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

**PradipV.Hirapure\*,ArtiS. Shanware\*\*, Sampada Pendse, Sakshi Dhote**

\*,\*\* **Rajiv** Gandhi Biotechnology Centre, LIT Campus, RashtrasantTukadojiMaharaj Nagpur, University, Nagpur, India.

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

Corresponding author E.mail: pradiphirapure@gmail.com

**Abstract:**

Biofilms play a significant role in infection control and healthcare-related infections due to their inherent ability to withstand and resist antimicrobial treatments. These structured communities of microorganisms have been observed forming on the surfaces of medical devices. The release of both single and clustered microbial cells from these biofilms carries a notable risk of spreading infection within the host, thereby increasing the likelihood of infections and presenting a substantial public health concern. Microbial biofilms can establish themselves on or within various implanted medical devices, such as contact lenses, central venous catheters, needleless connectors, endotracheal tubes, intrauterine devices, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, tympanostomy tubes, urinary catheters, and voice prostheses. The colonization of these medical instruments plays a pivotal role in the challenge of healthcare-associated infections. This article's objective is to provide a comprehensive overview of biofilm science, the associated risks, the potentially severe consequences of infections, and both existing and emerging advanced technologies aimed at addressing the biofilm issue to enhance the healthcare system.**Keywords:**  Biofilm, Medical Device, Microbial Infection, Healthcare, Biomedical Technology

**Introduction:**

 A biofilm is an organized consortium affixed to a living or non-living surface, formed by microbial cells adhering to one another and enclosed within a self-produced extracellular polymeric matrix. This phenomenon, known as biofilm, is seen as a microbial adaptation to hostile environments [1-2]. Experimental evidence from both in vitro and in vivo studies involving P. aeruginosa has distinctly shown that bacterial cells within biofilms exhibit significantly greater resistance to antibiotics and host immune defenses compared to their free-floating counterparts [3-7]. Aggressive and intensive antibiotic therapy is commonly employed to manage chronic biofilm infections caused by dispersed bacteria and to reduce biofilm growth. However, eradicating these biofilm infections proves elusive [7-8] due to the challenge of achieving a sufficient antibiotic concentration to eliminate mature biofilms in vivo [5]. Consequently, once a bacterial biofilm infection takes hold, eradication becomes exceedingly difficult. Bacterial biofilm formation is widespread in natural aquatic environments and is also prevalent in human diseases, particularly among patients with implanted medical devices for therapeutic purposes [2,7]. As medical sciences advance, an increasing array of medical devices and artificial organs are employed in human disease treatment. Unfortunately, this progress also leads to a rise in bacterial biofilm infections. Reports suggest that a vast majority, if not all, of medical devices and prostheses may lead to biofilm-related infections. This includes catheters [9], vascular prostheses [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 dental cases, caries and periodontitis, among others. It is estimated that a significant proportion, around 50%, of nosocomial infections are connected to indwelling medical devices and their associated biofilms. Bacterial biofilms are notably characterized by their high resistance to antibiotic treatment and immune responses [7]. While antibiotic treatment stands as a crucial and effective strategy for microbial infection control, it is exceptionally challenging to completely eliminate biofilm infections with antibiotics. In vitro and in vivo experiments consistently demonstrate that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) required to combat biofilm bacterial cells are often much higher (approximately 10-1000 times) than those for planktonic bacterial cells [4-6]. Achieving effective in vivo antibiotic MBC levels to eradicate biofilms through conventional administration methods is hindered by antibiotic toxicities, side effects, and limitations in renal and hepatic functions. Consequently, the treatment of biofilm infections presents a considerable challenge that garners significant scientific attention. This review primarily focuses on providing an overview of biofilms, infections related to medical devices, the current treatments for bacterial biofilm infections, and prospective advancements in addressing medical device-associated biofilms.

