Indian Journal of Biotechnology
Vol 8, April 2009, pp 159-168

Biofilm: Importance and applications

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Received 24 December 2007; revised 18 August 2008; accepted 2 November 2008

Biofilm is an assemblage of the microbial cells that is irreversibly associated with a surface and usually enclosed in a matrix of polysaccharide material. Biofilm is composed primarily of microbial cells and extracellular polymeric substance (EPS). Extracellular 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. Anthony van Leeuwenhoek, who discovered microbial attachment to his own tooth surface, is credited with the discovery of biofilm. 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 article provides an overview of the formation of biofilm, structure, role in microbial communities and its applications.

Keywords: Biofilm, polymeric substance, hydrodynamics, probes, pathogenesis

Introduction

A 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 counterparts by reduced growth rate, up and down regulation of gene and generation of extracellular polymeric matrix[1]. Genetic studies have revealed that biofilms are formed through multiple steps. They require intracellular signaling 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].

The bacterial growth and activity is substantially enhanced by the incorporation of surfaces to which microorganisms could attach (Bottle effect). Some researcherers also observed that number of bacteria at surfaces was dramatically higher than surrounding medium. With the use of polysaccharide stains, Ruthenium red and Osmium tetroxide, researchers were also able to show that extracellular polymeric substance (EPS) is made up of polysaccharides. Much of the work in the last two decades has increased our understanding about biofilm such as use of electron microscope and investigation of genes involved in cell adhesion and biofilm formation[1].

Biofilm formation occurs step by step, such as formation of conditioning layer, bacterial adhesion**,** bacterial growth (Fig. 1) 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].

Biofilm Structure

The confocal scanning laser microscope (CSLM) has been effectively used to monitor biofilm development in flow cells that allows direct observation of the biofilm without disrupting the community. CSLM, which allows the visualization of fully hydrated sample, has revealed the elaborate 3-dimensional structure of biofilm[5]. Biofilm is composed primarily of micro colonies 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

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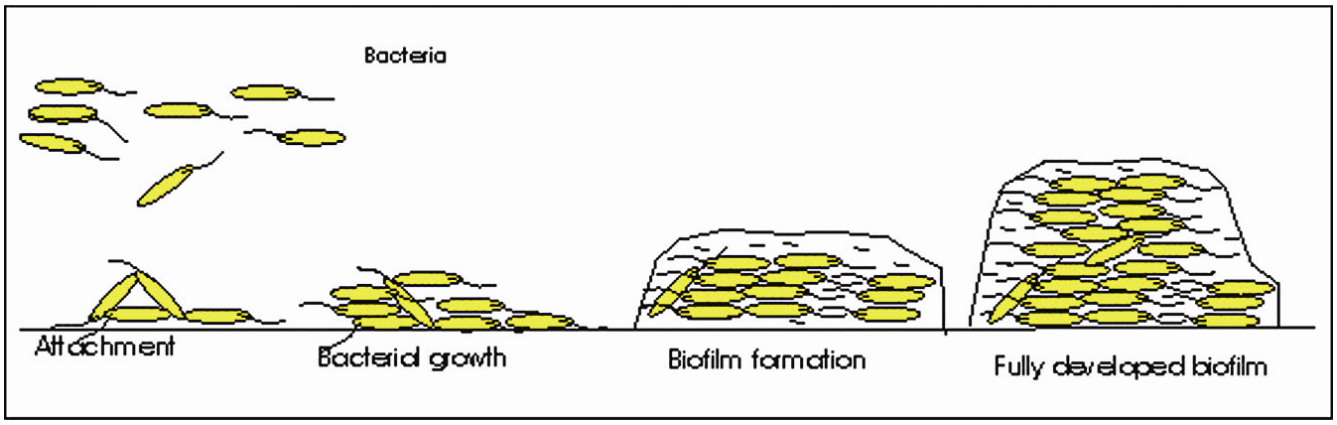

Fig. 1—Biofilm formation

(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 biofilm[6]. Backbone of EPS contains 1,3- or 1,4-β linked hexose residues. The amount of EPS produced by different organisms may vary and the amount of EPS increases with the age of biofilm. EPS may associate with metal ions, divalent cations and macro molecules (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 promote the EPS synthesis[7].

Role of Biofilm in Microbial Communities

Biofilm formation is prevailing in bacterial life style. Here, we have described why biofilm strategy has been adopted by so many microbes[2].

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[7]. 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[7,8]. 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 *p*H shift, UV radiation, osmotic shock and desiccation.

Nutrient Availability

The water channel provides effective means of exchanging nutrient and metabolites with the bulk aqueous phase, enhancing the nutrient availability as well as removal of potentially toxic metabolites[11]. Micro colonies in biofilm quite often consist of different microbial communities. These multispecies micro consortia can result from association between metabolically co-operative organisms. Their close proximity facilitates interspecies substrate exchange and 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. Fermentive bacteria initiate the catabolism 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].

