

## Microbial Biofilms: Pathogenicity and Treatment strategies

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### ABSTRACT

Microbial biofilms are complex structures wherein the planktonic cells change their growth mode to the sessile form. This kind of growth is assisted by the formation of a matrix of extracellular polymeric substances (EPS) which encapsulates the bacterial cells within it and thus, provides an additional protection. These biofilms are highly resistant to high concentration of antibiotics and poses a great threat towards public health. These biofilms are even beyond the access of a normal human immune system and are involved in infections of teeth, lungs and many other diseases. There lies an immediate need to replace the extensive use of antibiotics with new emerging strategies. The review intends to provide an insight on the various perspectives of microbial biofilms including their formation, composition, mechanism of communication (Quorum sensing) and pathogenicity. Recent emerging strategies have also been discussed that can be considered for successful eradication or inhibition of biofilms and related infections.

**Keywords:** Biofilm, Extracellular polymeric substances, antibiotics, microorganisms, Quorum sensing, Resistance

### INTRODUCTION

Biofilm is an association of microorganisms in which microbial cells adhere to each other on a living or non-living surfaces encased in a matrix of extracellular polymeric substance (EPS) produced by the microbial cells itself (Hall-Stoodey et al., 2004). Dutch researcher, Antoni van Leeuwenhoek, for the first time observed 'animalcule' on surfaces of tooth by using a simple microscope and this was considered as the discovery of microbial biofilm. Costerton coined the term 'Biofilm' in 1978 (Costerton et al., 1999). Research carried out has confirmed that the bacterial biofilms are beyond the access of antibiotics and are also not inhibited by the human immune system. The microorganisms responsible for producing biofilms have enhanced potential to resist or neutralize antimicrobial agents, thus making the treatment process prolonged. Some genes in the biofilm forming bacteria are switched on leading to activation of stress related genes, thus converting the bacteria into resistant phenotypes due to changes in cell density, pH, osmolarity, nutrition or temperature (Fux et al., 2005). Sekhar et al., 2009 reported that almost all the microorganisms except a very few have the ability to form biofilm in almost all types of surfaces (Sekhar et al., 2009). Biofilm poses a great threat for public health which

may be attributed to the diseases caused by it and the resistance it offers towards many antibiotics (Khan et al., 2014). The exopolymer in biofilms have been reported to limit the ability of leucocytes to penetrate the biofilm (Thurlow et al., 2011), hampers their movement through the biofilms, checking their ability to degranulate and produce reactive oxygen species, thus preventing the phagocytosis of bacteria (Bayer et al., 1991; Malic et al., 2011). Studies have reported large number of bacterial species such as *P. aeruginosa*, *S. epidermidis*, *E. coli*, *S. aureus*, *E. cloacae*, *K. pneumonia* to have the capability of forming biofilms (Fux et al., 2005; Parsek and Singh, 2003; Miller and Bassler, 2001; Ma et al., 2009). Biofilms have been found to spread infections by colonizing implanted medical devices (Habash et al., 1999; Wolcott et al., 2010) such as central venous catheters, urinary catheters, joint prostheses, pacemakers. Biofilm has also been reported to cause dental caries, lung infections in cystic fibrosis patients (Okada et al., 2005) and chronic wounds (Wolcott et al., 2010). The review tries to provide an overview on biofilm formation, composition and their resistance to anti-bacterial compounds as well as negative impact on human health. The authors have also tried to discuss possible methods for prevention and eradication of biofilms.

## FORMATION OF BIOFILM

Bacterial cells generally exhibit two different types of growth – planktonic cells and sessile aggregate. The sessile aggregates are known as biofilms. The formation of biofilm is a highly complex process in which the planktonic cells of the microorganisms transfer to sessile growth mode (Okada et al., 2005). Bacterial biofilms are formed so as to protect the

bacterial cells from adverse environmental conditions and nutrient deficiency (Rajan and Saiman, 2002). Biofilm formation occurs through a multiple step cyclic process. It consists of five different stages (Figure.1) namely- cell attachment, cell to cell adhesion, cell proliferation and growth, maturation and detachment or dispersal (Costerton et al., 1999).

