Medical biofilms – Its formation and prevention using organic molecules

S. S. Prasanna AND Mukesh Doble

Abstract | Biofilms play a significant role in the area of clinical medicine. Currently research is in progress to understand their formation with a view to develop preventive measures to fight the infections caused due to biofilms that are formed on implanted medical devices. The determination of biofilm architecture, particularly the spatial arrangement of micro colonies (clusters of cells) relative to one another, has profound implications for the function of these complex communities. Moreover, standard antimicrobial treatment fails to eradicate biofilms, due to the organisms adaptive resistance towards antibiotics. The need of the hour is the development of antimicrobial molecules or various combination techniques to counter biofilm infections. This review explains the mechanism of biofilm development and the reasons for its resistance to antimicrobial agents. Also various possible preventive measures, in particular development of anti microbial small molecules, are also discussed.

Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, India
mukeshd@iitm.ac.in

Keywords: Biofilms, antimicrobial resistance, EPS, Quorum sensing, adaptive phenotype, antimicrobial coatings.

Introduction

About 3.6 billions years ago the first bacteria appeared on earth which happens to be about 100,000 years before the *Homo sapiens*[1]. Today microorganisms are found to live in any environment due to their survival mechanism. It was only in 1884 that Robert Koch identified that microorganisms are the cause of diseases[2]. These microorganisms can attach to living and non-living surfaces like medical devices which include urinary, venous, and arterial catheters, shunts, heart valves and tubes. The attached microorganisms aggregate and multiply into mushroom like shape which is held together by glycocalyx, an extra cellular "polysaccharide matrix" which is known as biofilm.

Microbial biofilms, which often are formed by antimicrobial-resistant organisms, are responsible for 65% of infections treated in the developed world[3]. About 24% of adults have lost atleast 4 mm of periodontal attachment, and 60% of 15-year-olds and 40–50% of adults have some form of gingival (biofilm) infection[4,5] due to biofilm related infection. In a study of 4,000 infants who were given cerebrospinal-fluid shunts, 15–20% infants were found to be infected by biofilm[6]. Most of the infections are related to urinary catheters and intravascular devices. In fact, 95% of urinary tract infections are associated with a urinary catheter, 86% of pneumonias are associated with mechanical ventilation, and 85% of bloodstream infections are associated with an intravascular device[7].

The high percentage of human infection results from the fact that the microorganisms present in the biofilm are highly resistant to killing, and to treatment with microbial agents[8]. The acute illness can also be caused due to the shedding of the bacteria from the biofilms into the surrounding tissue and the circulation system. Some of the common microorganisms causing human infection are listed in Table 1. Most of the biofilm related infections are caused by E. *coli*, S. *aureus*, S. *epidermidis*, and *Pseudomonas*.

Table 1: Some of the common microorganisms which cause human infections through the formation of biofilms.

Body site species	Implant or device	Incidence (%)	Common bacteria
Urinary tract	UT catheters	10–20	*Escherichia coli*
	CV catheters	4–12	*Staphylococcus epidermidis* *Staphylococcus aureus*
	Temporary pacemaker	4	*Staphylococcus epidermidis* *Staphylococcus aureus*
Percutaneous	Peritoneal dialysis catheters	3–5	*Staphylococcus epidermidis*
	Orthopedic pins	50	*Staphylococcus aureus*
Subcutaneous	Cardiac pacemaker	1	*Staphylococcus epidermidis*
	Mammary prosthesis	1–7	*Staphylococcus aureus*
Soft tissue	Intraocular lenses	0.13	*Pseudomonas* *Staphylococcus epidermidis*
	Prosthetic heart valve	1.88	*Staphylococcus aureus* *viridans streptococci* *Staphylococcus epidermidis*
Circulatory system	Vascular graft	1.5	*Staphylococcus aureus* Gram negative bacteria
Bones	Prosthetic hip	2.6–4.0	*Staphylococcus epidermidis* *Staphylococcus aureus*
	Total knee	3.5–4	*Staphylococcus epidermidis* *Staphylococcus aureus*

Development of biofilms

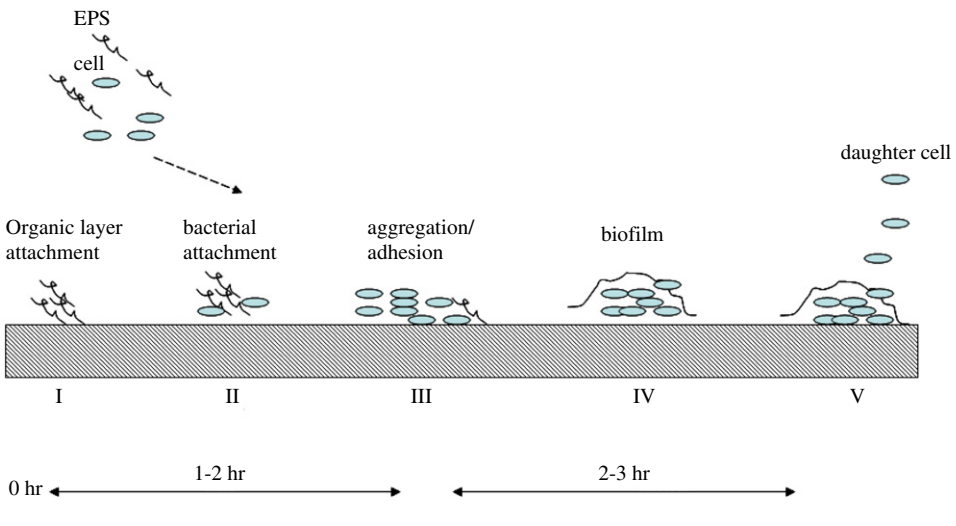
In the past two decades sophisticated imaging techniques have identified the structural, developmental complexity and mechanism of the formation of biofilm on surfaces. One of the advanced techniques to observe the biofilm formation is the use of confocal laser microscopy. Sessile bacteria growing in heterogeneous matrix, which were enclosed by micro colonies interspersed with open water channels facilitate efficient uptake of nutrient from the bulk phase into the biofilm. Thus the exchange of optimum nutrient and waste product provide the first link between formation and its function[9,10].

