Innovare Academic Sciences

Journal of Critical Reviews

ISSN- 2394-5125 Vol 2, Issue 4, 2015

Review Article

PERSPECTIVE STUDY OF BIO FILM AND ITS APPLICATIONS

SAVITHA T.

Department of Microbiology, Tiruppur Kumaran College for Women, Tiruppur, Tamilnadu, India
Email: selvipoovizhi23@gmail.com

Received: 30 Jun 2015 Revised and Accepted: 14 Aug 2015

ABSTRACT

Biofilm is an assembly of the microbial cells that is irreversibly associated with a surface and usually enclosed in a matrix of polysaccharide material. Biofilm is composed of microbial cells and extra cellular polymeric substance (EPS). Extra cellular polymeric matrix plays various roles in structure and function of different biofilm communities. Adhesion to the surface provides considerable advantages such as protection against antimicrobial agents, acquisition of new genetic traits, and the nutrient availability and metabolic co-operability. The formation of biofilm takes place in three steps. Biofilm is responsible for chronic bacterial infection, infection on medical devices, deterioration of water quality and the contamination of food. This review article provides an overview of the formation of biofilm, structure, role in microbial communities and its applications.

Keywords: Bio film, Polymeric substance, Adhesion, Pathogenesis.

INTRODUCTION

Biofilm is a well organized, cooperating community of microorganisms. Microbial cells attach to the surfaces and develop a biofilm. Biofilm associated cell is differentiated from suspended counterpart by the reduced growth rate, up and down regulation of gene and generation of extra cellular polymeric matrix [1]. Genetic studies have revealed that biofilms are formed through multiple steps. They require intracellular signalling and transcribe different set of genes different from planktonic cell. Therefore, biofilm formation can be viewed as a developmental process, which shares some of the features of other bacterial developmental processes [2]. Biofilm formation occurs step by step, such as formation of conditioning layer, bacterial adhesion, bacterial growth and biofilm expansion [3]. Biofilm can exist on all types of surfaces such as plastic, metal, glass, soil particles, wood, medical implant materials, tissue and food products. Bacterial attachment is mediated by fimbriae, pilli, flagella and EPS that act to form a bridge between bacteria and the conditioning film. Biofilms, in nature, can have a high level of organization and they may exist in single or multiple species communities and form a single layer or 3-dimensional structure [3, 4]. The presence, of indwelling medical devices increases the risk for bio film formation and subsequent infection. Transitioning from acute to chronic infection is frequently associated with bio film formation. Bacteria in bio films are innately more resistant to antimicrobial agents. The current antibiotic therapies are of limited effectiveness in resolving biofilms infection. This review attempts to discuss the stages in bio film formation, their pathogenic mechanisms, effect of antimicrobial agents, detection and eradication of the biofilms.

Biofilm structure

Biofilm is composed primarily of microcolonies of different species of microbial cells (+15% by volume) and of matrix material (+85%). EPS may vary in chemical and physical properties but it primarily consists of polysaccharides. Some of the polysaccharides are neutral or polyanionic. The presence of uronic acids (D-glucuronic, D-galactouronic and mannuronic) or ketal linked pyruvate confers the anionic properties. This property helps in the association of divalent cations such as calcium and magnesium, which have been shown to cross-link with the polymer strands and provide greater binding force in a developed bio film [5]. Backbone of EPS contains 1, 3-or 1, 4-βlinked hexose residues. The amount of EPS produced by different organisms may vary and the amount of EPS increases with the age of bio film. EPS may associate with metal ions, divalent cations and macromolecules (proteins, DNA, lipids and even humic substances). EPS production is known to be affected by nutrient status of the growth medium, excess available carbon; however, limitation of nitrogen, potassium, phosphate promotes the EPS synthesis [6]. The confocal scanning laser microscope (CSLM) has been effectively used to monitor bio film development in flow cells that allows direct observation of the bio film without disrupting the community. CSLM, which allows the visualization of fully hydrated sample, has revealed the elaborate 3-dimensional structure of bio film [7].

