Bacterial adherence and biofilm formation on medical implants- A review

SuganthanVeerachamy[a] *,Tejasri Yarlagadda*[b] *Geetha Manivasagam*[c]*,*Prasad KDV Yarlagadda*[d]

[a] Department of Biomedical Engineering, School of Biosciences& Technology, VIT University, Vellore.

[b]Yarlagadda, Tejasri: Newcastle University of Medicine, Malaysia

[c*] Centre for Biomaterials Science and Technology, School of Mechanical & Building Sciences, VIT University, Vellore. [geethamanivasagam@vit.ac.in]

d School of Chemistry, Physics and Mechanical Engineering, Science and Engineering Faculty, Queensland University of Technology, Brisbane.

Abstract

Biofilms are a complex group of microbial cells that adheres to the exo-polysaccharide matrix present on the surface of medical devices. Biofilm associated infections in the medical devices pose a serious problem to the public health and adversely affect the function of the device. Medical implants used in oral and orthopedic surgery are fabricated using alloys such as stainless steel and titanium. The biological behavior, likeOsseo-integration and its antibacterial activity, essentially depends on both the chemical composition and the morphology of the surface of the device. Surface treatment of medical implants by various physical and chemical techniques are attempted in order to improve their surface properties so as to facilitate bio-integration and prevent bacterial adhesion.The potential source of infection of the surrounding tissue and antimicrobial strategiesare from bacteria adherent to or in a biofilm onthe implant which should prevent both biofilm formation and tissue colonization.This article provides an overview of bacterial biofilm formation and methods adopted for the inhibition of bacterial adhesion on medical implants

Keywords: Biofilm, Bacteria, Medical implants, Infections, Nano polymer coatings.

1. Introduction

The skyrocketing increase in number of joint replacement surgeries and their associated failures have raised serious concern in the field of medicine. Amongst various reasons, failure of medical devices due to infection has resulted in increase in number of revision surgeries, and sometimes fatality.Biomaterial associated infection (BAI) is due to the bacteria in the skin of the patients. These infections are mostly caused by staphylococci, in particular by Staphylococcus epidermidis and Staphylococcus aureus, and by streptococci, Gram-negative bacilli, enterococci and anaerobes like Propionibacterium acnes. It also involves high medical costs and the insurance companies have procured the medical device related infections are preventable in USA. Infection is seen to occur either immediately after the surgery or post-surgery and the reasons for the same are such as i) implant surface, ii) surgical theater, surgical equipment's and surgeon iii) from the patient iv) contaminated disinfectants v) and from other persons. Apart from mal-nutrient and obesity, the patients with clinical problems such as rheumatoid arthritis, diabetes mellitus and immune-compromised status are frequently in more risk of infection. In the cases where the infections are not treatable using conventional techniques such as short and long term antibiotics treatment, two stage revision surgeries is preferred. In the first stage the infected implant is removed and the patient is treated for infection and in the second stage a new device is implanted. Thus understanding the causes for the biofilm formation and associated and its prevention has become one of the most important field in medical device research.

Biofilms are the most major form of microbial life and that are biologically active matrix of cells and extra-cellular substances in association with a solid surface (1). Biofilms are attached to a substrate and consists of many bacteria implanted in an organic polymeric substance. The extra-cellular polysaccharides (EPS) are an insoluble and slimy secretion that is released by bacterial cells, encases millions of adjoining cells in a well-organized and structured matrix (2). The EPS encapsulation offers three important advantages to cells that reside in biofilms. First, the EPS can assist in dissemination of nutrients that are necessary for cell growth (3). Second, due to the diverse composition of charged polysaccharide groups that can easily bind nutrient molecules, EPS traps the external nutrients that are required for cell sustenance and growth (3). Third, the

cells that are encapsulated in the EPS matrix receive better protection from external environmental stresses compared to planktonic bacteria (4). The advantages of biofilm formation includes protection from antibiotics (5), disinfectants (6) and from dynamic environments (7). Biofilm growth is governed by number of physical, chemical and biological factors.

