**MEDICAL DEVICE TECHNOLOGIES: POTENTIAL TO TREAT AND PREVENT BIOFILM RELATED INFECTIONS**

**Pradip V. Hirapure\*, Arti S. Shanware\*\*, Sampada Pendse\*\*\*, Sakshi Dhote\*\*\***

\*,\*\* **Rajiv** Gandhi Biotechnology Centre, LIT Campus, Rashtrasant Tukadoji Maharaj Nagpur, University, Nagpur, India.

\*\*\*,\*\*\*\*Department of Biochemistry and Biotechnology, Dr. Ambedkar College, Deekshabhoomi, Nagpur

Corresponding author E.mail: pradiphirapure@gmail.com

**Abstract:**

Biofilms are of great importance in infection control and healthcare-associated infections owing to their inherent tolerance and ‘resistance’ to antimicrobial therapies. Biofilms have been shown to develop on medical device surfaces, and dispersal of single and clustered cells implies a significant risk of microbial dissemination within the host and increased risk of infection and therefore pose a serious public health problem. Microbial biofilms develop on or within indwelling medical devices like contact lenses, central venous catheters and needleless connectors, endotracheal tubes, intrauterine devices, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, tympanostomy tubes, urinary catheters, and voice prostheses. Colonization of medical devices plays a key role in the problem of healthcare-associated infections. This article aims to provide an overview of the science of biofilms, the risks associated with them, the potentially serious outcomes of infections, current and new advanced technologies in terms of addressing the biofilm problem for the improvement of healthcare system.

**Keywords:**  Biofilm, Medical Device, Microbial Infection, Healthcare, Biomedical Technology

**Introduction:** A structured consortium attached on a living or inert surface formed by microbial cells sticked to each other and surrounded by the selfproduced extracellular polymeric matrix is known as biofilm. The formation of biofilm is considered an adaptation of microbes to hostile environments.[**1–2** ] Experimental evidences of P. aeruginosa in vitro and in vivo demonstrated clearly that biofilm bacterial cells are significantly more resistant to antibiotics and host immune defense than their planktonic counterparts.[3–7] Aggressive and intensive antibiotic treatment is usually helpful to control the exacerbations of chronic biofilm infections induced by dispersed bacteria and reduce the biofilms, but can not eradicate the biofilm infections,[7–8] because the minimal concentration of antibiotic for eradication of mature biofilm is difficult to reach in vivo. [5 ] Therefore, once a bacterial biofilm infection established, it becomes difficult to eradicate. Bacterial biofilm formation is widely found in natural environments with water, and also in human diseases, especially in the patients with indwelling devices for the purpose of medical treatments.[2,7] With the progress of medical sciences, more and more medical devices and/or artificial organs are applied in the treatment of human diseases. However, as a consequence, bacterial biofilm infections become also frequent. It has been reported that vast majority, if not all, of the medical devices or prostheses may result in biofilm infections, catheters,[9] vascular prosthesis,[10] cerebrospinal fluid shunts,[11] prosthetic heart valves,[12] urinary catheters,[12] joint prostheses and orthopedic fixation devices,[13] cardiac pacemakers,[14] peritoneal dialysis catheters,[15] intrauterine devices, biliary tract stents, dentures, breast implants, contact lenses and in the dental area caries and periodontitis, and so on. It has been estimated that most bacterial infections in human are correlated with biofilm and about 50% of the nosocomial infections are indwelling devices-associated Bacterial biofilms are characterized as highly resistant to antibiotic treatment and immune responses.[7] Although it is well known that antibiotic treatment is currently most important and effective measure for the control of microbial infections, however, antibiotic treatments are almost impossible to eradicate biofilm infections. In vitro and in vivo experiments demonstrated that the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for biofilm bacterial cells were usually much higher (approximately 10–1000 times) than the planktonic bacterial cells.[4–6] The effective antibiotic MBC in vivo for biofilm eradication are therefore impossible to reach by conventional antibiotic administrations due to the toxicities and the side effects of antibiotics and the limitation of renal and hepatic functions. Treatment of biofilm infections becomes therefore challenging and attracts significantly scientific attention. this review would focus mainly on the overview of biofilm . Medical device related infections, current treatment of bacterial biofilm infections, future new advance technologies to combat medical device Biofilm.