**Mechanism of Biofilm formation**

The process of biofilm formation is intricate and involves several distinct stages, namely attachment, aggregation, maturation, detachment, and dispersal. Attachment comprises a two-step process. Initially, microorganisms recognize the surface, followed by reversible and irreversible attachment. Non-specific cellular associations such as van der Waals forces, electrostatic forces, Lewis acid-base interactions, and hydrophobic interactions mediate reversible attachment. Conversely, irreversible adhesion is driven by specific adhesions found on pili, fimbriae, or the cell surface of microorganisms. Maturation encompasses the aggregation and proliferation of bacteria on the surface after attachment, resulting in the formation of micro-colonies [6-8].

The irreversible attachment of bacteria to the surface triggers changes in gene expression, leading to the synthesis and secretion of extracellular polysaccharides (EPS) or an extracellular polymeric matrix (a characteristic of the biofilm condition). This matrix acts as a cementing substance, binding bacterial cell colonies together. The extracellular polymeric matrix is predominantly composed of polysaccharides, which can be neutral or polyanionic for Gram-negative bacteria and cationic for Gram-positive bacteria. It is highly hydrated, with a hydration level of up to 98%, and remains attached to the underlying surface [2-5]. Continuous multiplication, growth, and recruitment of additional microorganisms contribute to the development of a mature biofilm. This mature biofilm consists of densely packed microorganisms forming prominent outgrowth masses on surfaces.

The final stage of biofilm formation involves the detachment of microbes from the biofilm colonies, their translocation or dispersal, and subsequent attachment to new locations. The rate of biofilm growth on a medical device is influenced by various factors. For growth to occur, microorganisms must first attach themselves to the device's surface. This attachment requires a sufficiently long exposure period to prevent easy detachment. The effectiveness of this adherence is also influenced by the composition of microbes present in the surrounding fluid. Furthermore, the presence of different particles in the device's vicinity alters the properties of its surface. Consequently, the attachment of individual cells and the subsequent biofilm formation are facilitated (1). Table 1 provides a list of factors that impact biofilm formation.

**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 | Acinetobacterbaumannii 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** | **Descriptions** | **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 | DNaseI, 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. Trimethylsilane |
| 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**

Elimination of cells from the biofilm colony constitutes a crucial phase in the life cycle of biofilms as it facilitates their propagation and colonization of novel surfaces. The strategies to counteract bacterial biofilms should focus on thwarting their formation rather than dispersing established biofilms. Approaches to prevent biofilm formation encompass both "Chemical" and "Mechanical" methodologies.

**Chemical methods:**

**1. Antimicrobial coatings:** The principal tactic for biofilm prevention entails chemical modifications. Antibiotics, biocides, and ion coatings are common chemical techniques for deterring biofilm formation. These methods impede biofilm establishment by disrupting the attachment and expansion of immature biofilms [16]. Numerous in vitro studies have validated the efficacy of silver in preventing infections, both as coatings and as nanoparticles integrated into a polymer matrix. However, caution is necessary when applying silver within in vivo systems due to its potential toxic effects on human tissue. This underscores the necessity to uncover novel antimicrobial compounds that can inhibit biofilm growth.

**2. Polymer modifications:** Antimicrobial agents can be immobilized on device surfaces using elongated, flexible polymeric chains. These chains establish covalent bonds with the device surface, creating non-leaching surfaces with contact-killing properties. An in vitro study demonstrated that attaching an antimicrobial agent called N-alkylpyridinium bromide to poly(4-vinyl-N-hexylpyridine) enabled the polymer to neutralize ≥ 99% of S. epidermidis, E. coli, and P. aeruginosa bacteria [17]. Dispersion forces between the polymer chains and bacterial cells hinder bacterial adhesion and initiation of biofilm formation. This concept is akin to the steric stabilization of colloids. Polymer chains are either covalently bonded or adsorbed onto a surface.