In general the mechanism of biofilm formation on medical devices can be understood through a sequence of five stages (fig 1). The first and the second stages are the identification and association with a surface followed by strong adhesion. These two stages consist of reversible cellular association with the surface with in the first 1–2 hrs of post-implantation. This non-specific association is mediated through long (e.g., gravitational, van der Waals, and electrostatic interactions) and short (e.g., hydrogen bonding, dipole–dipole, ionic, and hydrophobic interactions) range forces.

Stage three and four involve the aggregation of cells into micro colonies and subsequent growth and maturation of the biofilm. The third and the fourth stage take approximately 2–3 h and are characterized by stronger adhesion between the bacteria and the foreign material. Specific chemical reactions between compounds on the cell and substrate surfaces result in irreversible molecular bridging. Both polysaccharides on the bacterial membrane surface and adhesin proteins within facilitate attachment to substrate surfaces. The shape of the biofilm can be flat or mushroom shaped depending upon the nutrient source. Stage five is known as "transient motility" where the biofilms are sloughed or shed leading to the formation of daughter cells[11]. These cells travel down stream to form new attachment sites.

The aggregation and bacterial adhesion to the surface is due to the extra cellular polysaccharide (EPS) produced by them. This matrix can be considered as "house" of the microorganisms which allows for the formation of stable communities ("micro consortia") of synergistic strains and enables them to degrade recalcitrant substances. EPS retains water and prevents desiccation. They can be classified as exopolysaccharides and capsular polysaccharides. The distinction between them is that when the bacteria are grown in a liquid culture and then centrifuged the extracellular polysaccharide that remains associated with the cell is known as capsule, whereas those that remain in the supernatant are known as exopolysaccharides. It is this latter that plays an important role in determining the architecture of the biofilm. In most of the cases it is difficult to distinguish the polysaccharides as they are not easily separable form the biofilm which makes it more complicated to precisely determine their chemical structures. The exopolysaccharides synthesized by microbial cells vary greatly in their composition and also in

Figure 1: Mechanism of formation of biofilm



their chemical and physical properties. Some are neutral macromolecules, but the majority of them are polyanionic due to the presence of either uronic acids (D-glucuronic acid being the most common, although D-galacturonic and D-mannuronic acids are also found) or ketal-linked pyruvate. Inorganic residues, such as phosphate or rarely sulphate, may also confer polyanionic status[12]. The composition and structure of the polysaccharides determine their primary conformation. Further, ordered secondary configuration frequently takes the form of aggregated helices[13]. In some of these polymers, the backbone composition of sequences of 1,4-ß- or 1,3-ß-linkages may confer considerable rigidity, as seen in the cellulosic backbone of xanthene from *Xanthomonas campestris* [12–13]. Thus, biofilms can be considered as a natural example for sustainable use of nutrients. Figure 2 shows a scanning electron micrograph (SEM) of a biofilm, EPS and microorganism (S. *auerus*).

There are several advantages for microorganisms to form biofilms. They provide enclosed surface space which is occupied and can provide a degree of stability in the growth environment. They might have catalytic functions through the localizing cells in close proximity. They can afford protection from various environmental challenges, such as metal toxicity[11], dehydration, salinity, UV[14], antibiotics and antimicrobial agents[15].

