

Biofilms: A Review

Abstract

Formation of biofilm is the unusual way of survival for microbes forming the biofilm; **pathogenic** microbes increase their survival rate by showing resistance to the various antimicrobial therapy as well to other destroying agent. Establishment of biofilm vary from living to non-living surface that also includes the medical device and health care setting. Majority of the hospital born infection are due to the **biofilm** this occur due **persistence** of the biofilm on the medical device that lead to the spread of the infection among the patients. **The** biofilm also try to destroy the innate immunity of the host to which it cause infection. This review article describes the process of formation and the composition of biofilm. In most bacteria, formation of biofilm seems to happen in response to specific environmental stimulus and results in, formation or termination of biofilm matrix via the secondary messenger molecule c-di-GMP.

In between formation and termination of biofilm development we have determined biofilm stages, but the currently available data seems to indicate that these transformations are usually monitored not by specific genetic machinery but by adaptive responses. The formation of biofilm can occur by multiple pathways. The structure of the biofilms is specific to a particular species as well as dependent on environmental and physiological conditions. Various subgroups of bacteria e.g., motile and non-motile bacteria can interact during biofilm formation, and this type of interactions during the formation can affect biofilm constitution.

Keywords: Biofilm, biofilm formation, cystic fibrosis, dental plaque, leptospirosis, ear infection, nasal infection.

Introduction:-

The formation of biofilm is a procedure by which a microorganism or group of microorganisms are stuck to one another on the surface. These bacterial cells which are stuck to each other are repeatedly lodged within an extracellular polymeric substance (EPS) matrix produced by them self as to serve in adverse environmental condition. EPS of biofilm, this is also known as slime (either as neither as always we refer slime to biofilm), it is a polymeric cluster usually consisting of proteins, extracellular DNA and polysaccharides. In the aqueous solution or in solid submerged substance usually biofilm form and reside there. At specific environmental condition like high climates; as they are able to form mats in form of floating mats on liquid surfaces and they are also capable to form floating mats on the surface of leaves. The prevalence of biofilms is wide in nature, industrial area to hospitals setup, the formation of biofilms can take place anywhere ranging from living surface to nonliving surface. The bacteria itself composed of mainly polysaccharides, proteins and extracellular DNA [1] produce the matrix of extracellular polymeric substance of biofilm.

Specific species or group of species together can form a biofilm. As the predominant rate of biofilm form by multiple species is more, single-species biofilm mainly exist in the infection and variety of medical device [2,3,4].

Composition and Formation of Biofilm:

Biofilm consists of microorganisms and extracellular polymeric substances (EPS) produced by them self. A fully formed biofilm consists of various layers including an EPS matrix with a conditioning film and vertical structures. Towers or mushroom like appearance of the vertical structure of the microorganism is separated by interstitial space. Interstitial space acts as passage of transport media for biofilm, as interstitial space help the biofilms to take nutrient fast and immediately without much obstruction from the surrounding liquid media and in removal of waste material of biofilm [5].

The biofilm formation is promoted by different medium conditions, which requires the specific gene that will code for specific environmental condition that may stimulate various type of niches to be colonized by normal colonizing flora [6]. The biofilm formation can be species specific or it may vary as different group of microorganisms may also form single biofilm that lead to altered structure of biofilm. Biofilm formation is under the control of the bacterial adaptive response to that specific environmental condition.

1. Conditioning film:

Microorganism are able to adhere to surface of living and non living subject due to altered surface properties to which microorganisms is adherent due to formation of the conditioning films which change the properties of the surface of the substratum. For example, when body fluid, protein, polysaccharides, and many other component are exposed to microorganisms free objects, medical implants they adhere to the surface and are capable of forming conditional film. Rough and water containing surface more easily favor the biofilm formation than the normal surface.

2. Adsorption and attachment: Till today, it is unknown process by which the microorganism are adherent, mechanism of attachment of microorganism is still idiopathic. Initial process of microorganism attachment to surface of substratum is explained with the help of DLVO theory

and thermodynamic interaction mechanisms [7]. Microorganism possess various cell processes such as pili, fimbriae, flagella or glycocalyx which help **attachment** of microorganisms to the surface of substratum.

3. Growth and colonization:

The bacteria, which are capable of producing polysaccharides, that are able to anchor to the surface and help the colonies to grow. The growth of colonies is most important process and play significant role in deposition of biofilm which accounting for biofilm mass.[8]

4. Biofilm formation: The biofilm **consists** of following stages such as **attachment**, maturation and **dispersion**. A study done on various microorganisms including pseudomonas, salmonella, Proteus, vibrio cholera it has found that initiation of biofilm formation begins with increase in intracellular secondary messenger. Main study is done on gram negative bacilli that does not require any specific environmental condition for growth that is pseudomonas aeruginosa. Biofilm is to be fully developed when biofilms consist of an EPS matrix and vertical structures that are arranged in the form of tower and they are separated by interstitial spaces. Biofilm formation may vary as it may be homogenous or a heterogeneous, internal mass transport depends on the structure of the biofilm. [5, 8] polysaccharide which is component of biofilm is taken from external environment. The extracellular matrix synthesis by the pathogenic microorganism constitutes the irreversible stage of the biofilm formation.

