%), RNA (<1%); ions (bound and free), and finally 97% of water. The flow of essential nutrients inside a biofilm is attributed to the water content.<sup>14,18</sup>

## 3. Steps in biofilm formation

Genetic studies tell us about the formation of biofilm that it occurs in many steps. It requires special type of signaling, known as quorum sensing, between the microorganism cells. Also, it requires transcription of different set of genes compared to those of planktonic forms of the same microbial organisms.<sup>15,16</sup> In addition, there are channels in the biofilm that separate the micro colonies. Mechanical stability of a biofilm is attributed to the viscoelastic features of the EPS matrix.<sup>17</sup> Formation of biofilm is complex but according to different researchers it occurs in few common steps: initial contact/attachment to the surface, followed by micro-colony formation, maturation and formation of the architecture of the biofilm, and finally detachment/dispersion of the biofilm. Each of these steps will be discussed below.<sup>18</sup>

### 3.1. Initial contact/attachment to the surface

In this step of biofilm formation, microbial cells attach to the surface through their appendages like pilli and flagella and may also get attached through other physical forces like van der Waal's forces, electrostatic interactions etc. Other factors are also greatly affecting the bacterial adhesion to a surface. Adhesion – the attachment of microbial cells to a surface, and cohesion – the interaction/attachment within the cells, occur in biofilm formation.<sup>19,20</sup> Solid–liquid interface can also be a reason for attachment and growth of microorganisms in biofilm

formation.<sup>21</sup> The fimbriae, pilli and flagella give strength to the interaction between bacteria and the surface of attachment. The hydrophobicity of the surface may also play a role in strengthening the attachment of microbes, because it reduces the force of repulsion between the bacteria and the surface.<sup>22,23</sup> Microorganisms attach more likely to the hydrophobic and non-polar surfaces like Teflon and other plastics, than to hydrophilic and polar surface like metals and glass.<sup>24–26</sup>

### 3.2. Micro-colony formation

After an attachment of microorganisms to a biotic or an abiotic surface occurs and this attachment becomes stable, a process of multiplication and division of microbial cells starts, initiated through particular chemical signaling within the EPS. This process then leads to the formation of micro-colonies.<sup>21,27</sup> Bacterial colonies in a biofilm usually consist of many types of micro-communities. These micro-communities coordinate with one another in multiple aspects. This coordination plays a crucial role in exchange of substrate, distribution of important metabolic products and excretion of metabolic end-products. For instance, during anaerobic digestion, when complex organic matter is converted into  $\text{CH}_4$  and  $\text{CO}_2$ , a minimum of three types of bacterial involvement is required: (i) fermentative bacteria start the production of acid and alcohol from organic compounds, depending upon the catabolism of complex organic compounds, (ii) these substrates are then consumed by acetogenic bacteria as their substrates, and (iii) methanogens get energy by converting the acetate, carbon dioxide and hydrogen into methane. Biofilm provides a complete environment for the development of syntrophic association, an association of two or more metabolically different bacteria depending on each other for utilization of certain substrates for their energy purposes.<sup>28</sup>

### 3.3. Maturation and architecture

In this stage of biofilm formation microbial cells communicate with one another through auto-inducer signals.<sup>29,30</sup> Cell-to-cell communication is an important process, during which the required microbial cell density is attained. This leads to the secretion of signaling molecules, known as auto-inducers. These auto inducers facilitate quorum sensing.<sup>16</sup> At this stage of maturation certain gene products are expressed, that are considered important for the formation of EPS. Since EPS is the main material in the biofilm's three-dimensional structure, interstitial voids are then produced in the matrix. These channels are filled with water and act as a circulatory system, used to distribute important nutrients and remove waste products from the communities of micro-colonies in the biofilm.<sup>31</sup>

### 3.4. Detachment/dispersion of biofilm

In this phase, microbial cells within the biofilm perform quick multiplication and dispersion in order to convert from sessile into motile form. Detachment then occurs in a natural

pattern.<sup>21</sup> However, some types of bacteria do not produce extracellular polysaccharide and the bacterial cells disperse directly into the environment, but sometimes mechanical stress may also be involved in this process.<sup>32</sup> During the detachment process microbial communities within the biofilm produce different saccharolytic enzymes that help to release the surface of the microbes into a new area for colonization. For instance, *Escherichia coli* produces *N*-acetyl-heparosan lyase, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* produce alginate lyase, and *Streptococcus equi* produces *hyaluronidase* for the lysis of the EPS matrix and subsequent detachment.<sup>33</sup> In this phase microbial cells upregulate the expression of proteins related to flagella formation, to let the bacteria move to a new site. Detachment of microbial cells and transfer to a new site aid in the spreading of infections.<sup>34</sup>