**Mechanism of Biofilm formation**

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.[6-8] 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.[2-5] 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. 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 in table 1.

**Table 1 factors which affect biofilm formation**

|  |  |
| --- | --- |
| 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 |

**Table 2 List of medical implants prone to biofilm formation with the causative agent.**

|  |  |
| --- | --- |
| Medical device | Bacteria |
| Dental implants | Staphylococcus aureus, Candida albicans, Streptococcus |
| Intra-urine devices | S. epidermidis, K. pneumoniae, Enterococcus, Proteus mirabilis, P. aeruginosa, E. coli and other gramnegative bacteria |
| Artificial hip prosthesis | S. aureus, S. epidermidis, P. aeruginosa, E. coli, Neisseria gonorrhoeae, Candida albicans and Candida dubliniesis |
| 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 |

**Table 3 Biological and chemical approaches for biofilm infection treatment in medical devices.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Technologies** | **Discription** | **Antibiofilm agents** | **Refrences** |
| Bacteriophage Therapy | Lytic phages utilized which results in rapid destruction of the bacterial cell, therapy is host specific and bactericidal | E.coli T4 phage, coliproteus 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, Nacetylcysteine | 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, tetrasodiumEDTA, 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 |

**Table 4 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 help to inhibit the biofilm |
| 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 to inhibit the biofilm formation. |
| 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 |

**New technologies to prevent biofilm formation in medical devices**

Removal of cells from the biofilm colony is an essential stage of the biofilm life cycle because it enables biofilms to spread and colonize new surfaces. Strategies to plan against bacterial biofilm must be achieved by prevention of biofilm formation rather than dispersal of the formed biofilm. Strategies for prevention of biofilm formation include both “Chemical” and “Mechanical” methods.

**Chemical methods:**

**1. Antimicrobial coatings:** Chemical modifications are the main strategy for biofilm prevention. Antibiotics, biocides, and ion coatings are commonly used chemical methods of biofilm prevention. These methods prevent biofilm formation by interfering with the attachment and expansion of immature biofilms [16].Several *in vitro* studies have confirmed the effectiveness of silver at preventing infection, both in coating form and as nanoparticles dispersed in a polymer matrix. However, application of silver in the *in vivo* system is associated with warnings due to the toxic effect of silver on human tissue therefore a need to discover new antimicrobial molecules to inhibit the biofilm.

**2.Polymer modifications:** Antimicrobial agents can be immobilized on device surfaces using long, flexible polymeric chains. These chains are anchored to the device surface by covalent bonds, producing non-leaching, contact-killing surfaces. One *in vitro* study found that when N-alkylpyridinium bromide, an antimicrobial agent, was attached to a poly(4-vinyl-N-hexylpyridine), the polymer was capable of inactivating ≥ 99% of *S. epidermidis, E. coli,* and *P. aeruginosa* bacteria [17]. Dispersion forces between the polymer chains and the bacterial cells prevent bacteria from binding to the surface and initiating biofilm growth. The concept is similar to that of steric stabilization of colloids. Polymer chains are grafting to a surface via covalent bonding or adsorption.

**Mechanical methods:**

**1. Hydrophobicity, Surface roughness, Surface charge:** Formation of a biofilm begins with the attachment of free-floating cells to a surface. These first colonists adhere to the surface initially through weak, reversible adhesion. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili . Hydrophobicity also plays an important role in determining the ability of bacteria to form biofilms. Some species are not able to attach to a surface and are sometimes able to establish themselves directly to earlier colonists [17]. On other hand, some bacteria are unable to successfully form biofilms due to their limited motility. Nonmotile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria. Modification of the surface charge of polymers has also proven to be an effective means of biofilm prevention. Based on the principles of electrostatics charged particles will repel other particles of like charge. The hydrophobicity and the charge of polymeric chains can be controlled by using several backbone compounds and antimicrobial agents. Positively-charged polycationic chains enable the molecule to stretch out and generate bactericidal activity [17]. Surface roughness can also affect biofilm adhesion. Rough, high-energy surfaces are more conducive to biofilm formation and maturation, while smooth surfaces are less susceptible to biofilm adhesion. The roughness of a surface can affect the hydrophobicity or hydrophilicity of the contacting substance, which in turn affects its ability to adhere [18]. It is, thus, desirable to maintain a smooth surface on any products that may come in contact with bacteria [18]