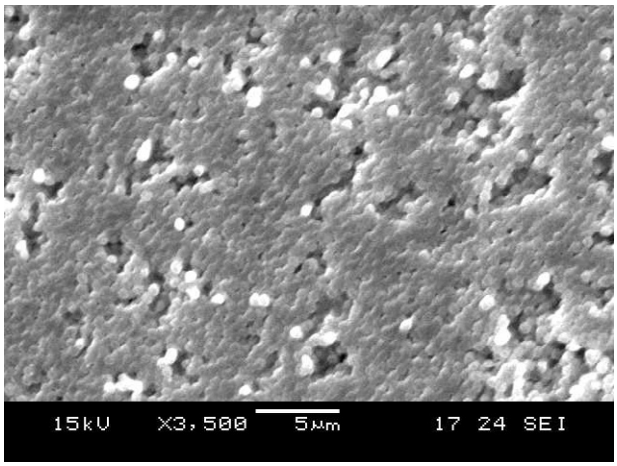
Properties of materials

The most important properties that enhance biofilm formation are surface area, the type of surface (rough or smooth), porosity, charge on the surface and surface hydrophobicity. Rough surface is more preferred by the microorganisms, because there will be stronger adhesion and crevices favour attachment. Hydrophobicity in case of polymeric materials enhances the biofilm formation. Hydrophobic organisms prefer hydrophobic surfaces. Organisms attach easily on porous surfaces. Electrostatic interactions contribute to biofilm cohesion and cations are significant cross linkers of the biofilm matrix. Divalent cations such as Mg^{2+} and Ca^{2+} can influence biofilm formation directly through their effect on electrostatic forces. Functional groups present on the surface of the material influences hydrophobicity and surface charge and this in turn affects the bacterial adhesion and its proliferation. There are several physical, chemical and biological techniques that can be adopted to modify the implant surface to decrease the attachment of the microorganism and make the material more biocompatible (figure 3). Each of these techniques has its own advantages and disadvantages and can be adopted depending on the site of implantation, its duration and other requirements.

Quorum sensing

Cell–cell communication in bacterial biofilms is performed through chemical signaling. Small, diffusible molecules belonging to the class of N-acylated homoserine lactones (AHLs) — are released by biofilm bacteria into their local environment, where they can interact with neighboring cells. These AHLs are known to associate with a cognate DNA binding protein. As the bacterial densities increase, accumulation of the AHLs to a threshold concentration occurs, and induces the transcription of specific gene throughout the

Figure 2: SEM of biofilm, EPS and microorganism



population. Regulation of this type are referred to as "quorum sensing", since it suggests the requirement for a 'quorate' population of bacterial cells that is necessary for the activation of AHL-responsive genes. This facilitates the coordination of bacterial behavior, and ensures that the bacteria respond as a group to carry out special and specific functions[16,17]. It is advantageous for the bacteria to act as a group. The role of quorum sensing has not yet been completely characterized and fully understood. Deeper study into this field can yield the development of new anti-infective chemotherapies which can prevent the initiation of the group behaviour of the microorganism.

Inhibition of biofilms by small molecules

It was previously the concern of industrial and environmental microbiologist in understanding the formation of biofilms and biofouling, but now there is a wide recognition of the contribution of biofilms to human infections. Research is underway to inhibit the formation of biofilm on medical devices. Some of the techniques which are followed to achieve this goal using small molecules are described in the subsequent sections (see figure 4).

Antibiotics and anti-microbial molecules

The MIC (minimum inhibitory concentration) has long been the standard for testing the susceptibility of microorganisms towards antibiotic. The MIC measures the actions of antibiotics against planktonic organisms and serves as an important reference in the treatment of many acute infections. Generally the bacterial infection is treated with many antibiotics such as gentamicin, carbenicillin, co-trimoxazole, tetracycline, and ceftizoxime. But the major problem in this case is the resistance of the biofilm towards the antibiotics. Reports show that *P. aeruginosa* which is found to be one of the most common causes for wound infection (about 73.9%) was 95% resistant to all the above mentioned antibiotics[18]. Some of the antibiotics like ampicillin, ceftiofur, cloxacillin, oxytetracycline, penicillin G, streptomycin, tetracycline, enrofloxacin, erythromycin, gentamicin, tilmicosin and trimethoprim-sulphadoxine are able to act on cultures like *Actinomyces pyogenes*, *Corynebacterium renale*, C. *pseudotuberculosis*, *Staphylococcus aureus*, *S. hyicus* and *Streptococcus agalactiae*. However biofilms formed by all these organisms were found to be resistant[19]. Chlorine as sodium hypochlorite is an oxidizing biocide (most effective antimicrobial agent). A 600 fold increase in its concentration is required to treat the biofilm formed by *Staphylococcus aureus* biofilm when compared to the planktonic cells of the same species[20].

The mechanisms for the resistance of biofilms to biocidal agents can be broadly classified into three types 1) adaptive phenotype, 2) restricted entry of the drug molecules, and 3) reduced metabolism and slow growth.