Role of bio film in microbial communities

(i) Protection from environment

EPS of biofilm provides certain degree of shelter and homeostasis to the bacteria residing in biofilm. EPS plays various roles in structure and function of different biofilm communities. The EPS matrix also has the potential to physically prevent the access of certain antimicrobial agents into the biofilm by acting as an anion exchanger. It restricts the diffusion of compounds from surroundings into the biofilm [6]. These characteristics largely depend on the nature of both the agent and the EPS matrix. This effect appears to be more pronounced with the antibiotics that are hydrophilic and positively charged such as aminoglycosides [8]. EPS has also been reported to sequester metal ions, cations and toxins [9, 10] and reported to provide protection from variety of environmental stresses such as pH shift, UV radiation, osmotic shock and desiccation.

(ii) Nutrient availability

The water channel provides an effective means of exchanging nutrients and metabolites with the bulk aqueous phase, enhancing the nutrient availability as well as removal of potentially toxic metabolites [11]. Micro colonies in bio film quite often consist of different microbial communities. These multi species micro consortia can result from association between metabolically co-operative organisms. Their close proximity facilitates interspecies substrate exchange, removal and distribution of metabolic products. For example, degradation of complex organic matter into methane and carbon dioxide during anaerobic digestion requires interaction of at least three bacteria. Fermentative bacteria initiate the catabolism of producing acids and alcohols, which are then utilized as substrate by acetogenic bacteria. Methanogen obtains energy by converting acetate, carbon dioxide and hydrogen to methane. Biofilm provides an ideal environment for the establishment of syntrophic relationship. Syntrophism is a special case of symbiosis in which two metabolically distinct bacteria depend on each other to utilize certain substrates, typically for energy requirements. Syntrophism has been well studied with regard to methanogenic degradation [11, 12].

(iii) Acquisition of new genetic trait

Horizontal gene transfer is important for the evolution and genetic diversity of natural microbial community. Acquisition of new genetic

trait gives chances to the microbial communities to transcribe the necessary genes to become the active member of the biofilm communities. This is due to transcription of different genes by bio film forming communities and the phenotypic characters are the expression of a particular genotypic character [13].

(iv) **Penetration of antimicrobial agent**

The nature of biofilm structure and physiological attributes of biofilm organisms confer inherent resistance to the antimicrobial agents. To inactivate the biofilm forming microbial community by antimicrobial agents, diffusion is the rate limiting step. EPS acts as diffusion barrier for these molecules by influencing the rate of transport of the molecule to the biofilm interior or the reaction of antimicrobial agents with the matrix material. The penetration of ciprofloxacin to the normal sterile surface required 40 sec whereas penetration into the bio film containing surfaces took 21 min* [5]. Biofilm mode of growth gives advantages to the microbial community in the following ways:

(a) As the growth is restricted all the energy is used up by the bacteria in making the EPS that will give protection to the microbial community.

(b) As the growth is restricted, bacteria will remain in dormant stages that will give protection to the microbial community against antibiotics because most of the antibiotics are active against the growth phase of the bacteria.

Factors favouring bio film formation

Biofilm may be formed on a wide variety of surfaces including living tissues, indwelling medical devices, portable water system pipe line or natural water system piping. The water system biofilm is highly complex. It contains corrosion products, clay material, fresh water diatoms and filamentous bacteria. The biofilm on the medical devices composed of a single coccid organism and the associated extra cellular polymeric substances matrix [14]. Different factors may influence the formation of bio film are as follows:

(i) Substratum effect

The extent of microbial colonization appears to increase as the surface roughness increases due to the diminished surface area and higher surface area on rougher surfaces. Maximum attachment depends upon high surface free energy or wettability of surfaces. Surfaces with high surface free energies such as stainless steel and glass are more hydrophilic. These surfaces generally show greater bacterial attachment than hydrophobic surfaces such as Teflon, Buna-n rubber and fluorinated hydrocarbon.

(ii) Conditioning film

Solid surfaces which have been exposed in an aqueous medium become conditioned or coated with polymers from the medium. The chemical modification of surfaces affects the rate and extent of microbial attachment. The surface is converted to hydrophilic by cleaning with alkali or strong acid (4M nitric acid) of stainless steel surfaces. Once the stainless steel is exposed to air or water, it is passivate by the formation of a chromium oxide layer. Organic soil adheres to the oxide layer, producing a conditioned substratum to which bacteria adhere [15]. Another prime example is "acquired pellicle" which develops on tooth enamel surfaces in oral cavity. It consists of albumin, lysozyme, glycoprotein, phospho proteins, lipids and gingival crevice fluid. Bacteria, from oral cavity, colonize pellicle-conditioned surfaces within hours of exposure to these surfaces. A number of host-produced conditioning films such as blood, saliva, tears, urine, intravascular fluid and respiratory secretions influence the attachment of bacteria to biomaterials. The surface energy of the suspending medium may affect hydrodynamic interactions of the microbial cells with surfaces by altering the substratum effects [16].

