Microbial biofilms: development, treatment and prevention in medical implants

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A biofilm is an accumulation of microorganisms enclosed in extracellular polymeric matrix and attached to solid surface. Biofilms are of great impotance and challenge to medical science, as they accounts for approximately 65 percent of microbial infections in the human body, which include cystic fibrosis, urinary tract infections, chronic otitis media (middle ear infection), chronic osteomyelitis, catheter and medical implant associated infections (teeth and dental implants, intra-uterine device, cardiovascular implants and prosthesis such as pacemakers, heart valves, stents, vascular grafts, joint fixation, sutures, etc. During growth generally bacteria exist as planktonic cell in one form and are organized into sessile aggregates in other form. The sessile aggregates or colonies encased in extracelluar polymeric matrix are known as biofilm. If the bacteria succeed in forming a biofilm within the human host, the infection often turns out to be untreatable and will develop into a chronic state. Biofilm infections are characterized by extreme resistant to antibiotics, other antimicrobial agents, and capacity for evading the host defences. This chapter will summarize the biofilm historical perspective, mechanism of formation and dispersal, interactions with host tissues and medical implants, antibiotic resistance, treatment of biofilm associated infections and techniques to prevent biofilm.

1. Introduction

If sometimes, we try to go in a river or a stream, we have a tendency to slip over the rocks under the water. This is because of the slimy nature of the biofilm-coated rocks. The plaque that forms on the teeth and caused tooth decay is another type of bacterial biofilm. Biofilms are assemblage of microbes that are enclosed within a self-produced matrix of extracellular polymeric substance (EPS) on both biotic and abiotic surfaces. Quintessentially, biofilms represent an interdependent community-based existence. As well as microbial components, non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, may also be found in the biofilm matrix. Biofilms were first observed by Van Leeuwenhoek on tooth surface. Biofilm are characterised by an outer layer of EPS (Extra polymeric Substance). The polymeric matrix may also contain traces of particles present in its environment. Due to the formation of EPS the biofilms can endure host defence mechanism and strong antibiotics which would have killed the planktonic cells.

During the late 1900s, the importance of biofilm growth was recognized with microorganisms in a biofilm differ from free-floating cells in behavior, and metabolism. The change in physiology of biofilm bacterial cell significantly influences the virulence, pathogenecity of microorganisms with their susceptibility to antibiotics. Biofilms develop on wide range of surfaces, including natural aquatic systems living tissues, indwelling medical devices/ prosthesis, water supply piping, industrial food processing equipments, etc. Predominantly microbes grow as biofilms in aqueous conditions, pathogenic especially in case of medical devices or prosthesis, secret deleterious products and toxins which embedded within the biofilm matrix made up of extracellular polysaccharides. They manifest an altered gene transcription in comparison to planktonic organism (Thomas and Day, 2007). Microorganisms form biofilms due to elevation in beneficial genes expression, phenotypic changes, antibiotic resistance persistence through plasmid gene transfer via conjugation, production extracellular polysaccharides, increased access to nutrients, favorable environmental condition and close association among cells which allows mutualistic or synergistic associations and protection (Costerton and Lappin-Scott 1989; Toole et al, 2000 and Meng et al, 2013). In humans, approximately 75% of all nosocomial infections are of biofilm origin (Donlan 2001). Biofilm infections established in medical devices are very cumbersome to treat due to tremendous increase in antimicrobials resistance. The control of biofilm infection has thus become a pressing issue with time.