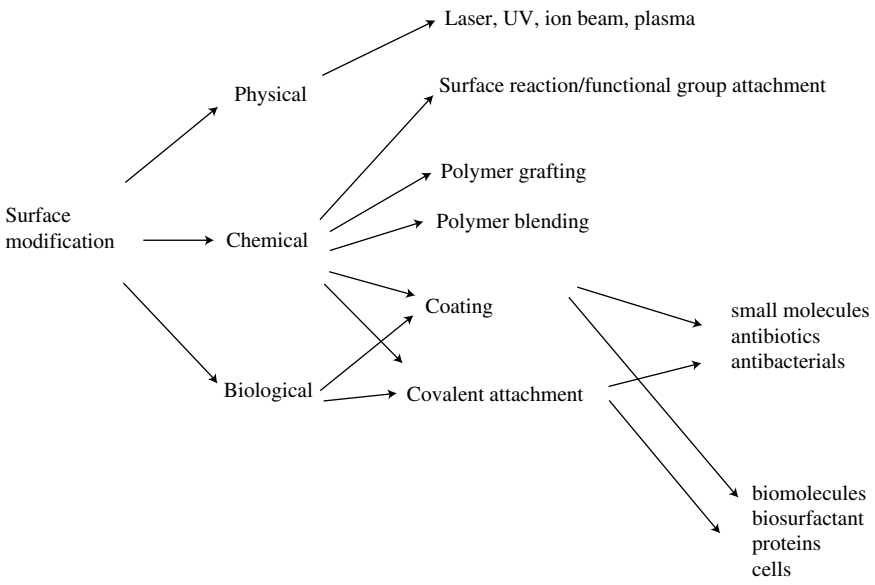
1. Adaptive phenotype

Persisters are small fraction of the entire population which have different phenotype or rather the most resistant cells in the biomass which makes it difficult for their complete elimination and hence the total removal of the biofilm, by the antibiotics or antimicrobial agents[21]. The population of persister cells has been estimated to reach about 0.1% to 10% of all cells in a biofilm[22]. When the antimicrobial therapy is discontinued, the unaffected persisters reverse the phenotype and become metabolically viable and active to regrow into a new biofilm. So the infection may reoccur at a later time and will exist for long time, or until the infected device is removed[23].

2. Restricted entry of drug molecules

The barrier properties of the EPS slime matrix contribute to the stability of the organisms to various treatments. The antimicrobial agent is adsorbed onto the EPS thus effectively diluting its concentration before it reaches to individual bacterial cells in the biofilm[24]. This can be one of the reasons for the need for increased input concentration. The substances in the EPS act as a diffusion barrier, either by limiting the rate of molecule transport to the interior of the biofilm or by chemically reacting with the molecules themselves[25]. The negatively charged EPS restricts penetration of the positively charged molecules of antibiotics, such as amino glycosides, by chemical interaction or molecular binding. If the antibiotic is inactivated or is attached ionically to the surface layers, its delivery to the depths of the biofilm can be profoundly retarded[26,27].

Figure 3: Surface modification trechniques



3. Reduced metabolism and slow growth

The cells deep within the biofilms have reduced metabolic activity and growth rates which make them inherently less susceptible to antibiotics[28,29]. The near complete consumption of oxygen and glucose in the surface layers creates anaerobic niches in the depths of the biofilm where in order to survive the cells down regulate into an extremely slow-growing or nongrowing state[30]. Therefore those antibiotics which readily diffuse through the biofilm are ineffective in killing those specific slow or nongrowing cells in the anaerobic regions of the biofilm. The susceptibility of the antibiotics also depends on the age of the biofilm. Older (10-day-old) biofilms are significantly more resistant than two-day-old biofilms[31]. This emphasizes the need for prompt diagnosis and treatment.

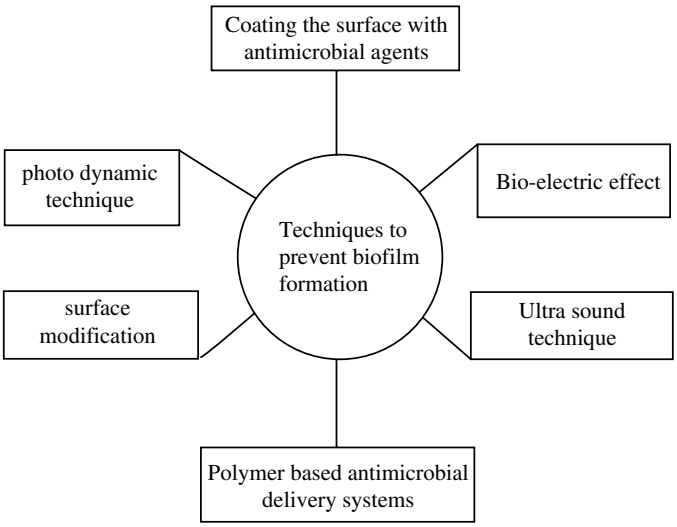
Although the relative contribution of the three mechanisms towards inhibition varies the final result is the development of resistance towards antimicrobial agents and antibiotics. Other angles of approaches to the problem are being looked into such as modifying the material properties of the medical devises. A few of these approaches that are being attempted are adsorption or coating of antimicrobials onto the surface, antimicrobial impregnated matrices or physico-chemical surface modification etc.

Coating with antimicrobial molecules

The tremendous resistance of biofilms to conventional antibiotic therapy has prompted a great deal of research on synthetic surfaces and coatings that resist bacterial colonization. The coatings include passive and active coatings. Coatings have been developed that reduce bacterial adhesion by altering the physicochemical properties of the substrate so that conditioning films do not form and bacteria–substrate interactions are not favorable. These coatings are referred to as "passive" and include surfaces modified with poly ethylene glycol[32] poly ethylene oxide[33] brushes, and hydrophilic polyurethane[34]. Unfortunately, the effectiveness of passive coatings for reducing bacterial adhesion is limited and varies greatly depending upon the bacterial species. The physicochemical properties of the surface (coating) can be masked by an adsorbed conditioning film, thereby diminishing their effectiveness.