(iii) Characteristics of aqueous medium

Physico-chemical characteristics of aqueous medium such as pH, nutrient levels, ionic strength, temperature, etc. may play an important role in the rate of microbial attachment to the surfaces. The bacterial attachment and biofilm formation in different aqueous systems are affected by season. This may be due to the temperature of water or other seasonally affected parameters. It is found that an increase in concentration of several cations such as sodium, calcium, lanthanum, ferric ions affects the attachment of *P. fluorescence* by reducing the repulsive forces between the cell and glass surfaces.

(iv) Horizontal gene transfer

The mobile genetic element mediates horizontal gene transfer between bacteria. These elements can be conjugative plasmids, transposons or bacteriophages. Bacteria in biofilm express different phenotypic characters from planktonic counterparts. This is due to different genes transcribed in the planktonic and bio film-associated phases of the bacterial life cycle. Some genes may be expressed in response to a specific surface on which bacterium has chosen to settle. Many marine *Vibrio* species survive by attachment and degradation to chitin. The structural genes responsible for attachment to chitin differ from those required for attachment to abiotic, non-nutritive surfaces such as plastics and glass [17]. *Bacillus subtilis* strain harbouring conjugative transposons which confers resistance to tetracycline was introduced to the system and resistance profile of bio film bacteria was assessed. It was found that transfer of the conjugative transposons occurred within a bio film resulting in *Streptococcus* species resistant to tetracycline [18]. This was the first demonstration of gene transfer in an oral microbe growing in a biofilm and these findings indicate that non-oral bacteria have the potential to transfer genes to oral commensally. The transfer of TOL plasmid, which carry the genes for the degradation of toluene and the benzyl alcohol has occurred in bio film community growing on benzyl alcohol as the sole carbon and the energy source [19]. Virus-mediated gene transduction is another mode of gene transfer in bio film associated microbial community.

(v) Quorum sensing

Cell to cell signalling has recently been demonstrated to play a role in cell attachment. Intracellular communication between bacteria is generally carried out by bacterial products that are able to diffuse away from one cell and enter into another cell. This method of intracellular signalling seems ideally suited for bacteria in a diffusion-limited environment. Production of quorum sensing molecules is known as acyl-homoserine lactone (acyl-HSL). *P. aeruginosa* is responsible for defining separation between bacterial pillars in the 3-dimen-sional structure of a biofilm. *P. aeruginosa* mutants that do not produce acyl-HSL form biofilms in which the cells are closely packed together and easily disrupted by sodium dodecyl sulphate. The role of intracellular signalling in multi species biofilms significantly differ from that observed in single species biofilms. These signals are broadly classified as any actively or passively transmitted bacterial products that alter the state of neighbouring microbes. These might include bacterial metabolites, acyl HSLs secreted proteins, genetic material such as DNA or RNA, etc. This signal may alter distribution of specific bacterial species in the biofilm, alter protein expression in neighbouring cells, introduce new genetic trait in neighbouring cells and incorporate bacteria in biofilm. In addition to above factors, properties of cell such as cell surface hydrophilicity, presence of fimbriae, and flagella and production of EPS l influence the rate and extent of attachment of microbial cell [2].

Applications of bio film

(i) Biofilm and devices associated infection

Biofilm on indwelling medical devices may be composed of Gram-positive or Gram-negative microorganisms. These organisms may originate from the skin of patient, or health-care workers, tap water to which entry ports are exposed or other sources in the environment. Biofilms may be composed of single species or multiple species, depending on the device and its duration of action.