Development of a biofilm involves five stages where in the first stage the cells get adhered to the surface and it produces extracellular polysaccharide which leads to the formation of biofilm. Once the bacterium is matured, they get detached and start dispersing single cells due to the unfavorable environmental conditions. The dispersed cells swims away from the biofilm and get adhered on the favorable surface matrix. The biofilm production causes serious threats in the medical field by infecting the medical implants and devices. In order to overcome all these ill-effects several surface modifications and treatments are attemptedto decrease the growth of biofilm formation on the medical implants. This review attempts to report some of the biomaterial associated infections and discusses in detail on the anti-biofilm coatings to overcome the challenges posed by the bacterial adhesion. The section 1.1 deals with the various aspects of biofilm formation ,while section 2 deals with the approaches to develop anti-biofilm and section 3 discusses on various biofilm models that inhibits bacterial formation.

1.1. Biofilm formation

Biofilms are medically important, accounting for over 80% of microbial infections in the body, including prostheses and internal fixation devices. *In vitro* study has shown that ***Pseudomonas aeruginosa, Staphylococcus aureus*** **and** ***Staphylococcus epidermidis*** can easily form biofilms on stainless steel and **titanium orthopedic screws**. Further *invitro* study demonstrated that *S. aureus, S. epidermidis* and *P. aeruginosa* possess strong forces of adhesion to foreign bodies.

The biofilm comprises of three layers and Fig, 1 shows the different layers of biofilm. The first layer , the linking or conditioning film which is attached to the surface of the tissue or biomaterial, the second is the biofilm base which contains microbes and the surface film acts as an outer layer where planktonic organisms are released as free floating and spreads to the surrounding compartment (8 -11).

1.2. Mechanism of biofilm formation in medical implants:

1.2.1. Initiation of biofilm formation:

The primary adhesion stage of biofilm involves the quick adhesion of microbes to the surface of the medical devices and proliferation of cells (Fig 2). During this formation of biofilm there are various factors which are responsible for the initial adhesion of the microbes such as polarity, London –van der Waal's forces (Fig 3) and hydrophobic interactions (12). There are various bacterial surface attached proteins(**not clear)** which contribute to the initial adhesion and biofilm formation. The biofilm consists of proteins, electrolytes and some unidentified molecules (8, 13). A polysaccharide structure known as capsular polysaccharide/adhesion (PS/A) leads to the initial adhesion and slime production (14). Initial attachment of *S.epidermidis* to a polymer surface may be mediated by AtlE, a surface associated autolysin (15).The biofilm associated protein Bap leads to the biofilm formation in *S.epidermidis* (16).

1.2.2 Aggregation and biofilm maturation:

The adherence of the bacteria to the inert surface leads to the formation of stable micro-colony. Bacterial cell proliferation and intercellular adhesion takes place once the microbes adhered to the surface of the implants. A polysaccharide antigen named polysaccharide intercellular adhesion (PIA) leads to the intercellular adhesion and biofilm accumulation in Staphylococci (17). During this accumulation phase, the microbes multiply and forms several layered cell clusters on the surface of the foreign body. Micro colonies further develop into macro colonies and are enclosed by an extracellular polysaccharide matrix (18). Extra polysaccharide substances (EPS) are produced in this phase which is responsible for the binding and cell adhesion to the surface (18, 19). The EPS matrix acts as a barrier and protects the microbes during adverse conditions. Within the EPS matrix intercellular signaling or quorum sensing takes place. Cell-cell signaling has been demonstrated to play a role in the cell attachment and detachment from biofilms (20, 21). Quorum sensing is based on the process of auto induction (22) and it provides a mechanism for self-organization and regulation of microbial cells (23). Microorganisms use the quorum sensing to coordinate their communal behavior such as biofilm formation, motility and production of EPS (24). For example, in *S. aureus*, the Agr quorum sensing system regulates the

production of virulence factors that enhance attachment to host cells, defensive factors to avoid elimination by the host, and factors that promote bacterial internalization and host cell apoptosis (25, 26). In gram negative bacteria, cell communication is achieved by the activity of acylated homoserine lactones (AHLs) (27). The accumulation of AHL in developing biofilm causes the transformation of individual cells from the planktonic to the biofilm phenotype and coordinates the behavior (28). The biofilm reaches a critical mass and generates planktonic microorganisms. These free floating organisms escape the biofilm and colonize on other surfaces. These bacterial cells becomes inactive or die due to the lack of nutrients, decrease in pH or accumulation of toxic metabolic by products (29). In this phase, matured biofilm is formed and the microorganisms are ready for the disruption from the surface.