Any surface such as polymer catheters or metal stents can be coated with antimicrobial organic molecules. This is known as "active" coating. The idea is to inhibit the bacterial adhesion at the first step itself. These "active" coatings have been designed to release high initial fluxes of antibacterial agents during the critical short-term post-implantation period (several hours) to inhibit the initial adhesion of the bacteria. This leads to a reversible and weak interaction between the microbe and the coated material[35]. It is reported that c-di-GMP treatment has an antimicrobial and antipathogenic activity *in vivo* and reduces, in a dose-dependent manner, colonization of biofilm of *S. aureus* strains in a mouse model of mastitis infection[36].

Figure 4: Techniques to prevent the formation of biofilm



However, given that many catheter-related infections are due to skin organisms acquired at the time of catheter insertion, then anti-colonization strategies are still worth exploring. For example, surfaces containing immobilized long-chain *N*-alklyated polyvinylpyridines and structurally unrelated *N*-alklyated polyethylenimines are lethal to *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*. The structure–activity analysis reveal that for these surfaces to be bactericidal the immobilized long polymeric chains have to be hydrophobic, but not excessively so, and positively charged[37]. In another case rifampin and amoxicillin have been adsorbed on polyurethane surfaces exhibiting acidic or basic properties. The binding affinity increased with the introduction of side-chain functional groups in the polymer and with matrix hydrophilicity. The zone of inhibition from amoxicillin-coated polymers lasted only a few hours, whereas that from the rifampin-coated polymer lasted for several months[38]. Emergence of multi-drug resistant pathogens is always a risk when antibiotics are used both in prophylaxis and long-term therapy as well as in conventional acute therapy. Hence, there is an interest in inhibiting biofilm formation on polymer surfaces using novel compounds that are not otherwise used. One such compound is usnic acid, a secondary lichen metabolite that possesses antimicrobial activity against a number of gram-positive bacteria, including *S. aureus*, *Enterococcus faecalis* and *E. faecium*. When loaded into modified polyurethane surfaces and placed in infection-simulating flow cell, confocal laser scanning microscopy indicated that whilst adhesion of *S. aureus* occurred, usnic acid inhibited the development of biofilm[39].

There are other organic molecules which are found to be potential lead compounds in inhibiting the biofilms and one such molecule is 3,4,5,3′,5′-pentabromo-2-(2′-hydroxybenzoyl) pyrrole[40]. Antimicrobial agents other than antibiotics have been investigated for the treatment of intraluminal biofilm infection. The use of taurolidine[41], ethanol[42], hydrochloric acid[43] and minocycline-EDTA[44](M-EDTA) has limited use and variable results. Out of the four, M-EDTA appears to be the most effective, but minocycline is no longer being produced. Another agent, tetrasodium EDTA, has been tested *in vitro* and *in vivo* with explanted infected hemodialysis catheters[42–44]. Within 24 hours of exposure, complete destruction of biofilm bacteria and yeast were observed. The ability to eradicate intraluminal biofilm within a few days would be quite advantageous over an extended and costly two week period. This agent is also a potent anticoagulant that could replace the use of heparin and eliminate the risk of heparin-induced thrombocytopenia.

There are various other effective physical techniques together with antibiotics or antibacterial being researched to inhibit the formation of the microbial biofilm. Some of them are discussed below.

Bio-electric effect

The advantage of this technique is, it prevents the biofilm formation and also enhances the activity of the antimicrobials against established biofilms. This technique refers to the simultaneous application of antibiotics and a weak electric field. It is found that when direct current electric fields between 1.5 and 20 V/cm is used, the concentrations of antibiotics needed to be effective against biofilm bacteria fall from approximately 5000 to 4 times greater than that necessary for planktonic bacteria in the absence of electricity[45].

Ultra sound technique

Ultrasound enhances antibiotic transport through the biofilm. It was found that low-frequency ultrasound (70 kHz) of low acoustic intensity increased the growth rate of *S. epidermidis*, *P. aeruginosa* and *E. coli* on polyethylene surfaces and it was hypothesized that ultrasound increases the transport of oxygen and nutrients to the cells[46].

Pulsed ultrasound over 24 h enhanced the activity of gentamicin against E. *coli* biofilms on polyethylene discs which were implanted subcutaneously into rabbits. The ultrasound was able to disrupt the biofilms without causing damage to the skin[47].

Table 2: Possible steps that could be inhibited by small molecule

Small molecules	Prevent
that alters the surface properties of the implant	Initial attachment and colonisation
that prevents quorum sensing	Group behaviour
that have higher activity towards 'persisters' or resistant cells	Complete killing of microorganism
slow releasing or long acting	
that prevent EPS formation	
that prevent escape of daughter cells	
that have high diffusion properties through the film barrier	
that silence virulent genes which help in biofilm formation	prevent biofilm

Photodynamic technique

The technique is found useful on pathogens associated with the skin and in the oral cavity and its application is limited to those regions where light can reach. Photosensitizing drugs produce reactive oxygen species which are difficult for the microorganisms to defend against leading to a breakdown of the biofilm. Similar findings have been made using multi-species biofilms of oral bacteria irradiated with light from a helium/neon laser in the presence of toluidine blue where greater than 95% of the biofilm bacteria were killed due to the laser[48]. Confocal microscopy revealed that a single photomechanical wave increased the penetration of methylene blue by 75% and enhanced the photo destruction of the biofilm[49].