(ii) Central venous catheter biofilm

All the indwelling central venous catheters are colonized by micro organisms embedded in a bio film matrix. The organisms, most commonly isolated from catheter biofilm, are *S. epidermidis*, *S.*

aureus, *C. albicans*, *P. aeruginosa*, *K. pneumoniae*, etc [20, 21]. Catheters may be inserted for administration of fluid, blood products, medications, nutritional solution, and hemodynamic monitoring. Biofilms have been reported to be universally present on central venous catheters using SEM and TEM and may be associated with either the outside of the catheter or inner lumen. These organisms originate from patient's skin micro flora, exogenous micro flora from health-care personnel. They gain access to the catheter by migration externally from skin along the exterior catheter surface or internally from the catheter port. During long term catheterization, there would be more formation of a bio film on the inner lumen of catheters. Biofilm on central venous catheters has routinely been detected by a semi quantitative procedure termed the roll plate technique. In this procedure, the distal tip of the catheter is removed aseptically and rolled over the surface of a non-selective medium. The roll plate technique has the limitation such as low diagnostic sensitivity and low predictive value for catheter-related infection. Therefore, researchers have attempted quantification of biofilm using sonication plus vortexing of catheter tips [6].

(iii) Prosthetic heart valves

Mechanical valves and bio prostheses are being currently used as prosthetic heart valves. The surgical implantation of the prosthetic valve results in tissue damage, leading to the accumulation of platelets and fibrin at the suture site and on the device. Micro organisms also have the greater tendency to colonize these locations. The resulting bio film more commonly develops on the tissue surrounding the prosthesis. The primary micro organisms responsible for this condition are *S. epidermidis*, *S. aureus*, *Streptococcus* sp, Gram-negative bacilli, diptheroids, enterococci and *Candida* sp. Antimicrobial agents are usually applied during valve replacement and whenever patient has dental work to prevent the initial attachment by killing the micro organisms introduced into the blood stream [1, 22].

(iv) Urinary catheters

Urinary catheters are tubular latex or silicone devices that are inserted through urethra into the bladder to measure the urine output and collect urine during surgery. Catheters may be open or closed systems. In the open system, the catheter drains into an open collection centre. On the other hand, in closed system, the catheter empties into a securely fastened plastic bag. In open system, catheter quickly gets contaminated and develops urinary tract infection (UTI). Patients using closed system are much less susceptible to UTI [23]. The organisms commonly contaminating these devices and developing biofilms are *S. epidermidis*, *Enterococcus faecalis*, *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *K. pneumoniae*, and other Gram-negative bacteria. Divalent cations (calcium and magnesium), increase the urinary pH and ionic strength, which results in enhancement of bacterial attachment. Some organisms of these biofilms produce urease, which hydrolyzes the urea to ammonium hydroxide. The higher pH responsible for bio film-urine interface results in precipitation of minerals such as struvite and hydroxyapatite. These mineral containing bio films, which form encrustations, may completely block the inner lumen [27]. Several strategies have been attempted to control the urinary catheter bio film such as antimicrobials, bladder irrigation, and antimicrobial agents in collection bags, and impregnation of catheter with antimicrobial agents such as silver oxide or systemic antibiotics [1].

(v) Contact lenses

Contact lenses have been classified as soft contact lenses and hard contact lenses according to material of construction, design, wear schedule, and frequency of disposal. Micro organisms readily adhere to the surface of both types of lenses. The degree of attachment to the lenses depends on the nature of substrate, water content, electrolyte concentration, polymer composition, type of bacterial strain, etc. Organisms mainly adhering to the contact lenses are *P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis*, *Proteus* sp., *Serratia* sp., *Candida* sp.,etc. Biofilms have been observed on the lenses removed from a patient with keratitis caused by *P. aeruginosa* using SEM. Biofilms have also been found to develop on contact lenses kept in storage cases [6].

(vi) Intrauterine devices

The intrauterine devices (IUDs) have a tail that facilitates locating the device for removal and it is composed of a plastic monofilament surrounded by a nylon sheath. The tail portion of the IUDs may be a primary source of contamination. Organisms which contaminate the IUDs are *L. plantarum*, *S. epidermidis*, *Candida albicans*, and *S. aureus*, *Corynebacterium* sp., and *Enterococcus* sp.,etc.

(vii) Biofilm and pathogenesis

The role of biofilm in implant infections has been established in numerous systems but their role in non-implant diseases is not well established. Here some of the examples of diseases, which are caused by micro organisms residing in the biofilms, have been reported.