**Strategies for Biofilm Dispersal**

Strategies for more effective biofilm dissolution treatments become fundamental. Mechanisms to understand the role of biofilms in chronic infections and antimicrobial resistance are important when design for new drug treatments [18]. Conventional antibiotics work by either preventing bacterial cell division (bacteriostatic) or killing the cell (bactericidal). Although over the years antibiotics have proven critical in eliminating bacterial pathogens, evidences indicate that they extensively damage the host microbiota, creating an environment where opportunistic pathogens can prevail [3]. Most recent advances in strategies are designed to prevent biofilm formation by killing the bacteria or targeting different biofilm developmental stages [18]. Some strategies and mechanisms for biofilm inhibition are discussed below.

**1. Bacterial Antibiofilm Polysaccharides**

Polysaccharides, as sugar polymers, have the capacity to act as lectin inhibitors. Lectins are proteins that specifically recognize and bind sugars without modifying these molecules. In bacteria, the primary function of lectins is to facilitate attachment or adherence of bacteria to host cells. These proteins play an important role in biofilm formation, and are essential for bacterial colonization and infection. Lectins are mainly located on the surface of bacteria cells where they can access and bind to the glycan substrates present on the surface of host cell. By competing for the sugar binding domain of lectins, polysaccharides can inhibit lectin-dependent adhesion of pathogens and biofilm formation. In fact, several plant, microbial and milk polysaccharides have been shown to block various lectins from human pathogenic bacteria by competitive inhibition [19]. Polysaccharides mediate cell-to-surface and cell-to-cell interactions that are critical for biofilm formation and stabilization. Recent evidence indicates that some bacterial exopolysaccharides inhibit or destabilize biofilm formation by other species [19]. Antibiofilm properties of polysaccharides are believed to lie on their ability to: a) alter the physical characteristics of bacterial cells or abiotic surfaces. b) act as signaling molecules that impact the gene expression patterns of susceptible bacteria. or c) competitively inhibit multivalent carbohydrate–protein interactions, thereby interfering with adhesion.

**2. Anti-biofilm enzymes**

Enzymes that degrade biofilm extracellular matrix may play a role in biofilm dispersal and may be useful as anti-biofilm agents. N-acetyl-D-glucosamine-1-phosphate acetyl transferase is an essential peptidoglycan and lipopolysaccharide precursor in Gram-positive and Gram-negative pathogens, respectively, is among the enzymes targeted for matrix disruption [18]. Treatment with such enzymes prevented *Staphylococcus* and *Enterococcus* biofilm formation and disperse preformed biofilms *in vitro* [18]. For example, Dispersin-B is a glycoside hydrolase that cleaves β 1–6 N-acetylglucosamine polymers in the bacterial peptidoglycan layer. Dispersin-B treatment has been shown to be effective against *S. aureus* and *S. epidermidis* biofilms and bacteria [19].

**3. Chelating Agents**

Metal cations, such as calcium, magnesium, and iron have been implicated in maintaining matrix integrity. Consistent with this observation, chelating agents have been shown to destabilize biofilm architecture besides interfering with bacterial membrane stability. For example, sodium citrate inhibited biofilm formation by several *Staphylococci* species *in vitro* [21]. In addition, tetrasodium-EDTA eradicated biofilms in an *in vitro* biofilm model and on explanted hemodialysis catheters, whereas disodium-EDTA, in combination with tigecyclin or gentamicin, reduced biofilm formation by *Staphylococcus* species and *P. aeruginosa.*

**4. Antimicrobial peptides**

Antimicrobial peptides are produced by the innate immune response system and have been proposed as attractive candidates for the development of novel types of antibiotics. However, their activity spectrum and mechanism of action need to be more precisely defined before they can be considered as possible therapeutic strategies [22]. A recent work, focused on reduced biofilm formation by multidrug-resistant *P. aeruginosa* strains isolated from patients with cystic fibrosis, revealed that the bacterium was killed within preformed biofilms. Lytic peptides are another group of antimicrobial peptides assessed for their inhibitory effects on biofilm formation. Lytic peptides bind the lipopolysaccharide moieties of the bacterial cell membrane, disrupting membrane stability [22].

