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Importance and concept of resistance versus tolerance in biofilm mode of bacterial proliferation

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Review Article

ABSTRACT

Biofilms play an important role in the pathogenesis of many microorganisms. A number of animal and clinical studies have presented importance and role of biofilms in various clinical conditions. This form of growth enables them to grow in form of microbial communities, which help them to grow and survive better. Resistance to antibiotics is usually linked and described as a reason for better survival of microbes in biofilms. In fact, it is not resistance to antibiotics in majority of cases; rather it is ‑tolerance‖ of these biofilm communities against antimicrobials. The layers of biofilm provide a protective cover around bacteria and hinder penetration of these agents deep. Furthermore, this tolerance also helps them to survive against immune system, as immune system cells and other components cannot penetrate through layers of biofilm. This review paper discusses important aspects of biofilm formation, clinical importance and the concept of resistance versus tolerance.

Keywords: Biofilm; Antibiotic resistance; Tolerance, Pathogenesis

INTRODUCTION

Biofilms play a vital role in pathogenesis and are strongly linked with clinical conditions including chronic conditions (Hussain *et al*., 2017; Rohde *et al*., 2006). Bacterial growth, in the form of biofilm, has been found to be an organized form of growth where bacteria proliferate in different parts or sections of biofilm and communicate through various means, such as quorum sensing (QS) (Camilli & Bassler, 2006; Donlan & Costerton, 2002). Quorum sensing is a mechanism of communication used by microorganisms. It involves secretion of substances which act as a messenger for other bacteria present in the surrounding environment and these secretions depend on the surrounding conditions (Mancl *et al*., 2013). The term, ‖functional equivalent pathogroups‖ has been used for different bacterial groups which grow together in an organized and calculated manner to form a pathogenic biofilm community (Dowd *et al*., 2008).

Mechanism of biofilm formation

The infection process itself has stages of attachment, adhesion, and aggregation. The spread of infection from a biofilm growth results from disruption of part of growth and spread to distant areas (Kaplan, 2010; O'Toole *et al*., 2000;Otto, 2009). Biofilm development therefore requires adhesive forces for both the colonization of surfaces and cell to cell interactions. Also, disruptive forces are required for the formation of channels (fluid-filled), which are important for nutrient delivery across all biofilm cells. The same disruptive forces cause detachment of clusters of cells from biofilms and might be a mechanism for the spread of bacteria and cause disseminated infection (O'Toole *et al*., 2000).

There are specific proteins which affect surface adhesion of bacteria. For example, *Staphylococcus epidermidis* has the protein called—Autolysin‖ (AtlE) (Heilmann, 1997) and the Bap protein (Tormo *et al*., 2005) for this purpose. These proteins are likely to contribute to the hydrophobic nature of the cell surface. Adhesion is the first step in the development of an infection. It has been reported that those strains of *S. epidermidis* which lack the ability of adherence or cluster formation, are less virulent (Rupp *et al*., 2001).

Biofilm development and its structure depend upon various factors such as the availability of nutrients and other environmental factors. Jesaitis *et al.* (2003) have observed in an *in vitro* study, that a flat biofilm structure is formed in the

presence of citrate while availability of glucose results in a mushroom like complex biofilm growth (Jesaitis *et al*., 2003). Stevens *et al.*(2009) has reported that the formation of biofilm occurs by the participation of components arising from both, the host and the bacteria(Stevens *et al*., 2009). There is a view that before the actual process of biofilm development and formation, the surfaces of indwelling devices are -conditioned *in vivo*. Different components present in body secretions such as saliva, mucus, urine form a coat by adsorbing to the surfaces of devices to form a conditioning layer or film upon which actual bacterial growth and biofilm formation occurs (Choong & Whitfield, 2000). This conditioning film acts as an attaching surface for bacteria (Fitzpatrick *et al*., 2005). Biofilms are made up of bacterial cells and their products known as extracellular polymeric substance (EPS) which is about 75–95% of overall structure and remaining 5–25% are bacteria (Chen & Wen, 2011; Hoiby *et al*., 2011).

In addition, biofilm formation can have different mechanisms such as *ica*-dependent (intracellular adhesion) genes and protein-dependent formation. This means components such as accumulation associated protein (Aap) are not activated if *ica*genes are present, but when *ica*operon is not active, other mechanisms of biofilm formation start operating. Likewise, *Pseudomonas aeruginosa* causes biofilm related clinical infections. PEL, PSL and alginate polysaccharides are produced by *P. aeruginosa* which harbour the *pel*, *psl* and *alg* genes, respectively. It has been shown that PEL and PSL polysaccharides are involved in biofilm production *in vitro*. *In vivo*, either one or a combination of these operons control biofilm production. It is interesting that strains lacking these genes can still form biofilm *in vivo* through mechanisms not requiring these polysaccharides or genes (Cole *et al*., 2014). Thus it is clear from these examples that there are various mechanisms of biofilms production.