(viii) Native valve endocarditis

The interaction between the vascular endothelium, generally of mitral, aortic, tricuspid, and the pulmonic valves of the heart and microbes circulating in the blood stream causes native valve endocarditis (NVE). The species of *Streptococcus*, *Staphylococcus*, *Pneumococci*, *Candida*, *Aspergillus* and some Gram-negative bacteria have been found responsible for NVE. These organisms mainly enter into the blood stream primarily via oropharnyx, gastrointestinal tract and genitourinary tract. Micro organisms adhere poorly to intact endothelium. But when the endothelium is damaged, non-bacterial thrombotic endocarditis (NBTE) is developed at the point of injury. It is the accumulation of platelets, fibrin and occasionally red blood cells. Fibronectin, which is secreted by endothelial cells, platelets and the fibroblast in response to vascular injury, has been identified in thrombotic lesion of heart valve. Fibronectin can simultaneously bind to fibrin, collagen, human cell and bacteria. Many bacterial species have fibronectin receptors including Staphylococcus and Streptococcus species. Bio films formed by microbe can damages valve tissues. Depending on the organisms involved, various antibiotic therapies are recommended such as penicillin is the normal treatment for Streptococcal endocarditis and it may be supplemented with gentamycin to produce synergistic killing.

(ix) Otitis media

Otitis media is a chronic ear infection that involves the inflammation of the mucoperiosteal lining. Otitis media is caused by a number of different organisms including *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *S. epidermidis*, *P. aeruginosa*, etc. Low concentration of antibiotic is present in middle ear fluid due to the limited penetration of antibiotic. Therefore, powerful antibiotics are used to combat the otitis media such as amoxicillin, cefaclor, erythromycin and clarithromycin [24].

(x) Chronic bacterial prostitis

The prostate gland may be infected with micro organisms that have ascended from the urethra or by reflux of infected urine into the prostatic ducts emptying into the posterior urethra [25]. Once the bacteria enter the prostatic duct, they multiply rapidly and elicit a host response. These bacteria can form sporadic micro colonies and bio films that adhere to the epithelial cells of the duct system. Micro organisms which are responsible for chronic bacterial prostitis include *E. coli*, *P. aeruginosa*, and species of *Klebsiella*, *Proteus*, *Serratia, Bacteroides* etc.

(xi) Cystic fibrosis

Cystic fibrosis (CF) is a chronic disease of the lower respiratory tract. The normal mucociliary clearance system that cleanses the bronchopulmonary epithelium of inhaled particles depends on an upward directional flow of a mucus layer on the tips of cilia that move freely in the underlying water layer. This is due to the net deficiency of water, which hinders the upward flow of the mucous layer. Decreased secretion and increased absorption of electrolytes lead to the dehydration and thickening of secretions covering the respiratory epithelium. CF patients are mainly defective in cystic fibrosis transmembrane conductance regulator protein (CFTR) which results in altered secretions in the secretary epithelia. The

hyper viscous mucus is responsible for increasing the incidence of bacterial lung infections in CF patients. *S. aureus* is the first pulmonary isolate from CF patients. *S. aureus*, *H. influenzae* infections usually predispose the CF affected lung to colonization with *P. aeruginosa*. The possibilities for successful treatment of CF may ultimately hinge on early antimicrobial treatment to prevent or delay chronic infection with *P. aeruginosa*. Early treatment with oral ciprofloxacin and colistin may postpone chronic infection with *P. aeruginosa* for several years. Vaccines are effective in preventing the initial Colonization of lungs of patients with CF [6].

(xii) Biofilm and food industry

Growth of biofilms in the food processing environment leads to increased opportunity for microbial contamination of the processed food. Micro organisms within the bio film are protected from sanitizer; therefore the survival of micro organisms and the chances of contamination of foods are also increased. Extra cellular polymeric substances which give several beneficial effects to the micro organisms are not removed by cleaning. This gives the attachment sites to the micro organisms newly arrived to the cleaned systems [3, 15]. Surface tension value is the critical factor to determine the extent of attachment of micro organisms to the surface. Maximum attachment depends upon the high surface energy or wet ability of the surface. Generally hydrophilic surfaces have greater surface free energy rather than the hydrophobic surfaces. The surfaces are abraded with repeated uses and increasing their ability to entrap bacteria and the soil. The most prevalent strain of *L. monocytogens* found in the food processing environment has good adhesion ability and requires only a short contact time for attachment. The organism is found in raw milk and has been associated with outbreaks involving dairy products. *L. monocytogenes* forms biofilm on stainless steel, plastic and other materials. This species is well suited for growth and survival in various micro niches found in food processing facilities. Bio film formation in food may be avoided by equipment design, temperature control and by reduction of nutrients and water. Biofilm control efforts most often focus on effective cleaning of potential growth sites. The cleaning agents used in food industry are alkali compounds. They can be used in combination with sequestrant or chelators and anionic wetting agents. The sanitizers used in the food industry are halogens, acids, peroxydens and quaternary ammonium compounds. Quaternary ammonium compounds are cationic surfactant sanitizers and also have cleaning activity. They are effective against bacteria as well as fungi. Hence, it is often recommended for floors, walls, storage containers and surfaces. Hydrogen peroxide is a broad spectrum sanitizer. It is most effective against *L. monocytogenes* and *Salmonella* species in a biofilm matrix.