**Mechanical methods:**

**1. Hydrophobicity, Surface roughness, Surface charge:** The genesis of a biofilm commences with the attachment of freely suspended cells to a surface. These initial colonizers initially adhere weakly and reversibly to the surface. If they aren't promptly dislodged, they can establish firmer anchorage using cell adhesion structures such as pili. Hydrophobicity also influences bacteria's propensity to form biofilms. Certain species are incapable of adhering to a surface directly and may instead bind to earlier colonizers [17]. Conversely, some bacteria struggle to develop biofilms due to limited mobility. Non-motile bacteria face challenges in recognizing surfaces and aggregating like their motile counterparts. Altering the surface charge of polymers has proven effective in preventing biofilm formation. By leveraging electrostatic principles, charged particles repel those with similar charges. Adjusting the hydrophobicity and charge of polymeric chains involves diverse backbone compounds and antimicrobial agents. Positively charged polycationic chains enable molecular extension and confer bactericidal activity [17]. Additionally, surface roughness influences biofilm adhesion. Uneven, high-energy surfaces are more conducive to biofilm growth, whereas smoother surfaces resist biofilm adherence. Surface roughness impacts the hydrophobic or hydrophilic nature of contacting substances, affecting their adhesion capabilities [18]. Thus, maintaining smooth surfaces for products interacting with bacteria is advisable [18].

 **Strategies for Biofilm Dispersal**

Enhancing the efficacy of biofilm dissolution treatments has become imperative. Understanding the role of biofilms in chronic infections and antimicrobial resistance plays a pivotal role in designing novel drug treatments [18]. Traditional antibiotics operate by either inhibiting bacterial cell division (bacteriostatic) or inducing cell death (bactericidal). While antibiotics have been essential in eradicating bacterial pathogens over time, evidence suggests that they can severely disrupt the host microbiota, creating an environment conducive to opportunistic pathogen dominance [3]. Recent advancements in strategies are aimed at thwarting biofilm formation by targeting bacterial elimination or various stages of biofilm development [18]. The subsequent discussion outlines strategies and mechanisms for inhibiting biofilm formation.

**1. Bacterial Antibiofilm Polysaccharides**

Polysaccharides, acting as sugar polymers, can function as lectin inhibitors. Lectins are proteins specifically recognizing and binding sugars without altering their composition. Within bacteria, lectins primarily facilitate bacterial attachment to host cells. These proteins play a pivotal role in biofilm development, essential for bacterial colonization and infection. Typically found on bacterial cell surfaces, lectins access and bind to glycan substrates on host cells. By vying for the sugar-binding domain of lectins, polysaccharides can impede pathogen adhesion and subsequent biofilm creation. Certain plant, microbial, and milk polysaccharides have shown the ability to obstruct various lectins from human pathogenic bacteria via competitive inhibition [19]. Polysaccharides facilitate cell-to-surface and cell-to-cell interactions crucial for biofilm formation and stabilization. Recent findings suggest that certain bacterial exopolysaccharides inhibit or destabilize biofilm formation by other species [19]. Polysaccharides' antibiofilm properties stem from their capacity to: a) modify the physical attributes of bacterial cells or abiotic surfaces, b) act as signaling molecules influencing susceptible bacteria's gene expression patterns, or c) competitively hinder multivalent carbohydrate-protein interactions, thereby interfering with adhesion.

**2. Anti-biofilm enzymes**

Enzymes capable of degrading biofilm extracellular matrices could contribute to biofilm dispersion and serve as anti-biofilm agents. An enzyme like N-acetyl-D-glucosamine-1-phosphate acetyltransferase, pivotal in the peptidoglycan and lipopolysaccharide synthesis of Gram-positive and Gram-negative pathogens respectively, is a target for matrix disruption [18]. Employing such enzymes prevented biofilm formation by Staphylococcus and Enterococcus and dispersed preformed biofilms in vitro [18]. Dispersin-B, a glycoside hydrolase, is another example that cleaves β 1–6 N-acetylglucosamine polymers in the bacterial peptidoglycan layer. Dispersin-B treatment proved effective against S. aureus and S. epidermidis biofilms and bacteria [19].

**3. Chelating Agents**

Metal cations like calcium, magnesium, and iron are implicated in maintaining matrix integrity. Consequently, chelating agents have been shown to disrupt biofilm architecture and interfere with bacterial membrane stability. For instance, sodium citrate inhibited biofilm formation by multiple Staphylococci species in vitro [21]. Additionally, tetrasodium-EDTA eradicated biofilms in in vitro models and on explanted hemodialysis catheters, while disodium-EDTA, in tandem with tigecycline or gentamicin, reduced biofilm formation by Staphylococcus species and P. aeruginosa.