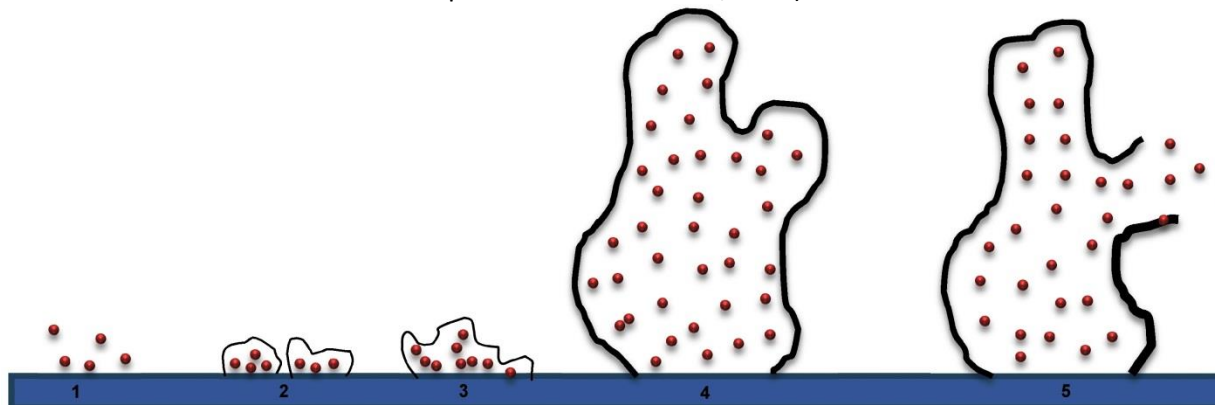


Figure.1. Different stages of biofilm formation: (1) Cell attachment (2) Cell to cell adhesion (3) Cell proliferation and growth (4) Cell maturation and (5) Cell detachment and dispersal.

### Cell attachment

In this step the bacterial cells come and attach to a surface or to any other microorganism already present on it. Rough, hydrophilic and coated surface favors the attachment of cells and formation of biofilm. A solid – liquid interface can provide an ideal environment for attachment (Costerton et al., 1999). Presence of nutrients, favorable temperature, locomotor structures, proteins and carbohydrates can also accelerate attachment (Donlan and Costerton, 2002).

### Cell to cell adhesion

In this step the individual bacterial cells adhere to each other thus forming stable micro colonies.

### Cell proliferation and growth

The bacterial cells in the micro colonies start to multiply as a result of signals in the form of chemicals. These chemical signals when cross a certain threshold, activate the production of exopolysaccharide. The bacterial cells continue to divide within the embedded exopolysaccharide matrix (Mckenney et al., 1998).

### Cell maturation

The cells grow and accumulate to form large three dimensional structures. Expression of certain genes related to biofilm formation along with formation of water filled channels for the transport of nutrients within the biofilm takes place in this step. These water channels not only distribute nutrients but are proposed to have ability to remove waste materials from the bacterial communities (Parsek and Singh, 2003).

### Cell detachment and dispersal

Detachment of bacterial cells from the biofilm is a programmed process. The bacteria stop production of EPS detaching themselves from the biofilm. Dispersion of the cells also occur either by detachment of new formed cells from the growing cells or dispersion of aggregates of biofilm due to quorum sensing. The cells dispersed from the biofilm retain certain characteristics of the biofilm like antibiotic resistance. The cells dispersed from the biofilm may quickly return to their original planktonic phenotype (Baselga et al., 1994).

## COMPOSITION OF BIOFILM

Biofilm is composed of many extracellular polymeric substances secreted by the microbes present in it. These extracellular polymeric substances are

proteins including enzymes, DNA, RNA, polysaccharides and water. Water constitutes the major part of the biofilm (up to 97%) and is responsible for nutrient flow inside the biofilm matrix. Architecturally the biofilm consists of two main distinct components. The first component is the water channel for nutrient transport and the second is a densely packed region of cells lacking prominent pores in it (Gavin and Gillian, 2012). The water channels present in the biofilm when compared to the circulatory system showed that the biofilms are primitive multicellular organisms (Gilbert et al., 1997).

### COMMUNICATION BETWEEN MICROORGANISMS (THE QUORUM SENSING MECHANISM)

Quorum sensing is the mechanism through which many bacterial species are able to communicate with each other during the formation of biofilm (Naves et al., 2010). The mechanism helps in communication between intraspecies and interspecies during biofilm formation, environmental stress conditions such as antibiotics, food shortages and much more (Ji et al., 1995; Sreenivasan et al., 2013). Quorum sensing stimulates the coordination of gene expression with other cells and density of the local cells. The signaling molecules in quorum sensing attach to the receptor of other bacteria and helps in the transcription of genes either in between single bacterial species and/or between bacteria of

different species (Miller and Bassler, 2001). A single celled bacterium is always more inclined to join dense population of other pathogens and as such the mechanism of quorum sensing lets the bacterium to perceive how many other bacteria are present in close proximity. The bacteria emit chemical signals which are recognized by other bacteria and thus they come together to form a complex biofilm structure (Percival et al., 2011; Singh et al., 2000), possessing high pathogenicity.