(xiii) Impact of biofilm on deterioration of water quality

Deterioration of the water quality during storage and in distribution system remains one of the major difficulties experienced by the potable water suppliers. The treated water, when flows through the distribution system, is adversely affected by its various conditions. There are two major factors which contribute heavily in the deterioration of the water quality. These bacteria can introduce into the distribution network from the external source by number of ways such as open reservoirs, breakage due to the new pipeline construction that may disturb the existing distribution system. The bacterial number may increase due to the internal regrowth or after growth of the bacteria and the associated formation of the bio film. Bio film formation is usually encouraged on the surface of a plumbing material if that material is able to supply the required nutrients for bacterial growth. There are various factors which will influence the formation of bio film in the water distribution system [26] such as type of piping material, temperature, type of disinfectants, resistance of bacteria to disinfectants, etc. Disinfectants used in appropriate concentrations are quite effective in the removal of micro organisms. The use of disinfectants also enhances the formation of easily biodegradable substances. These biodegradable substances can be used by micro organisms as energy source and promote the bio film formation in the distribution system. Micro organisms develop resistance towards the disinfectants used and they can survive and multiply despite the presence of measurable concentrations of disinfectants. Micro organisms develop resistance due to the indiscriminate use of disinfectants and acquisition of gene responsible for resistance by horizontal gene transfer. Regrowth of micro organisms in the drinking water distribution systems is caused by the use of biodegradable compounds. These compounds are either present in drinking water or originate from the materials in contact with drinking water. Disinfectants such as chlorine, chloramines, ozone or hydrogen peroxide are most commonly used for treating bio film forming microbes. Mono chloramine or hydrogen peroxide are maintained a longer disinfectant residual concentration throughout the distribution system, it results in more effective control of biofilm formation rather than free chlorine. Chlorine is used for final disinfection stage to ensure adequate protection. This is followed by the use of mono chloramine to ensure persistent concentration of disinfectant residual throughout the distribution system.

Biofilm examination and measurement

Biofilm development and structure has been analyzed using various methods. Light, fluorescence, differential interference contrast (DICM), transmission electron (TEM), scanning electron (SEM), atomic force (AFM), and confocal laser scanning microscopy (CLSM) are used to analyze and study the structure of biofilms. The use of TEM and specific polysaccharide stains like ruthenium red allowed researchers to both identify the nature of extra cellular fibres in biofilm and to better elucidate their association with cells. As mentioned earlier, the importance of CLSM in the 1980s provided researchers with the ability to examine biofilms in situ without the limitation encountered with the SEM. Electron microscopy has been used for the examination and characterization of biofilm on medical devices and in human infections. Fluorescent in-situ hybridization (FISH) and 16-23S rRNA hybridization with CLSM are used to observe microstructure and metabolism of biofilm. The FISH method was used to confirm decrease in the viability of cells as the biofilm ages [6]. The use of CLSM and epifluorescence microscopy requires the organisms in biofilms to be stained with fluorescent stains. These stains are designed to emit light at specific wavelengths and can be used to probe specific cellular functions. The most commonly used procedure for measurement of bio film is the viable plate count method. The re suspended and dispersed biofilm cells are plated onto a solid medium, incubated and counted.