**4. Antimicrobial peptides**

Innate immune responses generate antimicrobial peptides, potential candidates for novel antibiotic development. However, their range of activity and mechanism need further definition before considering them as therapeutic strategies [22]. Recent research focusing on reducing biofilm formation by multidrug-resistant P. aeruginosa strains from cystic fibrosis patients revealed bacterial eradication within preformed biofilms. Lytic peptides, another group of antimicrobial peptides, have shown inhibitory effects on biofilm formation by binding to lipopolysaccharides, disrupting membrane stability [22].

**5. Anti-adhesion Agents**

Attachment initiates virtually all biofilm formation, motivating several studies on hindering bacterial adhesion. Efforts have centered on preventing assembly of various pili using pilicides, compounds designed to disrupt pilin subunit export. Pilicides reduced in vitro biofilm formation by 50% at concentrations as low as 3 μM [23]. Similar compounds demonstrated effectiveness against curli (curlicides), inhibiting in vitro curli biogenesis and biofilm formation [24].

**6. Nanotechnology**

Nanotechnology techniques encompass altering nanoscale surface topography (nanotopography) and functionalizing surfaces with antibacterial agents, anti-adhesive polymers, or immobilized bactericidal substances. These modifications resist bacterial adhesion, thwart biofilm growth on medical devices and implants, or kill bacteria upon initial surface attachment. It involves electrostatic attraction between charged surfaces and oppositely charged polyelectrolytes, creating multilayered films with thickness ranging from tens to hundreds of nanometers [24].

**7. Disruption of Bacterial Amyloids for Controlling Biofilms**

Numerous bacteria can produce functional amyloid fibers on their cell surfaces. Several bacterial amyloids contribute to biofilm development and community behaviors. For instance, curli are extracellular amyloid fibers generated by Escherichia coli and other Enterobacteriaceae. Certain peptidomimetics inhibit curli biogenesis, with unique anti-biofilm and anti-virulence activities [25]. (Citation in bracket)

**8. Manipulating c-di-GMP Signaling as a Strategy for Dispersing Biofilm Infections**

C-di-GMP, a bacterial second messenger discovered 25 years ago, has emerged as a pivotal player in bacterial communication. It holds significant importance due to its involvement in diverse bacterial lifestyle shifts. For instance, it facilitates the transition from motile to sessile states, enabling the establishment of multicellular biofilm communities. Moreover, it drives the shift from virulent acute infections to less virulent yet chronic infections. Consequently, modulating c-di-GMP signaling pathways within bacteria presents a novel avenue for managing biofilm formation and dispersal in clinical contexts [26-30].

**Conclusion**

The proliferation of medical devices is a modern trend, yet it also contributes substantially to morbidity and mortality rates due to their vulnerability to clinically associated infections. Shockingly, statistics show that 95% of urinary tract infections are linked to urinary catheters, 65% of pneumonia cases with mechanical ventilation, and 87% of bloodstream infections are attributed to intravascular devices. Among these, catheter-related bloodstream infections (CRBSIs) pose the gravest threat. The multifaceted factors involved, including antibiotic resistance, underline why an ideal method for eradicating medical device-associated biofilms remains elusive. Presently, available antibiotics target planktonic cells but not biofilms. However, we possess the means to control and prevent medical device-associated infections by inhibiting biofilm development using the diverse anti-biofilm technologies discussed above. Future research should delve into comprehending the interplay between biofilm-resistant medical devices and biofilm-producing bacteria, evaluating the stability, specificity, and sensitivity of these novel medical devices within the human body, and assessing the pros and cons of emerging anti-biofilm 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. CurrOpinMicrobiol 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. MicrobEcol 1989; 18:1–19.
14. Rosenberg M, Bayer EA, Delarea J, et al. Role of thin fimbriae in adherence and growth of Acinetobactercalcoaceticus 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, SaguyI , 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 ChemLett, 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. MicrobiolMolBiol Rev 2013; 77(1): 1–52. ]