### Pathogenicity of microbial biofilms

Bacterial biofilms spread their pathogenicity through a number of mechanisms (Figure.2) such as attaching to a solid surface, evading host defenses like phagocytosis, production of high concentration of toxins, resisting anti-microbial agents, exchanging genes resulting in more virulent strains and dispersal of microbial aggregates thus transmitting the microorganisms to other places (Ward et al., 1992; Cochrane, 1988). Biofilms are responsible for a large number of microbial infections in our body such as in formation of dental plaques, child middle ear infections, urinary tract infections (Parsek and Singh, 2003), gingivitis, and contact lenses related infections. They even can be lethal and lead to diseases like endocarditis, infections in cystic fibrosis patients and can also infect permanent indwelling devices such as heart valves and joint prostheses (Hall-Stoodley et al., 2006).

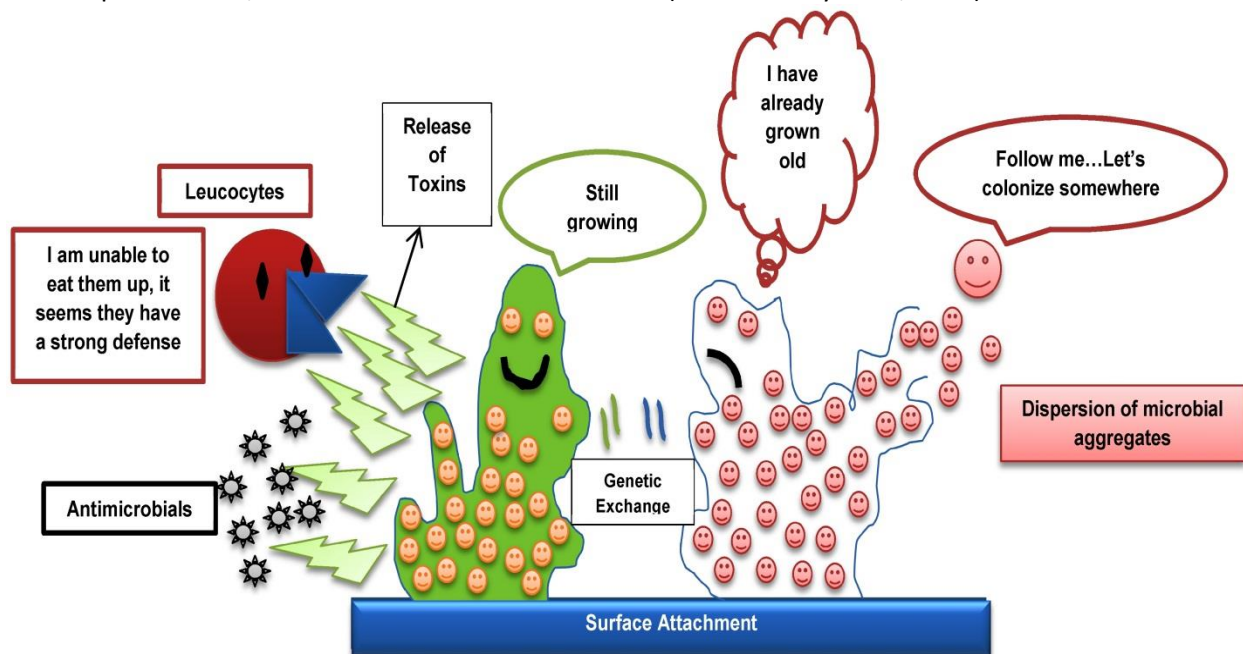


Figure.2. Mechanisms behind pathogenicity of microbial biofilms

### **Mechanism of antimicrobial resistance in biofilms**

Biofilms have been reported to possess resistance against various antimicrobial agents, thus helping them survive (Parsek and Singh, 2003). Studies have reported resistance of *Pseudomonas aeruginosa* biofilms towards ciprofloxacin (Kraigsley et al., 2017), *E.coli* towards ceftrimide (Lewis, 2001) and that of *Staphylococcus aureus* towards tobramycin (Sritharan and Sritharan, 2004). The antimicrobial resistance of biofilm can be attributed to factors like – Low penetration of antibiotics, neutralization of antibiotics by enzymes (Mah and O'toole, 2001), heterogeneous nature of the biofilm (Stewart and Franklin, 2008), slow growth rate of the cells in the biofilm (Durack and Beeson, 1972), presence of efflux pumps (De Kievit et al., 2001), alterations of membrane proteins (Hancock, 1997), production of diverse phenotypes within the biofilm (Neut et al., 2007) and the existence of persistent cells (Lewis, 2008).