2. Biofilm formation mechanism

Biofilm formation is a complex process consisting distinct stages i.e. attachment, aggregation, maturation, detachment and dispersal. Attachemt is a two-step process which involves the identification of surface by micro-organisms followed by reversible and irreversible attachment. The reversible attachment is mediated by non-specific cellular association viz. van der waals forces, electrostatic forces, lewis acid-base, hydrophobic interaction, etc. while, irreversible adhesion occurs due to specific adhesions present on the pili, fimbriae or cell surface of micro-oraganisms. Maturation involves the aggregation and multiplication of bacteria on surface after attachment to form micro-colonies. The bacterial irreversible attachment with surface leads to change in gene expression, resulting in the synthesis and secretion of extracellular polysaccharide (EPS) or extracellular polymeric matrix (characteristic of biofilm condition) which acts as

cementing substance and holds the colonies of bacterial cell together. Extracellular polymeric matrix primarily composed of polyssacharides (neutral or polyanionic for Gram negative bacteria and cationin for Gram positive bacteria), highly hydrated upto 98% and always bound to underlying surface. Continuous multiplication, growth and recruitment of additional micro-organism leads to mature biofilm development, consisting tightly packed large number of micro-organism into an outgrowth masses protruding from the surfaces. The last stage of biofilm includes the detachment of microbes from biofilm colonies, their translocation or dispersal and again attachment to new location.

2.1 Factors affecting rate of formation

Rate of growth of biofilms on a medical device depends on numerous components. For growth firstly the microorganism must attach itself to the device's surface. This exposure must be for a considerable amount of time so that it may not detach easily. This adherence also depends on the bunch of microbes present in the fluid in which the device is immersed. The properties of the surface of the device are altered by the presence of various particles present in its surrounding. Thus attachment of one of these cells and correspondingly the formation of biofilm occurs (1). Some factors which affect biofilm formation are listed below.

Substratum	Texture, hydrophobicity, conditoning film, surface charge
Aqueous medium	Velocity of medium, temperature, pH, cations, nutrients availability, antibacterial agents
cell	Cell surface, hydrophobicity, fimbriae, flagella, pili, adhesions, other surface appendages, EPS

3. Role of biofilm in clinical infections/ Medical implants

Microorganisms are known to attach and grow on universal surfaces. This ubiquitous nature of microorganisms compromises the properties of the material on which it grows and becomes the main cause of infection. The study of these microorganisms led to the discovery of surface associated microorganisms that showed a higher rate of gene transcription and growth. Such colonies of surface-related microorganisms are known as biofilms. Biofilms are of great impotance and challenge to medical science, as they accounts for approximately 75 percent of microbial infections in the human body and specially the main cause of infection in many cases such as cystic fibrosis, urinary tract infections, chronic otitis media (middle ear infection), chronic osteomyelitis, catheter and medical implant associated infections (teeth and dental implants, intra-uterine device, cardiovascular implants and prosthesis such as pacemakers, heart valves, stents, vascular grafts, joint fixation, sutures, etc.). The chronicity of infection is due to the fact that these biofilm exhibit high resistance against antimicrobial agents and defence against the phagocytic nature of host defence mechanism.

Both Gram-negative and Gram-positive bacteria form biofilm on in-vivo medical implants. The bacteria which are commonly aggregate on the devices are *Klebsiella pnuemoniae, Strptococcus viridans, Enterococcus faecalis, Stapylococcus aureus, Staphylococcus epidermis, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa.* These microbes may enter the patient's blood stream through their skin which comes in contact with a numerous substance present in the surroundings. Depending upon the device which is being infected, the formation of biofilm may be the work of one or more than one species of microbes. It may also depend on the amount of time for which the device was inserted (Dolan, 2001).

Biofilms on central venous catheters are primarily formed by *P. aeruginosa, K. pneumonia, E. faecalis, S. aureus, C. albicans, S. epidermis*. These microbes enter into the catheter environment from the skin and colonize rapidly within 24 hours due to the host produced environment of platelets, plasma and tissue protein (Maki et al, 1994). The degree of colonization depends upon the time the catheter was being administered. It was reported that those catheters which showed more formation of biofilms on their outer surface were being used for less than even 10 days. While those which showed intense formations on the inner lumen were administered for almost 30 days (Raad et al, 1993 and Ryder, 2005). The amount of biofilm formed was also affected by the type of fluid which was given with the help of these central venous catheters.