Clinical importance of biofilm and animal studies

Biofilms play a vital role in pathogenesis and are strongly linked to patient morbidity and mortality (Rohde *et al*., 2006). More recently, studies have focused on the *in vivo* role of biofilm in conditions such as chronic otitis media, prostate infection, bone infection, chronic rhinosinisitis and onychomycosis (Chen & Wen, 2011). Moreover, the role of biofilm in prosthetic device related infections is also reported (Zhao *et al*., 2013). Recent research in this area is suggesting a very important role of bacterial biofilm in chronicity of wounds (Ngo *et al*., 2012).Biofilm is also present in skin conditions such as bullous disease, atopic dermatitis, acne and candidiasis (Nusbaum *et al*., 2012; Vlassova *et al*., 2011). Characteristic infection signs are usually absent in the case of biofilm (Wolcott *et al*., 2008). With the ability to evade host immune system and avoid harmful effects of antibiotics, bacterial growth in biofilm mode is involved in many conditions such as endocarditis, gum disease, bone and foreign material infections(Hall-Stoodley *et al*., 2004; Parsek & Singh, 2003).Biofilm development is also a problem in animals. Although biofilms are involved in various infections in animals but this section just focuses on wound infections to elaborate importance of biofilm in animals. For instance, chronic wounds in horses have been reported to have biofilm. Similarly, abiotic surfaces such as different types of needles and catheters used clinically also act as a base for development of biofilm (Morgan *et al*., 2009; Westgate *et al*., 2010). Biofilms delay wound healing and conventional therapies are not very effective in the presence of biofilms (Bradley & Cunningham, 2013). In an animal study model using mice, Zhao *et al.*(2012) have reported wound healing within four weeks in wounds which were not inoculated with bacteria (*P. aeruginosa*). On the other hand, wounds which were given bacterial challenge showed delay in healing by two weeks on average. A few of the wounds which were not inoculated artificially also showed delay in healing and culture results from these wounds indicated the presence of large numbers of *Staphylococcusaureus*. This further indicates the role of bacteria in delaying healing process (Zhao *et al*., 2012).

Resistance versus tolerance of biofilm

Biofilm production allows bacteria to cause infection even if their numbers are low. It protects them from antimicrobials as well as from immune system cells. Biofilm disruption, as such, is an effective method for treating such infections as it will improve antimicrobial therapy (Bjarnsholt *et al*., 2005; Vuong *et al*., 2004). Biofilm modifications are important in providing a safe environment for bacteria. Examples of such modifications are: (i) a limited access of harmful molecules such as antibiotics and immune system products to bacteria, (ii) lower levels of inflammation which reduce

chemotaxis of defense cells, such as neutrophils, to the area of infection and; (iii) fermentation as an energy source rather than aerobic processes and other metabolic changes (Yao *et al*., 2005). Furthermore, the role of structural components of a biofilm are not limited to providing support for bacteria but are also involved in enabling the transfer of resistance genes amongst various species via plasmids (Fux *et al*., 2005). The physical structure of a biofilm is enabled by substances deposited in the biofilm which can form new chambers and release bacteria into these chambers to expand the biofilm structure (Schierle *et al*., 2009).

Bacteria growing in the form of a biofilm have been reported to have ten times higher survival rate compared to their planktonic growth (Spiliopoulou *et al*., 2012). Bacteria present in a biofilm cluster are more resistant to antibiotics and host defense mechanisms. For planktonic bacteria it is the opposite (Black & Costerton, 2010; Singh *et al*., 2000). Davis *et al.* (2008) have studied the effect of single and multiple antibiotic containing ointments against *S. aureus* growth and reported that planktonic bacteria were effectively killed but the eradication response was less for *S. aureus* present in biofilm (Davis *et al*., 2008). The important concept being focused in this article is the resistance versus tolerance of biofilm community. In fact, bacteria in a biofilm are more tolerant to antimicrobials. This is completely different from being resistant to antibiotics by avoiding/destroying antibiotics. In the case of biofilm presence, bacteria aren't exposed enough to antibiotics and are protected against antibiotic actions as the biofilm matrix hinders penetration of drugs (Brady *et al*., 2007; Mancl *et al*., 2013). Bacterial tolerance to antimicrobials when they form biofilms has been linked to factors such as nutritional limitation, slow growth and metabolism, reduced antibiotic penetration through layers of biofilm and other phenotypic characteristics (Stewart, 2002). Gurjala *et al*. (2010) have used a term -Biofilm burden‖ to explain that normally the presence of biofilm is tolerated even in normal body parts such as the gut but it is the presence of excessive biofilm, such as in cases of biofilm related infections, which is not tolerated (Gurjala *et al*., 2010).

CONCLUSION

It is clear from above discussion that formation of biofilm is a dynamic process which is controlled by certain genes but there are other mechanisms of biofilm formation which do not depend on these genes. This form of bacterial growth plays an important role in growth, survival and pathogenesis of bacteria. The important concept discussed in this article is that the biofilm layers act as a shield to protect the bacterial community in biofilms. These layers do not let antimicrobials as well as immune system components to reach deep into biofilm layers. This means the bacteria present in deeper layers are not exposed to the antimicrobials and keep on surviving and growing. If same bacteria are exposed to these protective mechanisms, they will be removed. Thus, treatment strategies in such conditions would include biofilm disruption strategies for better clinical outcomes. For examples, surgical debridement is usually done to clean wounds to remove surface growth of bacteria and dead tissues. There are other methods also available for debridement such as autolytic, biological, enzymatic and chemical debridement.

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