### **STRATEGIES FOR ERADICATION OF MICROBIAL BIOFILMS**

Microbial biofilms are quite difficult to eradicate and they still persist at the site of infection even after prolonged treatment. The problem gets more worsened as the microbial biofilms may contain cells of multiple species at different stages of the growth cycle. Further, the cells lying deep within the biofilm exhibit slow growth rates pertaining to response towards general stress. Thus, slow growth protects the bacteria from effects like change in pH, chemical agents and other antibiotics which require active bacterial growth and division to act effectively (Percival et al., 2011). Hence, chronic infections related to biofilms are very difficult to eradicate as compared to the acute infections that are caused by planktonic cells. Many strategies are being tried out for complete eradication of the biofilm related infections; however, these are still in the initial stages of research. This review has tried to discuss some of the emerging strategies for treatment or eradication of biofilm related infections.

#### **Surgical debridement followed by antimicrobial treatment**

Biofilms have been observed to mature in chronic wounds within 10 hours of infection and persist for an indefinite period while the wound is open (Harrison-Balestra et al., 2003). Previously conducted clinical study found that surgical debridement of chronic wounds although removed the biofilm communities from wounds, but, the biofilm again resurfaced within 2 days of the debridement and the biofilm matured completely within 3 days of the debridement (Wolcott et al., 2010). However, after debridement the planktonic bacteria that resurface again are susceptible to antimicrobial agents. Thus, repeated antimicrobial treatment following the

surgical debridement of chronic wounds could help prevent the further growth of the microbial biofilm over the surface of the wound.

#### **Synthesis of new drugs attenuating the virulence factors**

Strategies are being considered to develop new drugs that will not kill the bacteria but will rather interfere with the virulence factor producing ability of the bacteria. The virulence factors include factors responsible for the growth of biofilm and factors that help confer resistance to other existing antibiotics (Rasko and Sperandio, 2010).

#### **Development of recombinant phages**

There has been development of recombinant phages that attacks the biofilm forming bacterial cells and produces an enzyme that degrades the extracellular polysaccharide matrix (Romero and Kolter, 2011). Combination of multiple phages commonly known as a "phage cocktail" can also be used for complete eradication of bacterial biofilm (Jamal et al., 2015).

#### **Use of quorum sensing inhibitors**

Use of compounds or molecules that could block or inhibit the quorum sensing pathway in the biofilm forming cells is a novel way to tackle the growth of biofilm (Bjarnsholt et al., 2007).

#### **Application of low intensity electrical current**

Study conducted have showed that application of low intensity of electrical current do bring about a reduction in the number of bacterial cells in the biofilm, thus hampering the biofilm growth (Caubet et al., 2004).

#### **Use of nano-particles**

Nano particles have completely a different mechanism for action against microbes and thus possess a promising option for the eradication of microbial biofilms. Use of TiO<sub>2</sub> in prevention of fungal biofilms formed by several strains of *Candida albicans* and that of silica nano particles against biofilms of *S. aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *E. coli*, and *C. albicans* (Hetrick et al., 2009) have been extensively studied. Addition of nanoparticles also prevented the colonization of internal surfaces of medical devices (Rai et al., 2012). The anti-bacterial activities of nano particles are in negative correlation with their size. Nanoparticles due to their larger surface area can easily disrupt microbial membrane and enter into the cells (Xiu et al., 2012). Some nanoparticles generate free radicals which in turn possess certain antimicrobial effects.

Keeping in mind the enormous scope and potential that the nanoparticles possess, further research can be carried out for successful eradication of biofilm forming bacteria and related infections at the root level.

### CONCLUSION

Bacterial biofilms are a topic of major concern which can be attributed to their ability to cause chronic infections and can sometimes prove to be fatal. Moreover, their capacity to resist higher concentration of antibiotics adds to the problem. There is an immediate need to replace these antibiotics with emerging and promising treatment strategies, so as to prevent the uncontrolled growth of the infectious microorganisms and eliminate infections.

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