Prosthetic valve endocarditis is the term used for infection and formation of biofilm on the prosthetic heart valve and the encircling heart tissue. The microbes reach the valve through migration from skin or other implants attached to the patient's body like catheters. *Candida, Enterococcus, Staphylococcus* and *Streptococcus* are the elemental microbes associated with this condition. Generally, the origin of the infection determines the causative species. It has been observed that in most of the cases if the cause of infection is contamination during surgery then most probably it is caused by *S. epidermis*. While infection originated from a dental procedure is due to *Streptococcus* and that from other implants like catheters is due to a large variance of species. Impregnation of the heart valve results in tissue damage and aggregation of fibrin and platelets giving favorable sites for the attachment and consequentially the growth of biofilms (Braunwald et al, 1997 and Maki et al 2006).

The primary microorganisms which form biofilm in urinary catheters are mainly gram-negative bacteria like *E. coli, Proteus mirabilis, S. epidermis, P. aeruginosa, Enterococcus faecalis,* and *K. Pneumoniae.* The urinary catheters are

generally made up of silicone or tubular latex giving an idle surface for the formation of biofilms. The growth rate in these catheters depends upon the time for which they were used. The longer the use, greater is the chance of formation of biofilm which leads to an infection of the urinary tract. It is was observed that those patients which used the tube for a week's time had a lower probability of getting an infection resulting from biofilms. While those patients which were catheterized for almost a month had a cent percent chance of incurring the infection (Stickler, 1996).

It was accounted that the affinity of the microorganism to the catheter material was due to their degree of hydrophobicity. It was examined that the hydrophobicity of the catheter and as well as the organism played a role in the degree of adherence. Therefore, materials which were had both hydrophilic and hydrophobic regions were the once which showed the maximum affinity to almost all kind of microbes (Brisset et al, 1996). Moreover, it was observed that the increase in concentration of calcium and magnesium ions and increase in urinary pH resulted in more adherence of the bacterial cells. In more severe cases of urinary tract biofilms, some components release urease which hydrolyses urea to ammonium hydroxide. This results in the higher pH at the biofilm- urine junction which causes accumulation of minerals such as struvite and hydroxyapatite. This accumulation of minerals blocks catheter inner lumen. Enabling bacteria to reach the urinary bladder within 1-3 days (Tunny, 1999). The accumulation of damaged particles, the microorganisms adhere more frequently to the tissue surrounding the implant and on the material used for attaching the implant than on the implant itself (Carel et al, 1998 and Illingworth et al, 1998).

Table 1 List of medical implants prone to biofilm formation with the causative agent.

Medical device	**Bacteria**
Dental implants	*Staphylococcus aureus*, *Candida albicans*, *Streptococcus*
Urinary catheters	*S. epidermidis, K. pneumoniae, Enterococcus, Proteus mirabilis, P. aeruginosa, E. coli* and other gram-negative bacteria
Intra-urine devices	S. aureus, *S. epidermidis, P. aeruginosa, E. coli, Neisseria gonorrhoeae, Candida albicans* and *Candida dubliniesis*
Artificial hip prosthesis	*Staphylococcus* spp., *P. acnes, Salmonella enterica*, *Shigella*
Prosthetic heart valves	*Enterococcus, S. epidermidis, S. aureus, Streptococci, Diphtheria*, *Candida albicans* and gram-negative bacilli,
Synthetic vascular grafts	*S. aureus, Candida, Enterococcus, Streptococcus*
Ventilator tubing	*Acinetobacter baumannii* and *Pseudomonas aeruginosa*
Artificial voice prosthesis	*Candida albicans, S. aureus, P. aeruginosa,*
central venous catheters	*S. epidermidis, Enterococcus faecalis, K. pneumoniae, Candida albicans, P. aeruginosa, S. aureus*
Orthopedic implants	*S. epidermidis, P. aeruginosa, Enterococcus, S. aureus*