Polymer-based antimicrobial delivery systems

Biodegradable nano or micro sized particle polyester, hydrogels, micelles and fibrous scaffolds act as effective drug carriers[50,51]. The antimicrobial molecule is encapsulated in the microspores of the polymer and the drug is released at the site of action in a controlled way. The difficulty in this technique is low encapsulation efficiency, low stability of the drug in the polymer matrix, and poor physico-chemical compatibility of the polymer and the drug. It is found that bio degradable poly (lactide) (PLA) and its co-polymers with glycoside (PLGA) can release encapsulated drug in a controlled way[52].

Future direction in biofilm research

Inhibiting the biofilm after its complete formation is difficult due to various factors which include poor understanding of the characteristics of the biofilm, its mode of growth especially its phenotype characteristics etc. Hence preventing its formation at early stages could be the most effective approach. The presence of 'persister' cells and different phenotypes for different bacterial population makes it more difficult for antibiotic treatment. Sensors which can detect the early formation of biofilm could prevent infections to a large extent and also warn the doctors about the onset of infection. The techniques such as coating the device with antimicrobial agents need to be more widely studied. Slow release antibiotics or antimicrobial agents using biodegradable polymers as carriers also have proved to be successful. Looking into the genomics and proteomics of the bacteria in the biofilm to understand the physiology can yield new targets, which would initiate the development of specific drugs for their inhibition. Computational techniques which can predict a priori the interaction of an implant with the environment which includes bacteria, proteins, blood components and other floating material can help in designing new biomaterials. Identifying the virulent factors and genes which lead to the colonization and biofilm formation could help in silencing those to prevent biofilms. Role of quorum sensing could lead in developing techniques that could prevent colonization and biofilm formation.

Designing small molecules that can inhibit/prevent a specific step in the entire process of biofilm formation and its growth leading to the infection could be the best approach. Table 2 lists the possible steps that could be inhibited.

Conclusions

Biofilm bacteria are 150 to 3,000 fold stronger than regular bacteria. It is resistant to disinfectants and antibiotics, making it difficult to remove and control. Biofilm-associated infections extend stay in hosptial by about three days and it is estimated that up to 65% of hospital acquired infections are due to this and the treatment runs to $1 billion per year (http://caramola.usc.edu). Up to 82% of bacterial contamination in hospitals is due to intravascular catheterizations. *Pseudomonas aeruginosa* (gram-negative bacterium that is known to cause infections in the lungs) was found to form biofilm under microgravity conditions in American space shuttle during flight. Several small molecules

and antibiotics have been tested to prevent the formation of biofilms or break a well formed one. Physical techniques such as ultra sound, current etc are found to enhance the activity of antibiotics or small molecules coated on the surface of the implant.

Received 02 February 2008; revised 28 March 2008.