CONCLUSION

The importance from a public health perspective is the role of biofilm in antimicrobial drug resistance. The resistance of microbes residing in the biofilms towards various types of antimicrobial agents poses a serious threat to the pharmaceutical industries. Therefore, it is recommended to prevent their formation rather than treatment. Further study on the biofilms include effective control strategies to prevent the formation of bio films, effective treatment strategies for complete eradication of biofilms and complete understanding of which make the bio film phenotype so different from planktonic counterparts. Micro organisms on wet surfaces have been observed to aggregate and grow into micro colonies form 3-dimensional structures, resulting in a complex biofilm. They are difficult to remove from blood processing surfaces and environments due to the production of EPS materials and the difficulties associated with cleaning complex processing equipment and processing environments.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Donlan RM. Bio film and device associated infections. Emerging Infect Dis 2001;7:227-81.
2. Watnick P, Kolter R. Biofilm, city of microbes. J Bacteriol 2000;182:2675-9.
3. Kumar CG. Significance of microbial biofilm in food industry: a review. Int J Food Microbiol 1998;42:9-27.
4. Deibel V. Biofilms. J Food Safety 2001;1:6-7.
5. Davey EM, Otoole AG. Microbial biofilm: From ecology to molecular genetics. Microbiol Mol Biol 2000;64:847-67.

6. Donlan RM, Costerton JW. Biofilm: Survival mechanism of clinically relevant microorganism. Clin Microbiol Rev 2002;15:167-93.
7. Kumar A, Prasad R. Biofilms. JK Sci 2006;8:14-7.
8. Gilbert P, Das J, Foley I. Bio film susceptibility to antimicrobials. Adv Dental Res 1997;11:162-7.
9. Nichols WW, Dorrington SM, Slack MPE, Walmsley HL. Inhibition of tobramycin diffusion by binding to alginate. Antimicrob Agent Chemother 1988;32:518-23.
10. Nichols WW, Evans MJ, Slack MPE, Walmsley HL. Penetration of antibiotic into aggregate of mucoid and non mucoid *Pseudomonas aeruginosa*. J Gen Microbiol 1989;135:1291-303.
11. Decho AW. Microbial exopolymer secretion in ocean environment: Their role(s) in food web and marine process. Oceanography Marine Biol Ann Rev 1990;28:73-153.
12. Flemming HC. Biofilm and environmental protection. Water Sci Technol 1993;27:1-10.
13. Costerton JW, Lewaldowski DE, Caldwell DR, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol 1995;49:711-45.
14. Donlan RM. Biofilms: microbial life on surfaces. Emerging Infect Dis 2002;8:881-90.
15. Allison PG, Ruiz B, Sanjose, Gillbert P. Extra cellular products as mediators of the formation an detachment of *Pseudomonas fluoroscens* biofilm. FEMS Microbiol Lett 1998;167:179-84.
16. Chmielewski RAN, Frank JF. Bio film formation and control in food processing facilities. Compr Rev Food Sci Food Saf 2003;2:22-32.
17. Keyhani NO, Roseman S. The chitin catabolic cascade in the marine bacterium *Vibrio furnissii*. J Biol Chem 1996;271:33414-24.
18. Roberts AP, Pratten J, Wilson M, Mullany P. Transfer of a conjugative transposon, Tn5397 in a model oral biofilm. FEMS Microbiol Lett 1999;177:63-6.
19. Christensen BB, Sternberg C, Andersen JB, Eberl L, Moller S. Establishment of new genetic traits in a microbial bio film community. Appl Environ Microbiol 1998;64:2247-55.
20. Costerton W, Veeh R, Shirtliff M, Pasmore M, Ehrlich G. The application of bio film science to the study and control of chronic bacterial infections. J Clin Invest 2003;112:1466-77.
21. Costerton JW, Stweart PS, Greenberg EP. Bacterial bio films: A common cause of persistent infection. Science 1999;284:1318-22.
22. Darouiche RO. Anti-infective efficacy of silver-coated medical prostheses. Clin Infect Dis 1999;29:1371-7.
23. Elliott TSJ, Moss HA, Tebbs SE, Wilson IC, Bonser RS. Novel approach to investigate a source of microbial contamination of central venous catheters. Eur J Clin Microbiol Infect Dis 1997;16:210-3.
24. Wells CJ, Leech GJ, Lever AM, Wansbrough-Jones MH. Treatment of native valve Candida endocarditis with fluconazole. J Infect Dis 1995;31:233-5.
25. Domingue GJ, Hellstrom WJG. Prostitis. Clin Microbiol Rev 1998;11:604-13.
26. Bishop PL. Biofilm structure and kinetics. Water Sci Technol 1997;36:287-94.
27. Stickler DJ. Bacterial bio films and encrustation of urethral catheters. Biofouling 1996;94:293-305.