4. Antibiotic Resistance Mechanism in Biofilms

The most alarming effect of bacterial biofilms is the increased resistance of its constituent microbes to antibiotics. Decreased antibiotic vulnerability has added to the perseverance of biofilm infections such as those allied with implanted devices. It holds sterned penalties for infection control, treatment regimes, and disease development. The defensive mechanisms in biofilms appear to be different from those which are accountable for conservative antibiotic resistance. In biofilms, poor antibiotic diffusion, nutrient inadequacy, sluggish augmentation, adaptive stress responses, and development of persister cells are hypothesized to comprise a multi-layered protection. The genetic and biochemical details of these biofilm protections are now commencing to come into view. Each gene and gene product causative to this resistance can be a potential target for the progress of new chemotherapeutic agents. Disabling biofilm resistance may augment the capability of obtainable antibiotics to remove infections concerning biofilms which are unmanageable to current treatment procedure.

4.1 Glycocalyx

The production of an exopolysaccharide matrix, or glycocalyx, is distinguished property of biofilms. It prevents the antibiotics entry to the bacterial cells encased in it and also responsible for providing adhesion to the solid surfaces. Glycocalyx thickness varies from 0.2 to 1.0μm in biofilm. Its composition is complex and is controlled by the environmental nature of biofilm growth. Glycocalyx induces effective resistance for biofilm bacteria against antimicrobial proteins, smaller peptides-defensins and their analogs (Philip, 2012).

4.2 Metabolic growth and heterogeneity

Nutrients and oxygen variations in biofilm affect growth and metabolism of bacteria. Concentrations fluctuation in metabolic substrates and products during biofilm formation revealed that surface attached communities contain cells at all bacterial growth phases and at different activity levels which results microbial population heterogeneity. This

problem is associated with both uni-species and multi-species bacterial biofilms. Enormous availability of nutrients and oxygen in the peripheral area of biofilm promotes good metabolic activity of cells and allow their proliferation. While in inner or deep core areas of biofilm the metabolic activities of bacteria is reduced by the poor diffusion of nutrients. As most of the antibiotics are effective against metabolically active bacteria, its suggested that bacteria at the dormant growth phase or metabolically less active in the deeper region of biofilm are less susceptible to antimicrobial agents (Thien-Fah C.Mah and George A.O' Toole, 2001)

4.3 Efflux Pumps

Efflux pumps induce intrinsic and acquired resistance to antibiotics via applying energy to limit the cytoplasmic compound concentration to subtoxic level. These efflux pumps in bacterial cell are involved in multidrug resistance along with other more or less specific resistance systems like target mutation, plasmids and enzymatic modification of antimicrobial agents. The efflux pumps mechanism in *micro-organisms* decrease the penetration of hydrophilic solutes and transmembrane diffusion of lipophilic solutes due to down regulation of porin production. Bacterial efflux pumps composed of five classes of systems i.e. major facilitator superfamily (MF), ATP-binding cassette family (ABC), resistance-nodulation-division family (RND), small multidrug resistance family (SMR) and multidrug and toxic compound extrusion family (MATE). The ABC family hydrolyses ATP, while the MF, RND and MATE family acts as secondary transporters for catalysing drug ion antiport to derive efflux of antimicrobial agent. In gram-negative bacteria it includes: a fusion membrane protein attached with cytoplasmic membrane, a transporter protein for substrates supply in the inner membrane, and an outer membrane factor (OMF) to facilitate the entry of the substrate throughout the outer membrane(Li & Nikaido, 2009). By Molecular analysis of efflux pumps we get to know the significance of this mechanism in biofilm resistance to antibiotics.

4.4 Quorum sensing

Bacteria in biofilm have a capability to sense increase in population density and subsequently responds via particular set of genes induction which is known as quorum sensing or cell-to-cell signaling. It involves the synthesis and secretion of an acyl homoserine lactones (AHL, signaling molecule) especially by Gram negative bacteria, which diffuse through the cell wall, followed by cell to the medium. Gram-positive bacteria quorum sensing utilize secreted peptides as signal molecules and a regulatory system (membrane bound histidine kinase receptor and intracellular response regulator) to detect the peptide and regulate the gene expression. Autoinducers-2 are secreted by both gram-negative and gram-positive bacteria for this mechanism.