#### 4. Infections associated with biofilm

It is estimated that about 65% of all bacterial infections are associated with bacterial biofilms.<sup>35</sup> These include both, device- and non-device-associated infections. Data for device-related infections have been estimated for several devices, such as: 2% for breast implants; 2% for joint prostheses; 4% for mechanical heart valves; 10% for ventricular shunts; 4% for pacemakers and defibrillator, and about 40% for ventricular-assisted devices.<sup>36</sup> Native valve endocarditis (NVE) is an inflammation caused by interaction of bacteria with the vascular endothelium and pulmonic valves of the heart. This is usually the result of *Streptococci*, *Staphylococci*, gram negative bacteria, and/or fungal infections.<sup>46</sup> In this condition microbial cells gain access to the heart and blood through the gastrointestinal tract, urinary tract and/or through the oropharynx. As the intact valve endothelium gets damaged by the microorganisms that attach to it, even after the bacteria have been cleared by the immune system a non-bacterial thrombotic endocarditis (NBTE) develops at the injury location, as a result a thrombus formation occurs, a condition where platelets, red blood cells and fibrin are aggregated.<sup>37</sup>

##### 4.1. Device-related biofilm infections

Biofilms usually occur on or within indwelling medical devices such as contact lenses, central venous catheters, mechanical heart valves, peritoneal dialysis catheters, prosthetic joints, pacemakers, urinary catheters and voice prostheses.<sup>38,39</sup> Biofilms may be composed of only a single or of different types of microbial species. This depends on the devices and their duration of action.<sup>15</sup>

Contact lenses are categorized as soft and hard contact lenses. Microorganisms can adhere to both types of lenses. Their classification is based on construction materials, frequency of disposal, wear schedule and design. The type of microorganisms which are attached to contact lenses are mainly *E. coli*, *P. aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, species of *Candida*, *Serratia* and *Proteus*, etc., but most importantly, the degree of adherence to the lenses depends on

the water content, substrate nature, electrolyte concentration, type of bacterial strain involved and lastly the composition of the polymer. Under scanning electron microscopy biofilm has been observed on contact lenses of a patient diagnosed with keratitis, produced by *P. aeruginosa*. Biofilms can also form more frequently on contact lenses that are usually kept in lens storage cases. The lens storage cases, therefore, have been declared as a source of lens contamination.<sup>37</sup>

Formation of biofilm is universal on central venous catheters, but the location and extent of biofilm formation depend on the duration of catheterization. For example, a short-term (<10 days) catheters have more biofilm formation on the external surface, whereas long-term catheters (30 days) have greater biofilm formation in the catheter lumen. The growth of microbes may be affected by the nature of the fluid which is administered through the central venous catheter. For example, gram positive bacteria, such as *S. epidermidis* and *S. aureus*, do not grow well in intravenous fluids, whereas gram-negative aquatic bacteria, such as *P. aeruginosa*, *Enterobacter* species and *Klebsiella* species sustain growth in such fluids.<sup>40,44</sup>

Microbial cells attach and produce biofilm on mechanical heart valves and surrounding tissues, a condition known as prosthetic valve endocarditis. The types of bacteria responsible for this unpleasant condition are *Streptococcus* species, *S. aureus*, *S. epidermidis*, gram-negative *Bacillus*, *Enterococcus* and *Candida* spp. The origin of these micro-organisms may be from the skin or from other indwelling devices like central venous catheters or dental work.<sup>45</sup> At the time of surgical implantation of prosthetic heart valves, tissue damage may occur as a result of accumulation of platelets and fibrin at the location of suture and on the devices. Microbial cells have better ability to colonize these locations.<sup>37</sup>