1.2.3. Dispersal of biofilm cells:

The bacterial cells from the biofilm disperse the cells from the surface and migrate because of the depletion in nutrients. Finally the microorganism detaches from the macro-colony and moves into the bloodstream and spreads infections and embolic complications. This detachment can be due to various factors including fluid dynamics and shear effects of the bulk fluid (30). The surface hydrophobicity characteristic of newly divided daughter cells disperses spontaneously either from *E. coli* or *P. aeruginosa* biofilms differs substantially from the chemo stat- intact biofilm or re-suspended biofilm cells (31). The dispersal mode differs in each bacteria and it affects the morphological characteristics of the organisms. *P. fluorescens* disperses and recolonizes a surface after approximately 5 h, *V. parahaemolyticus* after 4 h, and *V. harveyi* recolonizes only after 2 h (32). This process paves a path for cells to migrate from deeply colonized areas which has less surface adsorbed nutrients to the surface which is rich in nutrients. These aggregate cells retain certain biofilm characteristics and antimicrobial properties. The cells which have been detached due to undesirable condition revert back quickly and float freely in the surface. Some of these released microorganisms have a tendency to relocate and restart the biofilm process (33). Inside bacterial biofilm there is a high density of bacterial population that activates a cell-density dependent mechanism called quorum sensing (QS). There are QS systems in both Gram positive and Gram negative bacterial populations and these regulate the expression of adhesion mechanisms and virulent factors. It has been

demonstrated that QS also control the differentiation of the biofilm and can lead to killing of the leukocytes in some Gram negative bacteria. Due to the protection offered by the extracellular polymeric substances produced by bacteria themselves and the changed physiology of the biofilm bacteria, it is difficult for the immune system and antibiotics to eradicate the bacterial cells embedded in the biofilm, and, therefore, the biofilm infection becomes chronic. Further, the biofilm bacterial cells usually elicit less inflammatory response than the planktonic bacterial cells which makes it difficult for clinicians to diagnose such an infection.

1.3. Biofilm formation on biomedical implants:

Microbial infections in the biomedical implants pose a serious threat in modern medicine. A Biofilm infection remains a major cause of failure in biomaterial implants. All medical devices are susceptible to colonization of microbial infection. Medicaldevices are responsible for about 60–70% of hospital-acquired infections, particularly in critically ill patients (34, 35). It is known that bacterial biofilms can colonize the surfacesof both tissues and implanted medical devices. Bacterial biofilm infection consequentlyleads to tissue destruction, systemic dissemination of the pathogen and dysfunction of the device, resulting in serious illness and death (36). The main microorganisms responsiblefor biofilm formation on indwelling medical devices are, Gram-positive (*Enterococcusfaecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*)and Gram negative (*Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) bacteria, as well as yeasts (37). Titanium implants pre-seeded with *S. epidermidis* or carrying a pre-grown *S. epidermidis* biofilm, the bacteria were mostly present in the surrounding tissue and co-localized with macrophages. *S. epidermidis* were cultured from the tissue in large numbers, shows that many of the bacteria were viable. Thus, a contaminated implant can be a reservoir for infection of the surrounding tissue where bacteria can reside intracellular (178).Biomedical device associated infections are resistant to immune defense mechanisms and are difficult to treat with antimicrobial agents because the organisms are encased within a protected microenvironment hampering the prevention and treatment of established bacterial associated infections (13). Biomaterial associated infection occurs despite of many preventive measures and intervention with antibiotics often is ineffective. Diluted susceptibility of sessile bacteria present in biofilms and poor penetration of antibiotics through