**5.Anti-adhesion Agents**

Attachment constitutes the first step in virtually all types of biofilm formation, thus numerous studies have focused on preventing bacterial adherence. Efforts have been made to inhibit assembly of different types of pili, through the use of pilicides, which are compounds rationally designed to interfere with export of the corresponding pilin subunits. Pilicides were shown to inhibit biofilm formation *in vitro* by 50%, at concentrations as low as 3 μM [23]. Similar compounds have been shown to be effective against curli (curlicides), inhibiting *in vitro* curli biogenesis and biofilm formation [24]

**6. Nanotechnology**

These techniques include the modification of surface topographical features on the nanoscale (i.e. nano topography) as well as the functionalization of the surfaces with eluting antibacterial agents, anti-adhesive polymers or immobilized bactericidal moieties. Medical device surface modified to resist bacteria adhesion and formation of biofilm, and/or kill the bacteria during their initial attachment to the surface. This technique is based on the attractive electrostatic force between a charged surface and an oppositely charged polyelectrolyte and the subsequent build up of oppositely charged polyelectrolytes into a multilayer, typically with a film thickness which ranges from tens to hundreds of nanometers[24]. It is a versatile and efficient, yet facile, technique, and a wide range of materials including natural polymers, peptide and nanoparticles can be incorporated into the layered films to inhibit adhesion and growth of of bacterial biofilm on medical devices and implants.

#### 7.Disruption of bacterial amyloids to control bacterial biofilms

Many bacteria can constitute functional amyloid fibers on their cell surface. The majority of bacterial amyloids contribute to the development of biofilm as well as other community behaviors. Curli are functional extracellular amyloid fibers produced by Escherichia coli and other Enterobacteriaceae. Two analogs of FN075 and BibC6 of ring-fused 2-pyridones, the peptidomimetics that target essential protein–protein interactions in macromolecular assembly, inhibited curli biogenesis in E. coli. [25] The ability of FN075 to block the biogenesis of both curli and type 1 pili composes unique anti-biofilm and anti-virulence activities on these compounds

**8.Modification of c-di-GMP as target to disperse biofilm infections**

C-di-GMP was discovered 25 years ago, and has been emerged as one of the most common and important bacterial second messengers. C-di-GMP has been shown to play key roles in lifestyle changes of many bacteria, for example, transforming from the motile to the sessile state to establish multicellular biofilm communities, and change from the virulent state of acute infections to the less virulent but chronic infections. Therefore, modulating c-di-GMP signalling pathways in bacteria could offer a new way to manage the formation and dispersal of biofilms in clinic situations**.[26-30]**

**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 blood stream infection (CRBSI). Different factors involved and the antibiotic resistance is the main cause that still an ideal method for the eradication of medical devices associated biofilm has not been developed yet. Currently, antibiotics available work against planktonic cells but not on biofilms. We can control and prevent the medical devices associated infection by inhibiting the biofilm development using above discussed various antibiofilm technologies. In future there is need of further research to understand the interaction of biofilm resistance medical devices and biofim producing bacteria, stability and specificity and sensitivity of these new medical devices in human body and also to understand the advantages and disadvantages of new antibiofilm technologies.

