Review

BACTERIAL BIOFILMS: UNDERSTANDING STRUCTURES AND ROLES

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ABSTRACT:

The present study addressed bacterial biofilms in terms of concepts, structures, and roles. The various aspects related to formation of biofilms were discussed. The roles of bacterial biofilms in clinical settings were discussed. We also discussed the roles of bacterial biofilms in wounds.

KEYWORDS: Bacterial biofilm, Wounds, Healing resistance, Antibiotics

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INTRODUCTION

Bacterial Biofilms

Bacterial biofilms are considered as complicated structures that consist of micro colonies. Open water channels are working to separate these micro colonies to permit certain processes including the passing of nutrients, waste and signaling molecules. Researchers have identified what is called self-produced matrix which is made of Extracellular Polymeric Substances (EPS) in which the main constituents are polysaccharides, proteins and nucleic acids[1]. The idea of having living bacteria on surfaces has been reported since the 1930's [2-4]. Later, in the late 1970's, studies have demonstrated that surface-associated, sessile bacteria predominated over planktonic cells in an aquatic environment[5]. Other studies confirmed this trend through observing the same phenomenon in other natural environments and in medical and industrial settings [6].

Studies on bacterial biofilms showed the possibility to their formation in natural and clinical settings. Furthermore, it has been suggested that various factors related to bacterial biofilms including physical location of the biofilm, the composition and virulence state of the bacteria are generally predicting factors for the outcome of the biofilm interaction if will be positively or negatively directed for the local environment and/or host[7].

Biofilms are characterized by being ubiquitous, and can live if surface contact is provided. According to this context, biofilms are expected to have various roles from a medical point of view as well as having roles in ecological and industrial settings[7]. Biofilms have been considered to be associated with several problems including resistance toantibiotics, hydrodynamic shear forces, UV light, and chemical biocides. Other problems include high frequencies of genetic exchange, anddistorted biodegradation[8].

Several studies have pointed that 65% of infections are attributed to biofilms[9, 10].Furthermore, studies have put emphasis on the decreased effects of antimicrobial agents on bacterial biolfilms as an important issue in the treatment of chronic infections[9, 10].According to the study of Adnan[11], biofilms,made of a single species, can be encountered in various infections as well as the surface of medical implants. The same study indicated that the phenomenon of biofilm formation can be better understood at the molecular level throughstudying single species biofilms.

Several genes were suggested to be involved in the formation of biofilms[12, 13].Of important genes involved in *E. coli* are *rpoS* (RNA polymerase sigma factor) and morphogene*bolA*. *RpoS* can be induced and canreplace vegetative sigma factor *rpoD*under several stress conditions[11].In their study, Santos et al [14] were the first to report the involvement of morphogene*bolA*in adaptation to the stationarygrowth phase. Various forms of stress can induce high expression level of *bola* mRNA which, in turn, helps in the formation of biofilms.Actually, the role of microbes in inducing disease has been recognized since a long time. According to Latasa et al[15], the growth of bacteriaprefers being attached to surfaces and form what is called self-produced extracellular matrix.

Better understanding of biofilms has been gained through the use of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) in examining biofilms[16]. It has been shown that differentbacteria can produce the same exopolysaccharides (cellulose, poly-β-1,6-N-acetylglucosamine) tobuild the biofilm matrix and the same secondary messenger, c-di-GMP (cyclicdiguanosine monophosphate), an important bacterial signaling molecule, to regulate the production of biofilm matrix[15].

Biofilm Lifecycle

Biofilm is defined as: a community of microorganisms encased within a secreted EPS matrix and attached to a surface"[11]. The formation of biofilm is considered as an alternative "way of life" for microbial cells, which is different from the old consideration in which cells grow and exist only in a planktonic or single cell state[6].

There are five main steps in biofilm lifecycle that have been identified by proteomic studies[17] (Figure 1).

- ***Reversible attachment***

In this stage, microbial cells are reversibly associated with a surface anddisplay species specific behavior including rolling, creeping, aggregate formation[1].

- ***Irreversible attachment***

In this stage, there is a molecular binding between microbes and the surface. These bindings are regulated at the transcriptional level. This permits the rapidtransition between planktonic and sessile forms depending on environmental factors[11]. An illustrating example is the polysaccharide intercellular adhesion (PIA) which that facilitatesthe cell-cell interactions in some staphylococcal biofilms[18, 19].

- ***Aggregation and Maturation***

Within these stages, the surface attached bacteria start replicating, a matter that increasesthe overall density and complexity of the biofilms. It has been found that biofilm bacteria in these stages have different levels of genetic and protein expression when compared to their planktonic counterparts[1].