5. Biofilm Dispersion: biofilm dispersion **constitutes** the last stage of the biofilm formation. In this stage biofilm is set up and it can only change its shape and size without **altering** its nature or properties [8,9]. **The** movement and communication of the microorganism within the biofilm occurs. According to the quorum phenomenon, the pathogenic biofilm forming microorganism can communicate with each other and help in the metabolic pathway to each other within the biofilm. Still quorum phenomenon remains unrecognized. **Once** the bacteria had sense that there are other bacterial groups, which are in their close proximity to them then, they lead to biofilm formation in their association [6]. As biofilm consists of extracellular polymorphic substance, the polysaccharides can be extracellular or intracellular i.e. it is synthesized inside the biofilm or outer environment (i.e. outside of the biofilm).

Relation of Biofilm to infection and Disease

In a body wide variety of microbial infection occurs which involves the biofilm formation. As biofilm plays a major role in microbial infection. The survival of different varieties of pathogenic microorganisms has been possible due to biofilm formation, which provides an alternative pathway for their **survival** as it is documented. In some conditions within the biofilm, microorganisms in the plaque type of biofilm can also change their pathogenic potential of bacteria that is noticeable from cariogenic bacteria in plaque biofilms. As many microorganisms can increase their virulence factor within the biofilm that lead to the more acuteness of the disease they caused. As stated by the national institutes of health that more than 70% of infections are due to the biofilm forming microorganism. As the biofilm forming bacteria are capable of producing various infections ranging from our mouth to the toes, this number appears to be excessive, but if we focus on frequent infectious cases such as catheter associated infections (whose causative agents include *Staphylococcus aureus* and other gram-positive bacteria) or urinary tract infections (given rise by the *E. coli* and other bacteria), the common infections in children as middle-ear infections (for example infections caused by *Haemophilus influenzae*), frequently occurring dental plaque

formation and gingivitis, are all a result of biofilm forming bacteria, which are hard to manage and are frequently relapsing, this figure appears practical.[10]

Dental plaque

The symbiotic relationship between mammals and microorganism include the classical example of dental plaque. Dental plaque which is first place at which biofilm is found in the human body. As from maximum study, it is evidenced that dental plaque is nothing different. The dental plaque on the surface of teeth is actually biofilm caused by biofilm forming bacteria. The accumulation of metabolites of bacteria on gingival tissues and teeth results in dental diseases[11] Multiple species are involved in formation of dental plaque biofilm, As cell division occur continuously which lead to the formation of multiple layer biofilm formation. Most of world population is suffering from chronic disease such as dental Caries that is tooth decay which occur due to biofilm forming microorganism. In addition, this microorganism are capable of interferes bacteria and fermenting carbohydrate in mouth along with many host factor and saliva.

Chronic sinusitis, osteomyelitis and endocarditis

It has been evident from recent study done, that in-patient who had undergone surgery for chronic sinusitis when the tissue is removed during the surgery biofilm is found on the removed tissue of 80% of patient. According to Parsek, wide variety of bone infection including osteomyelitis, which involves bone marrow, is said to be caused by biofilms. As study was done by using microscopy from which it is evident that infected surface of human and animal models involves the biofilm formation. According to microscopic study conducted by Parsek indicates that biofilms are present on prostatic duct associated with pathological condition chronic prostatitis.[12] the inflammatory and disease condition such as toxic shock syndrome that are caused due to settlement microbes that are capable of forming biofilm on the surface of the vaginal tissue and tampon fibers .[9,13] some pathogenic biofilm forming microorganism are may responsible for kidney stone condition . due to such pathological defect of kidney (kidney stone) lead to the obstruction of urine flow that lead inflammation and repeated infection condition which lead to end stage that is kidney failure . As study state that approximately 10-25% of case, reason behind the kidney failure is urinary tract infection. Pathogenesis behind the kidney stone formation is that microbe interacts with mineral that were going to extract in the urine, which lead to biofilm formation. This biofilm composition includes pathogen biofilm forming microbes and their products, and stone composed of mineral components [9,13,14] the inflammation of the innermost layer of the heart called as endocarditis caused by the bacterial biofilm along with some host components involving the cardiac valves . biofilm that involves the endocarditis formation have three basic mechanism such as the vegetation phase is first mechanism that lead to the disrupt valve physical function as leakage of blood when wall is closed and hence it affect blood flow rate as turbulent and diminished when valve is open. According to the second mechanism during antibiotic treatment, also there is continuous blood stream infection, which lead to periodic pyremia, chronic systemic inflammation, and other infections. And last Third stage (embolism) involves break off of infected part of tissue pieces that comes in the circulation and through the blood stream carried to various other organ , and other systems of the of the body, It is carried to the terminal when there is detachment of infective vegetation.[14,15]