**References**

1. De Fuente-Nu´ n ez C, Reffuveille F, Fernandez L et al. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol 2013; 16(5): 580–589
2. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2(2): 95–108.
3. Yang L, Liu Y, Wu H et al. Combating biofilms. FEMS Immunol Med Microbiol 2012; 65(2): 146–157.
4. Høiby N, Ciofu O, Johansen HKet al. The clinical impact of bacterial biofilms.Int J Oral Sci 2011; 3(2): 55–65.
5. Hengzhuang W, Wu H, Ciofu O et al. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and nonmucoid Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother 2011; 55(9): 4469–4474.
6. Hengzhuang W, Wu H, Ciofu O et al. In vivo pharmacokinetics/pharmacodynamics of colistin and imipenem in Pseudomonas aeruginosa biofilm infection. Antimicrob Agents Chemother 2012; 56(5): 2683–2690.
7. Høiby N, Bjarnsholt T, Givskov M et al. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010; 35(4): 322–332. 8 Høiby N. Recent advances in the treatment of Pseudomonas aeruginosa infections in cystic fibrosis. BMC Med 2011; 9: 32
8. Tomas B, Oana C, Molin S, Michael G, Niels H (2013) Applying insights from biofilm biology to drug development -can a new approach be developed? Nature Reviews Drug Delivery 12: 791-808.
9. Fletcher M, Loeb GI. Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. Appl Environ Microbiol 1979; 37:67–72.
10. Pringle JH, Fletcher M. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. Appl Environ Microbiol 1983; 45:811–7.
11. Characklis WG, McFeters GA, Marshall KC. Physiological ecology in biofilm systems. In: Characklis WG, Marshall KC, eds. Biofilms. New York: John Wiley and Sons, 1990:341–94.
12. Quirynen M, Brecx M, van Steenberghe D. Biofilms in the oral cavity: impact of surface characteristics. In: Evans LV, ed. Biofilms: recent advances in their study and control. Amsterdam: Harwood Academic Publishers, 2000:167–87.
13. Korber DR, Lawrence JR, Sutton B, et al. Effect of laminar flow velocity on the kinetics of surface recolonization by Mot and Mot Pseudomonas fluorescens. Microb Ecol 1989; 18:1–19.
14. Rosenberg M, Bayer EA, Delarea J, et al. Role of thin fimbriae in adherence and growth of Acinetobacter calcoaceticus RAG-1 on hexadecane. Appl Environ Microbiol 1982; 44:929–37.
15. Christensen GD, Baldassarri L, Simpson WA. Colonization of medical devices by coagulase-negative staphylococci. In: Bisno AL, Waldvogel FA, eds. Infections associated with indwelling medical devices, 2nd ed. Washington, DC: American Society for Microbiology, 1994:45–78.
16. Murga R, Forster S, Brown E, Pruckler J, Fields B, et al. Role of biofilms in the survival of Legionella pneumophila in a model potable-water system. Microbiology , 2001,147: 3121–6.
17. Jansen B, Kohnen W, Prevention of biofilm formation by polymer modification. J Ind Microbiol,1995 15: 391-6.
18. Meiron T, Saguy I , Adhesion Modeling on Rough Low Linear Density Polyethylene. J Food Sci,2007;72: E485–91.
19. Maria K, Maria H, Scott J , Bacterial Biofilms: Development, Dispersal, and Therapeutic Strategies in the Dawn of the Postantibiotic Era. Cold Spring Harbor Laboratory Press.2014
20. Kaplan JB Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. J Dent Res,2010; 89: 205–18.
21. Shanks R, Sargent J, Martinez R, Graber M, O’Toole G, Catheter lock solutions influence staphylococcal biofilm formation on abiotic surfaces. Nephrol Dial Transpl,2006; 21: 2247–55.
22. Kharidia R, Liang J ,The activity of a small lytic peptide PTP-7 on Staphylococcus aureus biofilms. J Microbiol,2011 49: 663–8.
23. Berg V, Das P, Chorell E, Hedenstrom M, Pinkner JS, et al. Carboxylic acid isosteres improve the activity of ring-fused 2-pyridones that inhibit pilus biogenesis in E. coli. Bioorg Med Chem Lett, 2008;18: 3536–40.
24. Cegelski L, Pinkner J, Hammer N, Cusumano C, Hung C, et al.Small-molecule inhibitors target Escherichia coli amyloid biogenesis and biofilm formation. Nat Chem Biol,2009; 5: 913–9.
25. K.G. Neoh, R. Wang, E.T. Kang [Surface nanoengineering for combating biomaterials infections](http://www.sciencedirect.com/science/article/pii/B9780857095978500074) Biomaterials and Medical Device - Associated Infections, 2015, Pages 133-161
26. Romling U, Galperin MY, Gomelsky M. Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. Microbiol Mol Biol Rev 2013; 77(1): 1–52. ]