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 biofilm forming communities and the phenotypic characters are the expression of a particular genotypic character. Transcription of *alg*C gene involved in the production of alginate is increased approximately fourfold in biofilm associated cells as compared to planktonic cells[13,14]. Researchers have noted that the pulmonary isolates (*Pseudomonas aeruginosa*) are mucoid due to the synthesis of large amounts of alginate. In addition, by mutational analysis researchers have shown that alginate synthesis is positively regulated by sigma factors whereas sigma factor negatively regulates the synthesis of flagellum. This reveals that when synthesis of the exopolysaccharides and alginate are increased in the biofilm-associated cells, flagellar synthesis decreases. Thus, to become a effective member of a biofilm community, the bacterium must differentiate into biofilm-associated cells by repressing the synthesis of flagellum that destabilizes the biofilm and producing exopolysaccharides that will reinforce the biofilm structure[14,15]. The nature of biofilm structure and physiological attributes of biofilm organisms confer inherent resistance to the antimicrobial agents. The mechanisms responsible for the resistance to these antimicrobial agents, antibiotics, disinfectants or germicides are described below.

Penetration of Antimicrobial Agent

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 biofilm containing surfaces took 21 min[6]. 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. A prime example of this concept is that *P. fluoroscens* produces exopolysaccharide lyase that degrades the biofilm associated exopolysaccharides for consumption and release cells from biofilm scaffold to seek more favourable environment[16]; (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 Biofilm Formation

Biofilm may be formed on a wide variety of surfaces including living tissues, indwelling medical devices, indus or portable water system piping 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 extracellular polymeric substances matrix[1]. Different factors affecting the formation of biofilm are as follows:

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[1]. 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.

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 passivated by the formation of a chromium oxide layer. Organic soil adheres to the oxide layer, producing a conditioned substratum to which bacteria adhere[17]. Another prime example is "acquired pellicle" which develops on tooth enamel surfaces in oral cavity. It consists of albumin, lysozyme, glycoprotein, phosphoproteins, 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[1,18].

Hydrodynamics

Biofilms have also been examined under various hydrodynamic conditions such as laminar and turbulent flow. It is found that biofilm response is altered in flow conditions. Biofilms grown under laminar flow are found to be patchy and consist of rough cell aggregates separated by interstitial voids. Biofilms grown under turbulent flow cells are also patchy but are elongated "streamers" that oscillate in the bulk fluid[6]. Association of cells with the surface also depends on cell size and cell motility.

Characteristics of Aqueous Medium

Physico-chemical characteristics of aqueous medium such as *p*H, 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[1].

Horizontal Gene Transfer

Horizontal gene transfer is important for the evolution and genetic diversity of natural microbial communities. During the evolution, an adaptation of bacteria to new environment often results in the acquisition of new genetic traits via horizontal gene transfer rather than accumulation in modification of gene function by mutations[19]. 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 biofilm-associated phases of 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[20]. A *Bacillus subtilis* strain harboring conjugative transposons which confers resistance to tetracycline was introduced to the system and resistance profile of biofilm bacteria was assessed. It was found that transfer of the conjugative transposons occurred within a biofilm resulting in *Streptococcus* species resistant to tetracycline[21]. 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 biofilm community growing on benzyl alcohol as the sole carbon and the energy source[22]. Virus-mediated gene transduction is another mode of gene transfer in biofilm associated microbial community.

Quorum Sensing

Cell to cell signaling 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[2]. This method of intracellular signaling 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-dimensional 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 sulfate. The role of intracellular signaling in multispecies 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 1 influence the rate and extent of attachment of microbial cell[2].

Biofilm Examination and Measurement

Biofilm development and structure has been analyzed using various methods. Light, fluorescence, differential interference contrast (DIC), transmission electron (TE), scanning electron (SE), atomic force (AF), 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 extracellular fibers 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[7].

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 (Table 1). For example, nucleic acid stain such as DAPI (4′6′-diamidino-2-phenyl indole), acridine orange, and Syto9 will stain the DNA and RNA of all cells regardless of their viability[23]. The most commonly used procedure for measurement of biofilm is the viable plate count method. The resuspended and dispersed biofilm cells are plated onto a solid medium, incubated and counted.

Applications of Biofilm

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[1]. Microorganisms commonly associated with biofilm on indwelling devices are shown in Table 2.