Due to heterogeneity of biofilms and their capability to synthesize enzymes which deactivate biocides, it's reasonable to mention that biofilm antimicrobial agent's resistance can be influenced by quorum sensing. Likewise, coordinated expression of quorum sensing-mediated phenotypes is important in cells migration to favorable environment for survival and adaptation to new growth modes which may increase their protection from deleterious environment (Li & Nikaido, 2009).

4.5 General Stress Response

Response to stress is described by various alterations or modifications in bacterial morphology and metabolic activity which induce bacterial stress resistance (Lee et al., 2009). During unfavorable conditions, stress response acts as a factor to prevent cell injury instead of its repairing. The mechanism which works behind this induction is due to deficiency of nutrients which promotes the stationary phase in life cycle of bacteria, adverse temperature either low or high, change in osmolarity and pH. Exposure of *Escherichia coli* to adverse conditions can induce central regulator RNA polymerase sigma subunit (RpoS) which determine general stress tolerance, while others mediate the reprogramming of the bacterial metabolism in stress condition. The general stress response acts both as a rapid emergency response and as a long-term mechanism which enables the cell adaptation to unfavorable environmental conditions that cause changes in physiology of bacteria (Kaplan, 2010).

4.6 Persister Phenomena

Biofilms contain a petite reversible subpopulation of persister cells that adopt to slow generation time existence throughout the appearance of small colony variants and are extremely bearing to extracellular stresses, like antibiotic treatment.It has been noticed that many major antibiotics are less efficient against slow or non-growing cells compared with fast-growing ones. The reason is, growth-specific factors are targeted by such antibiotics. Thus, the sluggish growth rates of biofilm-growing cells will cause them to be less vulnerable to antibiotics. For example, β-lactams are only active against dividing bacterial cells, whilst fluoroquinolones are capable of killing non-growing cells, but are more effective in killing cells that are speedily budding and dividing.The efficacy of fluoroquinolones on biofilm-growing *P. aeruginosa* is superior when compared with β-lactams, whilst both fluoroquinolones and β-lactams are less effectual against biofilm-growing *P. aeruginosa* compared to planktonic cells. Only persister cells may survive after antibiotic treatment. Thus, creating the reservoirs of existing cells that may rejuvenate which may lead to a relapsing

chronic infection, which has been clearly seen in case of cystic fibrosis-associated lung infections caused by *P. aeruginosa* (Mulcahy LR et al., 2010) and for candidiasis by *C. albicans* (Lafleur MD et al., 2010). Activating cells to go into persister cell destiny is to overproduce the toxins that restrain cellular processes and augmentation, which is by toxin–antitoxin modules (Lewis 2010).

5. Treatment of biofilm infections

Biofilm bacteria are highly resistant to antibiotic due gene transfer between/among bacteria of resistance markers, decreased diffusion by the extracellular matrix formation, antibiotic inactivation by low pH, efflux pumps, metabolic stress response and persister cells that survive treatment. The combination of above mentioned mechanism allows biofilm bacteria to develop tremendous resistant against antibiotics than free floating cells (Hoiby et al. 2010). Therefore, there is an urgent need of more effective biofilm treatments. Some of the recent advancement developed to treat biofilm infections are listed in table 2.

Table 2 Biological and chemical approaches for biofilm infection treatment in medical devices.