the biofilm matrix are considered the predominant causes of the limited efficacy of antibiotics against biomaterial associated infection. Therefore the treatment often requires prolonged antibiotic therapy, because as antibiotics alone they are able to only suppress infection and not completely eliminate the infection caused. So in many cases ultimately antibiotics needs to be combined with adequate surgical intervention (178).The bacterium which grows on the medical implants forms a slimy layer of biofilm and the simplest way to treat the infections is to treat and modify the surface of the medical device. Several research efforts have been made to eliminate and reduce the infections in the medical implants. Microbial infections have been observed in almost all medical devices or implants like prosthetic heart valves, orthopedic implants, dental implants (Fig 4)intravascular catheters, artificial pump, left ventricular assist devices, cardiac pacemakers, vascular prostheses, cerebrospinal fluid shunts, urinary catheters, voice prostheses, ocular prostheses and contact lenses, and intrauterine contraceptive devices (35, 38, 39) (Table1). In non-surgical indwelling medical devices, such as central venous and urinarycatheters, colonization of biofilm may originate either from the skin at the point of insertion, or around the catheter once implanted. As for surgical devices, tissue damage and clot formation associated with surgical implantation are associated with the enhanced rates of microbial biofilm colonization (21, 40-42). Upon implantation, there is a competition between integration of the material into the surrounding tissue and adhesion of bacteria to the implant surface (43).

For a successful implant, tissue integration should occur prior to significant bacterial adhesion, thereby preventing the bacterial colonization at the implant. All medical devices or tissue engineering constructs are susceptible to microbial colonization and infection (44). Upon a strong adhesion to the surface, the bacterium begins to secreteand collect proteins, polysaccharides and DNA to formulate a biofilm (45, 46). Biofilminfections constitute a number of clinical challenges, including disease, chronic inflammation, impaired wound healing, rapidly acquired antibiotic resistance, and the spread of infectious emboli (34, 36, and 47). Infections related with biomaterial inorthopedic applications, lead to a condition of osteomyelitis with disturbing effects on bone and the environmental soft tissues. Such infection does not seem to respond to any conventional antibiotic treatments (48-51). Micro-organisms on the implant surface that contains adhesins which favors attachment leads to surface tension or hydrophobicity [non-specific factors]. In fracture fixation, infections occurs in a) Pre-operative

cases [Open Trauma], b) Intra-operative cases [Insertion of Fixation devices] c) Post-Operative Cases [Wound Healing]. In orthopedic surgery,the infection rate is reported more in stainless steel when compared to titanium alloys as the later favors easy formation of soft tissue (52). In revision surgery of prosthetic hips and knees, the presence of bacteria in peri-implant tissue indeed is a risk factor for reinfection. Bacteria may even persist in large numbers within host cells, including in macrophages (178).

It has been reported that many bacteria can cause prosthesis-related infections, such as *S. aureus*, including methicillin-resistant strain (MRSA), coagulase-negative staphylococci (CNS) (e.g. *S. epidermidis, S. haemolyticus, S hominis, S warneri*), *Propionibacterium acnes, P. aeruginosa, Haemophilusinfluenzae, Providencia, Enterococci, Streptococcus viridans, Escherichia coli, Citrobacter, Lactobacillus, Acinetobacter, Serratiamarcescens, Klebsiellapneumoniae,* and*Corynebacterium*. Amongst these pathogens, *S. aureus* and coagulase- negative *staphylococci* are the most common bacteria responsible for prosthesis-related infections, accounting for approximately half of the infections or more. Infections occurring in the first three months after surgery are usually caused by virulent microorganisms such as *S. aureus*, whereas delayed infections (3-24 months after surgery) are in most of the cases caused by low virulent microorganisms such as coagulase-negative staphylococci. Poly-saccharide intercellular adhesin (PIA) produced by staphylococci has been demonstrated to be a crucial virulent factor that helps staphylococci to form biofilm in implants or orthopedic biomaterials. The other important phenomenon in the pathogenesis of biomaterial associated infection is the survival of bacteria in the tissue surrounding implants, are observed. Though the macrophages and granulocytes are present around an implant, the microorganisms cannot be cleared, due to the frustrated phagocytosis caused by the implantation of a biomaterial.