Central Venous Catheter Biofilm

All the indwelling central venous catheters are colonized by microorganisms embedded in a biofilm matrix. The organisms, most commonly isolated from

Table 1 — Common fluorescent probes used in biofilm studies

Name	Excitation\emmision	Applications
FITC	490\520	Binds to proteins, may be conjugated to antibodies, lectins, dextrans, ficols
Acridine orange	490\530	Stains DNA and RNA
Fluoroscein diacetate	495\520	Indicates esterase activity
Nile red	450\530	Stains neutral lipid and phospholipids
Propidium iodide	530\615	RNA, DNA intercalating agent
Fluoroscein	490\520	Negative staining , *p*H indicator
TRITC	541/572	Bind to proteins, may be conjugated to antibodies, lectins and dextran
RITC	575/ 595	Bind to proteins, may be conjugated to antibodies, lectins and dextran
Fluo-3	506/ 526	Calcium indicator
NCECF	500/ 530or 620	*p*H indicator
Hoescht	362/ 470	Stains DNA

Table 2 — Microorganisms associated with biofilm on indwelling devices

Medical devices	Causative organism
Urinary catheter, Intra uterine devices, prosthetic heart valves, central venous catheter	Coagulase -negative *Staphylococci*
Central venous catheter, urinary catheter	*Klebsiella pneumoniae*
Artificial hip prosthesis, central venous catheter, urinary catheter	*Pseudomonas aeuginosa*
Artificial voice prosthesis, central venous catheter, intra uterine devices	*Candida albicans*
Artificial hip prosthesis, central venous catheter, intra uterine devices, prosthetic heart valve	*Staphylococcus aureus*
Artificial hip prosthesis, prosthetic heart valve, urinary catheter	*Enterococcus* spp.

catheter biofilm, are *S. epidermidis*, *S. aureus*, *C. albicans*, *P. aeruginosa*, *K. pneumoniae,* etc[24,25]. 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 microflora, exogenous microflora 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. Colonization and biofilm formation may occur within 3 d of catherization. Raad and Sherertz also showed that catheters in place for less than 10 d tended to have more extensive biofilm formation on the external surface of the catheter[26]. During long term catheterization there would be more formation of a biofilm on the inner lumen of catheters. Biofilm on central venous catheters have routinely been detected by a semiquantative procedure termed the roll plate technique. In this procedure, the distal tip of 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[7]. Several studies have examined the effect of various types of antimicrobial treatment in controlling the biofilm. Researchers have found that addition of sodium metabisulfite to dextrose heparin flush of left arterial catheter eliminated microbial colonization of this catheter. Other research group has found that catheter impregnated with minocycline and rifampicin were less likely to be colonized than those impregnated with chlorhexidine and silver sulfadiazine. Catheter impregnated with cationic surfactant, which is used to bond cephalosporin, will less likely to develop biofilm than untreated catheter[27].

Prosthetic Heart Valves

Mechanical valves and bioprostheses 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. Microorganisms also have greater tendency to colonize these locations[7]. The resulting biofilm more commonly develops on the tissue surrounding the prosthesis. The primary microorganisms responsible for this condition are *S. epidermidis*, *S. aureus, Streptococcus* spp, Gram-negative bacilli, diptheroids, enterococci and *Candida* spp. Antimicrobial agents are usually applied during valve replacement and whenever patient has dental work to prevent the initial attachment by killing the microorganisms introduced into the blood stream[27,28].

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) within 4 d. Patients using closed system are much less susceptible to UTI[29]. The longer the urinary catheter remains in place, greater the tendency of these organisms to develop biofilm and result in UTI. Only 10 to 20% of patients undergoing short-term catheterization (up to 7 d) but essentially all the patients undergoing long-term catheterization (more than 30 d) get infected with UTI[30]. 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 *p*H 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 *p*H responsible for biofilm-urine interface results in precipitation of minerals such as struvite and hydroxyapatite. These mineral containing biofilms, which form encrustations, may completely block the inner lumen[31]. Several strategies have been attempted to control the urinary catheter biofilm 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[27].

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. Microorganisms 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* and species of *Proteus*., *Serratia, Candida,* 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. In fact, the lens case has been implicated as the primary source for contamination[7].

Intrauterine Devicess

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 *Lactobacillus plantarum, S. epidermidis, Candida albicans,* and *S. aureus* and species of *Corynebacterium, Enterococcus,* etc. IUDs removed from women with pelvic inflammatory disease may also contain streptococci, *S. aureus*, *E. coli* and some anaerobic bacteria[7].

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 microorganisms residing in the biofilms has been reported.

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. Microorganisms 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. Biofilm formed by microbe's damages valve tissue.

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 gentamicin to produce synergistic killing[7]. *Candida endocarditis* has been successfully terminated with fluconazole[31].

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. pneumomoniae, Haemophilusinfluenzae, 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[7,32].