- ***Detachment***

After reaching of biofilms their critical mass, based on the availability and perfusion limit of nutrients and wastes, it is expected that the peripheral layer ofgrowth to start another stage, re-differentiate into planktonic organisms[20]. According to Davies et al[21], there was an evidence suggesting that all these stages of biofilm formation anddevelopment could be controlled by genes that respond to population density[21].

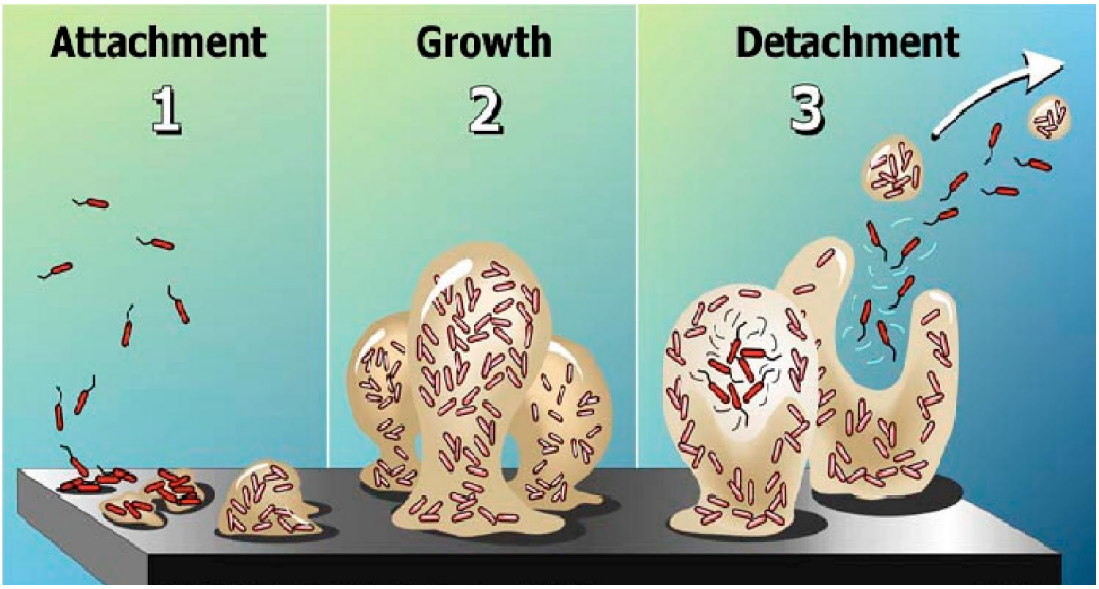

Figure 1: Biofilm life cycle in three steps[11].

Biofilms: Ultra structure and function

It was believed that biofilms had no oriented order for clumps ofbacteria that situated randomly. The studies of Flemming and Wingender[22] showed that biofilms are complicatedly arranged.A biofilm is mainly made of microbial cells,EPS and canals for nutrient circulation[23].

The researchers were able using confocalscanning laser microscope to determine the three-dimensional structure ofbiofilms[6, 7].It was found that biofilms formed bysingle species or mixed species exhibited similar structural properties[6, 24].

According to the study of Purevdorj et al[25], bacterial biofilms are mainly made of single species population or multimembercommunities, based on the environmental parameters under whichthey are formed. There are other factors including surface and interface properties, nutrientavailability, composition of microbial community and hydrodynamics that have their effects on the structure of biofilm.

The results of studies that targeted the effects of hydrodynamic situations including laminar and turbulent flows have shown that biofilm structures weredisrupted as a response to flow conditions. The results also showed that in biofilms exposed to laminar flow, aggregates were detached by interstitial voids[25], whereas in biofilms exposed to the turbulentflow, cells were not stable (Figure 2). According to the previous context, it is plausible to say that biofilmdevelopment has various aspects such as being polymorphic and its structure depends on changes in nutrientavailability[11].