References

1. N. Graves, "Economics and preventing hospital-acquired infection", *Emerg. Infect. Dis*. 2004, 10, pp 561–566.
2. R. Munch, R. Koch, *Microbes Infect*. 2003, 5, pp 69–74.
3. J. W. Costerton, P. S. Stewart, E. P. Greenberg, "Bacterial biofilms: a common cause of persistent infections", *Science* 1999, 284, pp 1318–1322.
4. National Institute of Dental Research. The Oral Health of United States Adults. The National Survey of Dental Caries in US Employed Adults and Seniors, 1985–1986, DHHS Pub. No. (NIH) 87–2868. (US Department of Health and Human Services, Bethesda, Maryland 1987).
5. National Institute of Dental Research. Oral health of United States children. The National Survey of Dental Caries in US School Children, 1986–1987. DHHS Pub. No. (NIH) 89–2247. (Department of Health and Human Services, Bethesda, Maryland 1989).
6. I. J. Pople, R. Bayston, R. D. Hayward, "Infection of cerebrospinal fluid shunts in infants: a study of etiological factors", *J. Neurosurg.* 1992, 77, pp 29–36.
7. M. J. Richards, J. R. Edwards, D. H. Culver, R. P. Gaynes, "Nosocomial infections in medical intensive care units in the United States", *National Nosocomial Infections Surveillance System. Crit. Care Med.* 1999, 27, pp 887–892.
8. J. W. Costerton, "Bacterial biofilms in nature and disease", *Annu. Rev. Microbiol. 1987,* 41, pp 435–464.
9. D. deBeer, P. Stoodley, F. Roe, Z. Lewandowski, "Effects of biofilm structures on oxygen distribution and mass transport", *Biotechnol. Bioeng. 1994,* 43, pp 1131–1138.
10. P. Stoodley, D. de Beer, Z. Lewandowski, "Liquid flow in biofilm systems", *Appl. Environ. Microbiol. 1994,* 60, pp 2711–2716.
11. K. Sauer, A. K. Camper, G. D. Ehrlich, J. W. Costerton, D. G. Davies, "*Pseudomonas aeruginosa* displays multiple phenotypes during development as a Biofilm", *J. Bacterio. 2002,* 184, pp 1140–1154.
12. I. W. Sutherland, "Biotechnology of Exopolysaccharides" Cambridge: *Cambridge University Press*, 1990.
13. I. Sutherland, "Biofilm exopolysaccharides: a strong and sticky framework", *Microbiology 2001,* 147(Pt 1), pp 3–9.
14. P. Gilbert, D. G. Allison, A. J. McBain, "Biofilms *in vitro* and *in vivo*: do singular mechanisms imply cross-resistance?" *J. Appl. Microbiol. 2002,* 92, S98–S110.
15. J. L. Hall-Stoodley, W. Costerton, P. Stoodley, "Bacterial biofilms: from the Natural environment to infectious diseases", *Nature Reviews Microbiology* 2004, 2 (2), pp 95–108.
16. A. Latifi, M. Foglino, K. Tanaka, P. Williams, A. Lazdunski, "A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhlR (VsmR) to expression of the stationary-phase sigma factor RpoS", *Mol. Microbiol. 1996,* 21, pp 1137–1146.
17. W. C. Fuqua, S. C. Winans, E. P. Greenberg, "Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators", *J. Bacteriol*. 1994, 176, pp 269–275.
18. L. A. Rastegar, H. H. Bahrami, R. Alaghebandan, "*Pseudomonas* infections in Tohid Burn Center", *Iran. Burns 1998,* 24, pp 637–641.
19. M. E. Olson, H. Ceri, D. W. Morck, A. G. Buret, R. R. Read, "Biofilm bacteria: formation and comparative susceptibility to antibiotics", *Can. J. Vet. Res.* 2002, 66, pp 86–92.
20. S. B. Luppens, M. W. Reij, R. W. van der Heijden, F. M. Rombouts, T. Abee, "Development of a standard test to assess the resistance of *Staphylococcus aureus* biofilm cells to disinfectants", *Appl. Environ. Microbiol.* 2002, 68, pp 4194–4200.
21. A. L. Spoering, K. Lewis, "Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials", *J. Bacteriol*. 2001, 183, pp 6746–6751.
22. M. E. Roberts, P. S. Stewart, "Modeling protection from antimicrobial agents in biofilms through the formation of persister cells", *Microbiology* 2005, 151, pp 75–80
23. P. S. Stewart, J. W. Costerton, "Antibiotic resistance of bacteria in biofilms". *Lancet* 2001, 358, pp 135–138.
24. G. H. Dibdin, S. J. Assinder, W. W. Nichols, P. A. Lambert, "Mathematical model of β-lactam penetration into a biofilm of *Pseudomonas aeruginosa* while undergoing simultaneous inactivation by released β-lactamases", *J. Antimicrob. Chemo. 1996,* 38, pp757–769.
25. R. M. Donlan, J. W. Costerton, "Biofilms: survival mechanisms of clinically relevant microorganisms", *Clin. Microbiol. Rev.* 2002, 15, pp 167–193.
26. K. Lewis, "Riddle of biofilm resistance" *Antimicrob. Agents Chemother.* 2001, 45, pp 999–1007.
27. P. S. Stewart "Mechanisms of antibiotic resistance in bacterial biofilms" *Int. J. Med. Microbiol.* 2002, 292, pp 107–113.
28. H. Anwar, J. L. Strap, J. W. Costerton, "Establishment of aging biofilms: possible mechanism of bacterial resistance to antimicrobial therapy", *Antimicrob. Agents Chemo. 1992,* 36, pp 1347–1351.
29. T. F. Mah, G. A. O'Toole, "Mechanisms of biofilm resistance to antimicrobial agents", *Trends Microbiol.* 2001, 9, pp 34–39.
30. J. G. Leid, M. E. Shirtliff, J. W. Costerton, A. P. Stoodley, "Human leukocytes adhere to, penetrate, and respond to Staphylococcus aureus biofilms", *Infect. Immun.* 2002, 70, pp 6339–6345.
31. R. M. Donlan, J. W. Costerton, "Biofilms: survival mechanisms of clinically relevant microorganisms" *Clin. Microbiol. Rev.* 2002, 15, pp 167–193.
32. P. Kingshott, J. Wei, D. Bagge-Ravn, N. Gadegaard, L. Gram, *Langmuir* 2003, 19, pp 6912–6915.
33. H. J. Kaper, H. J. Busscher, W. Norde, *J. Biomater. Sci., Polym. Ed.* 2003, 14, pp 313–318.
34. J. A. Nagel, R. B. Dickinson, S. L. Cooper, *J. Biomater. Sci., Polym. Ed.* 1996, 7, pp 769–774.
35. Y. H. An, R. B. Dickinson, R. J. Doyle, "Mechanisms of bacterial adhesion and pathogenesis of implant and tissue infections", Y. H. An, R. J. Friedman, Eds, *Handbook of Bacterial Adhesion: Principles, Methods, and Applications*, Humana Press Inc., Totowa, NJ (2000), pp 1–27.
36. E. Brouillette, M. Hyodo, H. Mamoru, K. Yoshihiro, K. R. David, F. Malouin, "3',5'-cyclic diguanylic acid reduces the virulence of biofilm-forming *Staphylococcus aureus* strains in a mouse model of mastitis infection", *Antimicrobial Agents and Chemotherapy* 2005, 49 (8), pp 3109–3113.
37. J. S. Lin, K. L. Qui, A. M. Klibanov, "Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines", *Biotechnol. Prog.* 2002, 18, pp 1082–1086.
38. A. Piozzi, I. Francolini, L. Occhiaperti, M. Venditti, W. Marconi, "Antimicrobial activity of polyurethanes coated with antibiotics: a new approach to the realization of medical devices exempt from microbial colonization", *Int. J. Pharm.* 2004, 280, pp 173–183.
39. I. Francolini, P. Norris, A. Piozzi, G. Donelli, P. Stoodley, "Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces", *Antimicrob. Agents Chemo.* 2004, 48, pp 4360–4365.
40. D. Schillaci, S. Petruso, V. Sciortino, "3,4,5,3′,5′-Pentabromo-2-(2′-hydroxybenzoyl)pyrrole: a potential lead compound as anti-Gram-positive and anti-biofilm agent", *International Journal of Antimicrobial Agents* 2005, 25(4), pp 338–340