Treatment	Description	Example	Reference
Bacteriophage Therapy	Lytic phages utilized which results in rapid destruction of the bacterial cell, therapy is host specific and bactericidal	*E.coli* T4 phage, coli-proteus bacteriophage	Burrowes et al. 2011
Antibacterial Peptides	Secreted by immune defense cells bears low MW, broad spectrum activity against bacteria and also proposed as novel antibiotics, bactericidal	lytic peptide PTP-7, cathelicidin peptides	Pompilio et al. 2011
Antimatrix Agents	Targets by disrupting components of the extracellular polysaccharide or glycocylax secreted by bacterial cell in biofilm, bactericidal	DNase I, Dispersin B, *N*-acetylcysteine	Burton et al. 2006
Signal Transduction Interference	Gene expression is hindered by interfering with signaling receptors involved in transduction and modify virulence selection, bacteriostatic	QseC kinase inhibitor, Siamycin I	Gotoh et al. 2010
Chelating Agents	Interfere with metal ions, destabilize biofilm architecture along with interfering with bacterial membrane dynamics, bactericidal	sodium citrate, tetrasodium-EDTA, aminocycline-EDTA	Donlan 2011
Antiadhesion Agents	Compounds interfere with the adhesive properties of glycocylax or bacterial cell surface appendages, bactericidal or bacteriostatic	Mannosides, pilicides	Cusumano et al. 2011
Modifying Dispersal Signals	Signal for biofilm dispersion is combined with an antibacterial agent for killing the dispersed organisms, novel therapy, bactericidal or bacteriostatic	D-Amino Acids	Ma et al. 2011b

6. Prevention of biofilm formation on medical devices

The microbes encapsulated in a biofilm will not respond to antibiotic treatment. Hence, it becomes evident that the key to prevention of device related infections and minimize the use of antibiotics, is to prevent colonization. For this various chemical and mechanical approches can be used viz. device coatings, immersion, polymer modification, altering hydrophobicity, surface charge, surface roughness, etc. (Romling et al, **2012** and Meng et al., 2013) among them coating of medical device is considered to the best approach as a preventive measure. Implants are coated with antibiotics, metals, signal interfering molecules, etc. through various coating, grafting and deposition techniques. Some recent advancement in device coatings to prevent biofilm are listed in table 3.

Table 3 Surface modification approaches to prevent biofilm formation in medical devices.

Method	Description
Silver treatment	Implant treated with sodium hydroxide and silver nitrate solutions after oxygen glow discharge treatment
Palladium/tin salt mixture treatment	Immersion and rinsing in a palladium/tin salt solution
Plasma treatment	Ionized gases generated artificially used to vaporize and redeposit metals for surface modification. eg. Trimethyl silane
Polymer modification	Antibiofilm compounds immobilized on implant surfaces via polymer chains through covalent coating which results in non-leachable, contact-killing surfaces. Eg. N-alkylpyridinium bromide attached to a poly(4-vinyl-N-hexylpyridine
Unique configuration of noble metals	Prevent colonization of bacteria on medical device surface, eg. Bactiguard
Perfluoro-alkylsiloxane (PAS) treatment	Surface oxidized and PAS were chemisorbed on medical devices

Quaternary ammonium silane coatings	Oxidized implant surfaces covered with QAS and left to react and dry, inhibits adhesion and viability property of bacterial cells
Ion implantation	Injects accelerated high-energy ions into the surface of a material to modify its physical, chemical and biological properties
Bulk surface photografting	Surface modification of hydrophobic and bioinert polymer. The radiation breaks chemical bonding on material surface to be grafted and form free radicals followed by exposure to monomers to start surface graft polymerization

7. Conclusion

Medical devices are an emerging modern day practice. But at the same time they are a major cause of morbidity and mortality due to their susceptibility towards most of the clinically associated infections. Statically, 95% of the urinary tract infection is related to urinary catheters, 65% of cases of pneumonia with mechanical ventilation and 87% of the infections related to blood are due to intravascular devices. While the most life threatening of all is the catheter-related bloodstream infection (CRBSI) (McLean et al 1995 and Ryder 2005). Different factors involved and the antibiotic resistance is the main cause that still an ideal method for the eradication of biofilm associated infection has not been developed yet. Currently, antibiotics available work against planktonic cells but not on biofilms.

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