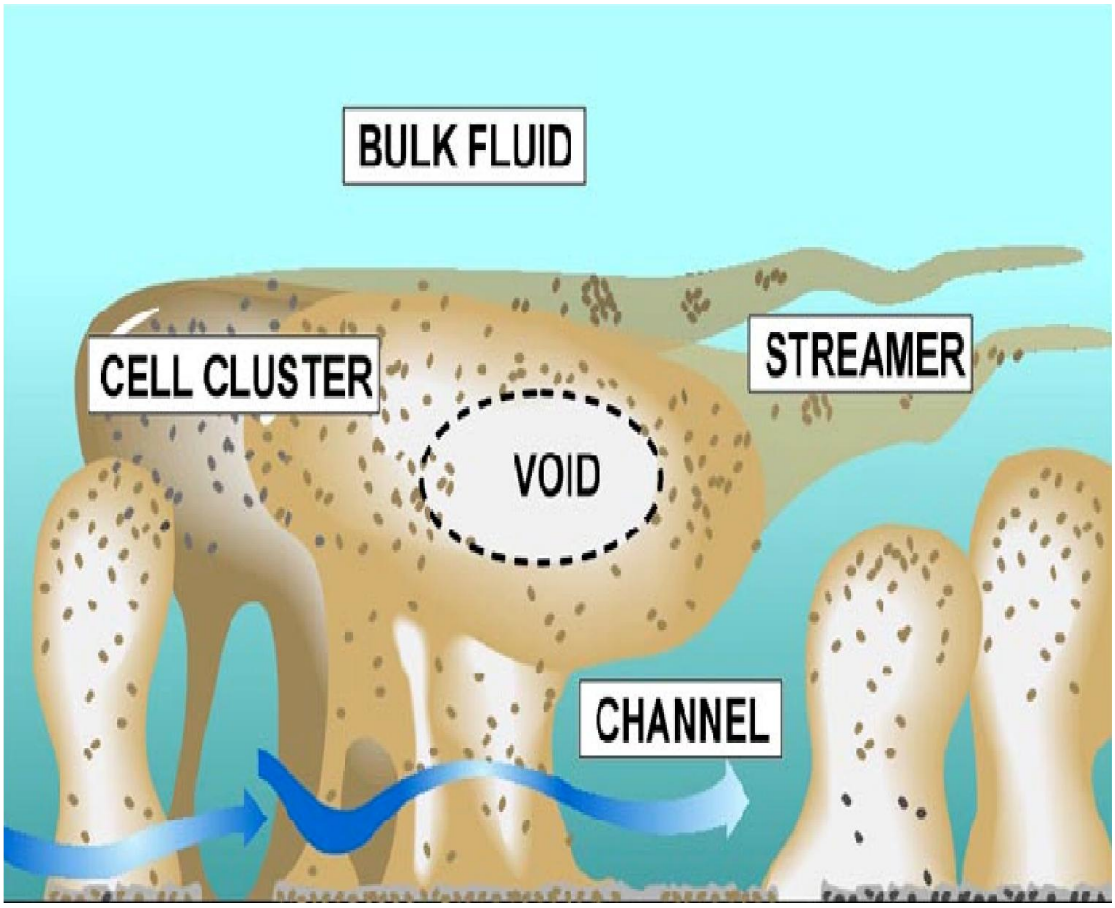

Figure 2: Heterogeneous structure of biofilm which includes cell cluster, void,

Channel and streamer[11].

It has been shown that structural organization is a characteristic of biofilm communities which, in turn, differentiates thisunusual mode of growth from usual forms. Biofilm structure involves the presence of interstitial channels as a maincomponent. These channels serve to supply nutrient transportation and exchangingof metabolic products. An illustrating example for the role of these channels has been shown through transferring the oxygenated bulk fluid throughout the biofilm[6, 26].

Biofilmdevelopment: Detailed mechanism

Research studies have pointed to difficulties in developing the three-dimensional structures in biofilms as this requires a coordinated series of molecular events including mechanisms for adhesion, aggregation and community expansion[27].

The first step that is crucial for bacterial colonization on a surface is the adhesion. Adhesion involves various structures including flagella, fimbriae, outer membrane proteins(OMPs), curli and EPS[24].Studies have indicated to the ability of bacteria to express several adhesions that give specific recognition and attachment to certain molecules ontarget surfaces including surface components of tissue or cell surfaces, surfaces of abiotic materialssuch as glass and plastic[18, 28]. Generally speaking, bacterial adhesions are considered as thin, thread like organelles referred toas fimbriae. There are several illustrating examples including type IV pili in Pseudomonas aeruginosa, thin aggregativefimbriae (SEF17) in Salmonella enteritidis, type I pili and curli in E .coli, theautolysin At1E and SSP adhesions in Staphylococcus epidermidis[18, 28-30]. These structures have been found to have various functions depending on species and environmental conditions[31].

Other studies addressed flagellar motility as being a fundamental component for bacteria to exceed the forces which prevent bacteria from reaching abiotic surfaces. Upon reaching surfaces, the activation of OMPs and curliis required to accomplish stable cellto cell and cell to surface attachment29.

It has been indicated that the expression ofbacterial adhesions is a phase reversible, and subjected to environmentalconditions. The motile bacteria has the ability to swim towards a nutrient and this process is called Chemotaxis. Motility and chemotaxispermit movement across thetarget surface to sites of increased nutrient availability[7, 14]. It has also been shown that surface bacterial attachment leads to the propagation into morecomplex microcolony structures and this process is facilitated by autoaggregation factors[32]

Cell-to-cell signalling mechanisms that observepopulation density play an important role in prevailing community structure[28, 33].