2. Novel anti biofilm control approaches in medical implants:

A variety of approaches have proven to be effective in reducing biofilm related infections by preventing the bacterial adhesion on medical devices, at least in high risk populations (53). The prevention of biofilm formation in medical implants can becontrolled by following various novel emergent strategies like Quorum sensing quenchers, polymer coatings, antimicrobial coatings, enzyme mediated approaches, phage therapy, immunotherapy, nano structured coatings, surface

modifications and bio surfactants (Table 2).

Bacterial adherence to silicone has been found to be significantly higher than to polyurethane or Teflon_R (54). Host factors, such as fibronectin, fibrinogen or platelets may be deposited on the foreign body material and provide specific ligands for bacterial adhesions (55). Bacterial biofilm infections on the medical implants can be prevented by device coatings, device immersion, anti-septic irrigation of the surgical site, antibiotic loaded cements in orthopedic surgery (42) and antibiotic catheter lock therapy containing vancomycin and heparin (56) or minocycline and EDTA (57). In antibiotic catheter lock therapy, a concentrated antibiotic solution is placed in a catheter in a volume adequate to fill the lumen. The catheter is then "locked" into place for an extended period while the catheter is not in use, with the goal of preventing it from becoming colonized and thereby reducing the risk of infection (41). Impregnation of catheter surfaces with antiseptics (58) or antibiotics (42) has been shown to delay bacterial colonization. Antibiotic lock therapy was used to prevent catheter related blood stream infections in hemodialysis patients (59, 60), identified a new lipid based formulations and incorporated antibiotics for anti-infective action in vascular grafts to prevent bacterial colonization. Bi-functional poly ethylene glycol with integrin active peptide RGD (arginine-glycine-aspartic acid) coatings on titanium oxide has the potential to prevent biofilm formation and supports the tissue integration (61).

2.1. Prevention of biofilm by surface modification and coatings:

Bio-inspired coatings are developed to reduce the bacterial adhesion and to prevent the microbial biofilm formation in the medical implants. Various strategies are made to produce anti-biofilm coatings using natural and synthetic materials.

2.1.1. Antibiotic - Hydroxyapatite based coatings:

The advantage of local delivery of antibiotics for a prolonged use of drug therapy inhibits medical implant-related infections. It is applicable in lower dose and less susceptibility to promote antibiotic resistance and has long term sustained release for different drugs with different kinetics (62). The antibiotics used widely in controlled-release of drugs are gentamicin,

amoxicillin, vancomycin, cephalothin and tobramycin. Among these drugs, the most recently used drug carrier for antibiotics and antibacterial material is polymethylmethacrylate (PMMA) (63). Equally the effect of controlled release of antibiotics at a very high rate was observed in biodegradable drug carriers like poly lactic- co- glycolic acid (PLGA), poly lactic acid (PLA) and poly ethylene glycol (PEG). Price et al., 1996 examined the bacterial growth *invitro* by coating biodegradable polymer PLGA with the antibiotic gentamicin in orthopedic implants which showed 99% efficiency to control the bacterial adhesion. Hydroxyapatite coating is most widely applied in the medical field. The synthetic hydroxyapatite and calcium phosphates are used for bone implants due to the ability to support the growth of new bone tissue and induces bone-tissue integration. These coatings can be altered by surface-adsorbed antibiotics by immersion in antibiotic solutions. Sol-gel spin-coating process is used to coat titanium alloy with HA and evaluated its efficiency as drug carrier by immersion with gentamicinesulphate (64). Enriched implant fixation also acts as an indicator to a reduced rate of infection. In the largest part of cases, the implants are preferably removed after fracture healing and excessive fixation makes this removal hard or even unbearable (65, 66).

2.1.2. Antiseptic based Coatings:

Antiseptics plays a major role in reducing the potential for enhancing resistant bacterial strains, compared to the use of antibiotics in implants (67, 68). Development of implant fixation and contaminated prophylaxis in external fixation in goat studies proved the efficacy of HA coatings containing antiseptic substances. The successful coating of chlorhexidine and chloroxylenol for intramedullary implants of a rabbit model was used in local drug delivery as a coating for catheters application (35, 69).