41. M. Koldehoff, J. L. Zakrzewski, "Taurolidine is effective in the treatment of central venous catheter-related bloodstream infections in cancer patients", *Int J Antimicrob Agents* 2004, 24, pp 491–495.
42. C. Dannenberg, U. Bierbach, A. Rothe, J. Beer, D. Korholz, "Ethanol-lock technique in the treatment of bloodstream infections in pediatric oncology patients with broviac catheter", *J Pediatr Hematol Oncol.* 2003, 25, pp 616–621.
43. D. Barbaric, J. Curtin, L. Pearson, P. J. Shaw, "Role of hydrochloric acid in the treatment of central venous catheter infections in children with cancer", *Cancer* 2004, 101, pp 1866–1872.
44. I. Chatzinikolaou, T.. F. Zipf, Hanna H., "Minocycline-ethylenediaminetetraacetate lock solution for the prevention of implantable port infections in children with cancer", *Clin. Infect. Dis.* 2003, 36, pp 116–119
45. W. Costerton, B. Ellis, K. Lam, F. Johnson, A. E. Khoury, "Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria", *Antimicrob. Agents Chemo.* 1994, 38, pp 2803–2809.
46. W. G. Pitt, S. A. Ross, "Ultrasound increases the rate of bacterial growth", *Biotechnol. Prog.* 2003, 19, pp 1038–1044.
47. A. M. Rediske, B. L. Roeder, J. L. Nelson, R. L. Robison, G. B. Schaalje, R. A. Robison, "Pulsed ultrasound enhances the killing of *Escherichia coli* biofilms by aminoglycoside antibiotics in vivo", *Antimicrob. Agents Chemo.* 2000, 44, pp 771–772.
48. J. F. O'Neill, C. F. Hope, M. Wilson, "Oral bacteria in multi-species biofilms can be killed by red light in the presence of toluidine blue", *Lasers Surg. Med.* 2002, 31, pp 86–90.
49. N. S. Soukos, S. S. Socransky, S. E. Mulholland, S. Lee, A. G. Doukas, "Photomechanical drug delivery into bacterial biofilms", *Pharm. Res.* 2000, 17, pp 405–409
50. N. K. Varde, D. W. Pack, "Microspheres for controlled drug delivery", *Expert Opin. Biol. Ther.* 2004, 4, pp 35–51.
51. M. A. Moses, H. Brem, R. Langer, "Advancing the field of drug delivery: taking aim at cancer", *Cancer Cell* 2003, 4, pp 337–341.
52. S. Freiberg S, X. X. Zhu, "Polymer microspheres for controlled drug release", *Int. J. Pharm.* 2004, 282, pp 1–18.

Mukesh Doble is a Professor in the Department of Biotechnology at IIT Madras, Chennai, 600036, India (mukeshd@iitm.ac.in). He has experience in Bioreactors, Molecular modelling, Drug design, Bioremediation/Biodegradation, Homogeneous, Heterogeneous and Enzyme Catalysis, Scale-up, Process Development and Statistical Process control and Six-Sigma.

He holds B. Tech and M. Tech degrees in Chemical Engineering from IIT, Madras and a Ph. D. from University of Aston in Birmingham, UK and has carried out postdoctoral research at University of Cambridge, U.K., and Texas A&M, U.S.A.

He has previously worked in the ICI and GE Technology research centres for 20 years in the areas of Pharmaceuticals, Rubber Chemicals, Polymers, Explosives, Specialty Chemicals and Paints.

He has authored or coauthored five books, 120 technical papers in International journals, filed 3 patents and presented in more than 50 National and International conferences. He is a recipient of Herdillia Award for "Excellence in Basic Research" from Indian Institute of Chemical Engineers. He is in the Editorial Boards of Chemical Engineering and The Open Petroleum Engineering journal and a member of American and Indian Institute of Chemical engineers.

Prasanna S S. has completed his M.Sc (Chemistry) from Sri Sathya Sai University. He has qualified the CSIR-JRF 2007. He has also secured the all India 85^{th} rank in the GATE exam 2007. He worked on "Isolation and structural studies on anti-fungal compound in *ACACIA NILOTICA* flowers" as part of his M.Sc project (2007) . He also did a project from NUS (National university of Singapore), worked on Bismuth Nano crystals in the Chemistry department from April–May 2006. He is currently working with professor Mukesh Doble I I T Madras (Indian Institute of Technology, Madras), on the topic of "Prevention of biofilm formation using organic molecules".