On the medical implant heart valve, joint prostheses and on the medical device found the variety of the pathogenic biofilm that can lead to major infection. When the medical device related infection were at pick then surface of the medical device was visualized under the electron microscope that shows the existence of huge number of the slime -encased bacteria. as stated that on electron microscopy when tissue examined from chronic infection that were not due medical device also shows exopolysaccharide matrix surrounding the biofilm.[16] the highest **proportion** of the corneal infection is due to the biofilm forming microorganism on the contact lens . The biofilm formation vary it may composed of single or different species or fungi.[17,18]

Leptospirosis

As we South East Asia and South America is suffering from major health problem of leptospirosis with severe cases that exceeds 500,000 that are reported each year. The fatal case range from 5% to 20% . The carrier of pathogens of leptospira involves the mammals such as Rats. **As their kidneys are interrogans by leptosporia.** As carrier of pathogen of laptospirosis bacteria as they undergoes the process of urination , that lead to contamination of surface of water with the bacteria , as suitable condition bacteria gets which prolonged their **survival** for decades. Sign of leptosporia may **include** fever, headache, body pain, as leptospirosis may lead to kidney and liver damaged. Previously, it was assumed by researchers that the biofilm forming bacteria were planktonic. However, Professor Picardeau and his team have showed that biofilms can be produced by the ***L. interrogans***, which could be considered one of the important factor, which controls disease transmission, and survival of biofilm forming bacteria. [19]

Cystic Fibrosis

Cystic fibrosis is inherited disorder that mainly affect the lung and digestive system. The reasons for death of cystic fibrosis patient is infection with microorganism such as ***Pseudomonas aeruginosa***. Tha main cause of lung tissue damage is chronic inflammation this occurs due to the infection with Pseudomonas, which are capable of forming biofilm and stick to the lungs surfaces for long period in cystic fibrosis patient. As seen earlier that body gives immune reaction against the any infection in the form of inflammation that lead to failure of respiratory system. There is presence of biofilm formed by ***P. aeruginosa*** in airway due to permanent infection phase, however the pathogenesis of infection is still not well known. [15,19] the main pathogenesis of Cystic fibrosis is impaired chloride level due to mutations in the proteins regulating chloride channels. Cystic fibrosis progression depend if the number of protein regulated chloride channel had undergone mutation responsible for infection by microbe. If this is the pathogenesis behind the formation of biofilm formation is a topic much debated by researchers. Nevertheless,the only fact accepted is that there is impairment of host defense mechanisms of respiratory system in patient suffering from cystic fibrosis. This is responsible for the formation of biofilm and its biofilm related infection. [20]

Infections of Ear

Majority of ear infection are due to the microorganisms, which are capable of forming biofilms. major pediatric group population visit the hospital with complaint of acute as well as chronic ear infection such as the otitis media to take the antibiotic treatment or surgery , as it is crucial problem of united state , children are more **prone** to the ear infection due small size of the Eustachian tube. After the study of ten decades, it is clear that biofilm was responsible for otitis

media. In a subsequent study by Ehrlich and Post obtained middle ear mucosa of patient undergoing otitis media and children undergoing cochlear implantation and then compare .[19]

Chronic infections

As study conducted states that the chronic infection and inflammatory condition are due wide microbe and chronic biofilm establishment (collectively called the Th1 pathogens). [21,22]

Treating Biofilm Infection

Survival of the pathogenic microbes and persistence of disease and inflammatory condition is due to development of the resistance by Biofilms to the antimicrobial agent / therapy as well as to the inhibitory substance. [12] For example ciprofloxacin to *Pseudomonas aeruginosa* biofilms. [8] cefrimide vulnerability is reduced in *E.coli*. [15] The factors which contribute to the resistance which is shown by bacteria due to biofilm formation includes inability of antimicrobials to penetrate biofilms, limitations of nutrients, and deposition of toxic metabolites and lower oxygen tension. [23-25].

Conclusion:

The major infection in human body caused by biofilm associated infectious or pathogenic microorganism includes the Infectious disease processes such as otitis media the middle ear infection, periodontitis, cystic fibrosis and chronic prostatitis of prostrates. In addition, indwelling medical devices have been shown to harbor biofilms, which have been implicated in infections. Most disinfectants and antibiotics fails to work against the microorganisms involving the bacteria. As biofilm act as repository, within biofilm bacteria that were earlier sensitive acquire resistance by transforming the resistant plasmid within biofilms and then bacteria dells resistances.

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