In their studies, Reisner et al[34] pointed to a metabolic interactionbetween different organisms to facilitate microcolony expansion by letting organisms to co-exist in a co-operative symbiotic manner. The biofilm architecture is an important step in biofilm development[22]. The architecture of biofilm can be analyzed depending on certain techniques such as a mutant of P.aeruginosa,which is unable to synthesize the key quorum-sensing molecules acylhomoserinelactones (acyl-HSLs), was used to develop a biofilm and the architecture was greatly altered[35, 36].

Bacterial biofilms are considered as complicated structures that consist of microcolonies. Open water channels are working to separate these microcolonies to permit certain processes including the passing of nutrients, waste and signaling molecules[1].

Researchers have identified what is called self-produced matrix which is made of extracellular polymeric substances (EPS) in which the mainconstituents are polysaccharides, proteins and nucleic acids[1].

Studies on bacterial biofilms showed the possibility to their formation in natural and clinical settings. Furthermore, it has been suggested that various factors related to bacterial biofilms including physical location of the biofilm, the composition and virulence state of the bacteria are generally predicting factors for the outcome of the biofilm interaction if will be positively or negatively directed for the local environment and/or host[7].

Bacterial biofilms in clinical settings

It has been shown that bacterial biofilms can be found on mucosal surfaces[6]. Furthermore, the status of disease (present or absent) depends on the bacterial constituents of such biofilms[37]. Examples of these biofilms include vagina[38] and gastrointestinal tract[39].

No exact mechanisms to explain factors involved in shifting bacterial biofilms from benign o harmful have identified. It is plausible that changes in bacterial composition including change of the resistance and virulence profile of the biofilm, changes in environmental signals generated from within the biofilm and exogenous sources areinvolved 7,40.

It has been indicated that bacterial biofilms are made of human commensal bacteria, or of opportunisticpathogens that colonize the host[40].If these biofilms interfere with physiological functions of the body, then they become a real problem. Several examples have been encountered including chronic lung biofilm infections which cause airway obstruction; urinary tract infectionsand infective kidney stones, which can obstruct urine flow, which, in turn, induce inflammation and recurrentinfection; and infective endocarditis leading to disruption of heart valve function[40].

Bacterial biofilms and chronic inflammation

According to the study of Costerton et al[41], the majority of bacterial biofilms induce chronic infections, in which the most involved features are persistent inflammation and tissue damage[42].In their study, Høiby et al[43] have pointed to the characteristics of these chronic infections such as wound and foreign body infections to include persisting existence irrespective to antibiotic therapy and the immunity of the host.

Several studies have argued that although traditionally biofilms were considered to be attached to surface, but later studies do not show the need of bacteria to be attached to surfaces to establish a chronic infection. Bacteria have been found to produce non-attached microcoloniesthrough gathering with their fellow bacteria through matrix components, and making an impenetrable barrier to host immune cells such as phagocytic cells[44-46].

Two studies have pointed to the challengesencountered with biofilms in chronic infections and found to associate with high tolerance to treatment with antibiotics and to the host's immune response[43].

Other studies showed that bacterial biofilm is made of a verity of bacteria that have different physiologies e.g., some are dormant and some are actively growing. In accordance with this context, treatment approaches using combinationsof different antibiotics have successfully been designedto target these different sub-populations[47].It has been shown that therapeutic approaches employing these combinations of antibiotics were successful partially in reducing the potential of biofilm infection, but it is not likely to eradicate biofilminfections and the infection is likely to appear after treatment had stopped [48, 49].

It has been shown that antiseptics have effective activities against planktonic bacteria andimmature biofilms[50, 51]. Other studies pointed to the consideration that he applications of antiseptics on mature biofilms with low growth rates and solid matrix can prevent further biofilm spreading while the infection still exists. This increasing tolerance with age is similar to antibiotics[52, 53].

The tolerance of Biofilmto the host immune reactions is one of the properties for chronic infections. Polymorphonuclearleukocytes (PMNs) have been found in acute infection to phagocytize microorganisms and foreign materials and also found surrounding the biofilms[54, 55].Studies conducted on *Pseudomonas aeruginosa*biofilms showed that PMNs are inactivated by a factor produced by *P. aeruginosa,* called rhamnolipids[56, 57].

Biofilms and wounds

Wounds are subjected to the infection, which is attributed to the loss of skin integrity that offers good environmental conditions such as wetness, warmness, and nutrients which favors microbial colonization. Bacterial infections work to inhibit wound healing by induction of ulcer enlargement anddelayed healing[58]. Several studies have recently demonstrated that biofilm-growing bacteria are identified in chronic wounds which explains the persistence of these wounds[59].

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