Chronic Bacterial Prostitis

The prostate gland may be infected with microorganisms that have ascended from the urethra or by reflux of infected urine into the prostatic ducts emptying into the posterior urethra[33]. Once the bacteria enter the prostatic duct, they multiply rapidly and elicit a host response. These bacteria can form sporadic microcolonies and biofilms that adhere to the epithelial cells of the duct system. Microorganisms which are responsible for chronic bacterial prostitis include *E. coli, P. aeruginosa,* and species of *Klebsiella, Proteus, Serratia, Bacteroides* etc. The role of biofilm in chronic bacterial prostitis has been studied by employing animal model or from biopsies collected from men with prostitis[7].

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 increaseing 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[7].

Periodontitis

Periodontal diseases are infections which involve the supporting tissues of teeth, gums (gingiva), and periodontal tissues (gingiva, alveolar bone, and periodontal ligament). Chronic periodontitis may lead to exfoliation of the teeth. Sub gingival crevice (channel between the tooth root and the gum) is the primary site of periodontal infection[34]. The main microbe associated with periodontitis is *Porphyromonas gingivalis*. Other organisms are *Fusabacterium nucleatum, Peptostreptococcus micros, Eubacterium timidum, E. brachy, Pseudomonas anerobicus*, etc. These bacteria can colonize a number of surfaces in the oral cavity, including various mucosal surfaces[35,36] and the tooth surfaces. The binding of one bacterium to another or co-aggregation is well studied aspect of tooth colonization. Colonization of surfaces may permit the bacteria to invade mucosal cells, alter calcium flux in epithelial cells and release toxins. A climax biofilm community (plaque) will develop within 2-3 wk. Plaque that becomes mineralized with calcium and phosphate ions is termed as calculus or tartar[37]. As the plaque mass increases in protected areas the antimicrobial properties of the saliva are less liable to penetrate and protect the tooth enamel that may lead to dental carries or periodontal diseases. The control of periodontitis includes removal of established biofilms from subgingival areas in combination with supplemental antimicrobial agents[7].

Biofilm and Food Industry

Growth of biofilms in the food processing environment leads to increased opportunity for microbial contamination of the processed food. Microorganisms within the biofilm are protected from sanitizer; therefore the survival of microorganisms and the chances of contamination of foods are also increased. Extracellular polymeric substances which give several beneficial effects to the microorganisms are not removed by cleaning. This gives the attachment sites to the microorganisms newly arrived to the cleaned systems[3,17]. Surface tension value is the critical factor to determine the extent of attachment of microorganisms to the surface. Maximum attachment depends upon the high surface energy or wettability 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 (Table 3). 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.

Biofilm 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, peroxygens and

Table 3 — Food borne pathogens and the spoilage organisms in biofilm

Food borne pathogen	Growing surface
Listeria monocytogens	Dairy processing plant, conveyor belt
Psudomonas spp.	Drain, vegetable meat surface
Bacillus spp.	Pipeline, joint in processing environment, hot fluid.
Salmonella spp.	Poultry processing environment

quaternary ammonium compounds. Quaternary ammonium compounds are cationic surfactant sanitizers[23] 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.

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. Distribution system is one of the vital importances in determining the final quality of potable water. The treated water, when flows through the distribution system, is adversely affected by the conditions in the distribution system. 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 biofilm. Biofilm 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 biofilm in the water distribution system[38] 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 microorganisms. The use of disinfectants also enhances the formation of easily biodegradable substances. These biodegradable substances can be used by microorganisms as energy source and promote the biofilm formation in the distribution system. Microorganisms develop resistantance towards the disinfectants used and they can survive and multiply despite the presence of measurable concentrations of disinfectants. Microorganisms develop resistance due to the indiscriminate use of disinfectants and acquisition of gene responsible for resistance by horizontal gene transfer. Regrowth of microorganisms 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 biofilm forming microbes. Monochloramine or hydrogen peroxide are maintained a longer disinfectant residual concentration[39,40] 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 monochloramine to ensure persistent concentration of disinfectant residual throughout the distribution system.

Conclusions

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 biofilms, effective treatment strategies for complete eradication of biofilms and complete understanding of which make the biofilm phenotype so different from planktonic counterparts.

Microorganisms on wet surfaces have been observed to aggregate and grow into micro colonies form 3-dimensional structures, resulting in a complex biofilm. Biofilms 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. Model systems should be developed and used to study biofilm processes on various indwelling medical devices. These systems should closely simulate the *in vivo* or *in situ* conditions for each device. This system design could be used to investigate and compare various biofilm control treatments, device design modifications or different media formulations.

Acknowledgement

Dr C R Kokare acknowledges All India Council for Technical Education (AICTE), HRD Ministry, New Delhi for financial support to this research project under the scheme of 'Career Award for Young Teachers, 2005-06'.

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