

## 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 themself to serve in adverse environmental conditions. EPS of biofilm, this is also known as slime (either as neither as always we refer to slime as biofilm), it is a polymeric cluster usually consisting of proteins, extracellular DNA and polysaccharides. In the aqueous solution or in solid submerged substances usually biofilm forms and resides there. At specific environmental conditions (high humidity) as they are able to form mats in the form of floating mats on liquid surfaces and they are also capable of forming floating mats on the surface of leaves. The prevalence of biofilms is wide in nature, industrial areas to hospital setups, the formation of biofilms can take place anywhere ranging from living surfaces to non-living surfaces of objects. The matrix of extracellular polymeric substance of biofilm is produced by the bacteria themselves and is composed of mainly polysaccharides, proteins and extracellular DNA [1].

Specific species or groups of species together can form a biofilm. It is seen that where multiple species groups are involved in the formation of biofilm, that biofilm predominates more in wide environmental conditions than biofilm formed by single species. As the predominant rate of biofilm formation by multiple species is more, single-species biofilms mainly exist in the infection and variety of medical devices [2,3,4].

## **Composition and Formation Of Biofilm-**

Biofilm consists of microorganisms and extracellular polymeric substances (EPS) produced by themselves. 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 a passage of transport media for biofilm, as interstitial space helps the biofilms to take nutrients fastly and immediately without much obstruction from the surrounding liquid media and also in the removal of waste material of biofilm [5].

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

### **1. Conditioning film:**

Microorganisms are able to adhere to the surface of living and non-living subjects due to altered surface properties to which microorganisms are adherent due to the formation of conditioning films which change the properties of the surface of the substratum. For example, when body fluids, proteins, polysaccharides, and many other components are exposed to microorganisms on free objects, medical implants they adhere to the surface and are capable of forming conditioning films. Rough and water-containing surfaces more easily favor biofilm formation than the normal surface.

**2. Adsorption and attachment :** Till today it is unknown process by which the microorganism are adherant , 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 posses various cell process such as pili , fimbriae, flagella or glycocalyx which help attacement 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:** Tthe biofilm consist of following stages such as attachment , maturation and disperation. As a study done on various microorganisms including pseudomonas , salmonella, Proteus, vibrio cholera it has been found than initiation of biofilm formation begin with increase in intracellular secondary messenger. Main study is done on gram negative rod shaped bacilli that does not requires any specific envormental 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. Biofilms formation may vary has it may be homogenous or a heterogeneous ,internal mass transport depend on the structure of the biofilm.[5, 8] polysaccharied 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 it shape and size without altering its nature or properties [8,9]. the movement and communication of the microorganism within the biofilm occurs. As study have shown that ,according to the quorum phenomenon the pathogenic biofilm forming microorganism can communicates with each other and help in the metabolic pathway to each other within the biofilm.but still quorum phenomenon remain unrecognized. Once the bacteria had sense that their are other bacterial group which are in their close proximity to them then bacteria which have sensitized ,they lead to biofilm formation in their association[6]. As biofilm consist of extracellular polymorphic substance, the polysaccharides can be extracellular or intracellular i.e it is synthesized inside the biofilm or outer enviroment (I.e outside of the biofilm).

### **Biofilm infection and Disease**

In a body wide variety of microbial infection occurs which invovles the biofilm formation. As biofilm play major role in microbial infection . The survial of different variety of pathogenic microorganisms has been possible due to biofilm formation which provide alternative pathway for their survial it is documented as. In Some condition within the biofilm, microorganisms in the plaque type of biofilm can also change their pathogenic potential of bacteria which is noticeable from cariogenic bacteria in plaque biofilms. As many microorganism 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 infection 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 catheter associated infections (whose causative agents includes 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 evident 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. And this microorganism are capable of interferes bacteria and fermenting carbohydrate in mouth along with many host factor and saliva.

### **Chronic sinusitis and osteomyelitis**

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 have interaction 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 their 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 porportion 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 which 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 survial for decades. Sign of leptosporia may includes 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. But Professor Picardeau and his team have showed that biofilms can be produced by the L. interrogans, which could be considered to be 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 microoorganism such as pseudomonas aeruginosa . Tha main cause of lung tissue damage is chronic inflammation this occur 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. The question was asked to many scientist were studying cystic fibrosis they said that it is i not possible to kill pseudomonas. 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 infections are due to the microorganisms which are capable of forming biofilms. A 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 a crucial problem of the United States, children are more prone to the ear infection due to the 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 patients undergoing otitis media and children undergoing cochlear implantation and then compared. [19]

### **Chronic infections**

As study conducted states that the chronic infection and inflammatory condition are due to 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] ceftriaxone 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 prostates. In addition, indwelling medical devices have been shown to harbour biofilms, which have been implicated in infections. Most disinfectants and antibiotics fail 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 develops resistances.

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