Urinary catheters are usually made of silicon or latex and are normally used during surgical operations to measure the urine generation and excretion. Urinary catheters are administered through the urethra up to the urinary bladder. They may have a closed system or an open system. In an open catheter system, urine is drained in an open collection center, but in this type of system chances of contamination are higher, that may lead to urinary tract infections (UTI) within days. In closed catheter systems urine accumulates in a plastic bag, thus this type of system provides less chances for UTIs.<sup>46</sup> Microbial cells that commonly contaminate and form biofilms on these devices are *E. coli*, *Enterococcus faecalis*, *S. epidermidis*, *P. aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and other gram-negative bacteria.<sup>47</sup>

##### 4.2. Non-device related biofilm infections

Periodontitis is an infection of the gums. In this infection damaging of soft tissues, as well as that of bones supporting the teeth occurs. Normally, it is caused by poor oral hygiene. Tooth-loss is also possible.<sup>48</sup> *P. aerobius* and *Fusobacterium nucleatum* are among the causative agents of periodontitis. These microbes also have the ability to form biofilms on a variety of surfaces, including mucosal surfaces in the oral

cavity.<sup>49</sup> Microbial colonization of teeth surfaces may permit them to invade mucosal cells and alter the flow of calcium in the epithelial cells, as well as to release toxins. A plaque can then develop within 2–3 weeks. The plaque may mineralize with calcium and phosphate ions, forming the so called tartar or calculus.<sup>50</sup>

Osteomyelitis is a disease of bones, which may be caused by bacterial cells or fungi. Bacteria enter the bones through the bloodstream, trauma or through previous infections.<sup>51</sup> When microbes enter through the bloodstream and the metaphysis of the bone becomes infected, this leads to the recruitment of white blood cells (WBCs) to the site. These WBCs attempt to phagocytose or lyse the pathogens by secreting enzymes. These enzymes may lyse the bone, which results in the formation of pus, and spread through the bone blood vessels, thus stopping the proper flow of blood and causing tissue damage and deterioration of the function of the affected bone areas.<sup>52</sup>

## 5. Advancements in biofilm research

Biofilm formation of infectious significance is mostly found on “implant devices”. Recently, in the last year, another saprophytic organism, the incidence of which is increased in nosocomial (hospital acquired) infections, is a risk factor for medical device-carrying patients.<sup>55</sup> *P. aeruginosa* is the 2nd most common reason for ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections (CAUTI). *P. aeruginosa* forms biofilms on endocardial tubes and catheters in CAUTI and VAP patients.<sup>56</sup>

Another recent advancement in biofilm research has been applied to control energy crises. This approach is using microbial fuel cells (MFCs). MFCs produce electricity by utilizing chemical energy found in organic and some in-organic compounds. Electrogenic microbes play a role in this process by accepting or donating electrons to an external object (electrode), while some non-electrogenic microbes are also involved as part of a synergistic electrogenic biofilm.<sup>57</sup>

Another biofilm-related problem is caused by *Asaia* species, which form biofilms on plants used for the production of soft drinks, and may thereby contaminate the soft drinks even in the presence of a preservative. Additionally, biofilm resistance against antibiotics has reached an alarming state. Antibiotic therapy is not effective once the biofilm is matured. A Chinese medical herb *Herba patreniae* degrades the mature biofilm of *P. aeruginosa* and its exopolysaccharide.<sup>58</sup> A biofilm-growing mucoid strain can play a role in the exacerbation of cystic fibrosis. There are several antimicrobial agents used to treat this biofilm-forming strain of *P. aeruginosa*. For example, Ciprofloxacin has been shown to kill the bacteria found on the surface of the biofilm, whereas Colistin was shown to kill the ones found in the depth of the biofilm. There are many other possibilities that can be applied in the journey of treatment of biofilm-related infections, such as inhibition of quorum sensing through breaking of matrix by *alginate lyase* or F-actin.<sup>59</sup> For bacterial biofilm formation quorum sensing activity is very important, as revealed by genetic analysis of biofilms. There are many identified molecules produced by

eukaryotes and prokaryotes to quench the quorum sensing, i.e. quorum quenching.<sup>55</sup>

In conclusion, biofilm formation on indwelling medical devices greatly affects surgical and instrumental procedures and public health as well. It also has implications in non-device-related human-health complications. There is a need for an in-depth research to optimize measures for its prevention. Good hygienic conditions and practices are very necessary to avoid biofilm formation. With the passage of time, and with the advent of new technologies, progress has been made to remove and control biofilm-associated infections. However, new anti-biofilm strategies are necessary to handle biofilm-associated chronic infections.

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