2.1.3. Nano -Silver Coatings:

Silver is known to have antimicrobial activity (70, 71) and by encapsulating silver nanoparticles either in implant coatings or directly with cements is used for joint replacement applications. Nano silver coatings have been prominently applied to several medical devices like catheters,

and wound dressings. The use of polymer coatings with antibacterial substance allows a choice of material to be used to adapt the kinetics by varying the formulation of the coating components. Rameshbabu et al.,2006, have used microwave processing of nanosized silver substituted HA particles (30 nm) and studied the effect of silver concentration (0.5–3%) on the antimicrobial effect against *E. coli* and *S. aureus*. Silver nanoparticles on the surface of medical implants prevent bacterial adhesion and formation of biofilm. The nanoparticles are either deposited directly on the surface of the device or applied in a polymeric surface coating. The silver is slowly released from the surface, thereby killing the bacteria present near the surface (72). As a result silver has been incorporated into the surface of a variety of medical devices, such as vascular, urinary and peritoneal catheters, vascular grafts, prosthetic heart valve sewing rings, surgical sutures and fracture-fixation devices (38, 73). A broad spectrum of pathogens found at implant sites, including *P. aeruginosa*, *E. coli, S. aureus*, and *S.epidermidis*, can be affected (40). A suggested mechanism considers that Ag+ reacts withand disrupts the function of bacterial cell membranes and crucial metabolic proteins and enzymes by binding to DNA and thiol groups in proteins (74). Nano silver crystalline HA demonstrates improved biological efficiency in terms of osteoblast adhesion, proliferation, Osseo-integration, and formation of new bone on its surface (75).

2.1.4. Photoactive based coatings:

A material which quickens a chemical reaction during its irradiation of an electromagnetic wave is a photo catalyst material. In this condition, the most considered semiconductor photo catalyst after the detection of its behavior is Anatase TiO_2 (76). In order to yield an effective ion process, the semiconductors were exposed upon with ultraviolet radiation, in which the photon energy generates its excitation. These ions are efficient in degrading organic contaminations and provide an antimicrobial function (77-78).

The photoactive Titanium films are obtained by electrophoretic deposition (79), plasma ion implantation (78, 80), direct oxidation of Ti (81), sol-gel and dip coating (82) and arc-ion plating methods. Similarly, the effect of UV light pre-treatment of titanium surface with its osteoconductive capacity reflects that on post-exposure of UV radiation, the hydrophilic surface was transformed to super-hydrophilic surface (83). There is also no substantial difference

between the antimicrobial behavior of TiO_2 – coated and uncoated CP-Ti implants; i.e., both materials reveals antibacterial activity without cytotoxicity on L-929 cells. Moreover, to deposit anatasetitania film on the surface of titanium implants, plasma ion implantation and post-annealing process are used (84). Later those implants were illuminated by UV radiation before in vitro testing using pluripotent mesenchymal precursor C2C12 cells. These observations show the effect of super hydrophilicity of TiO_2 on cell behavior and bone formation (80).

2.1.5. Nanostructured Coatings:

Nanomaterials show great promise in the fabrication of novel biomedical implants and its coatings. Nanofilms, nanocoatings, and nanostructured surfaces are being widely used for biomedical applications (85). Several mechanisms in controlled drug release from surface of implanted devices coated with nanostructure biofilm yields more advantages over conventional coatings. However, as this approach has not been used for orthopedic coatings, the usage of inorganic nanocoatings as drug delivery systems plays a major role in stents. Inorganic nanocoatings are also used for drug delivery in implantable sensors and a device has been achieved by fabrication of silicon nanowires. (86, 87). In addition, the loading and release efficiency from titania nanotube, using bovine serum albumin and lysozyme as model proteins are also being investigated as nanotubulartitania does not cause chronic inflammation or fibrosis *invivo* (88). Ceramic nanoparticles enhance bioactivity while the polymer component improves the fracture toughness and adhesion to the substrate without the need of high-temperature firing (89). Diamond nanoparticles or nanodiamonds (NDs) have recently attained significant interest for local drug release in the form of coatings. Owing to superior physical properties and biocompatibility,diamond-based nanostructures have emerged as an alternative promising material for biomedical applications such as diamond films for robust implant coatings (90).

2.2. Treatment of medical implant associated infections:

Biomaterials associated infections can be treated by prolonged and high- dose of antibiotic therapy. Bacterial biofilms are inherently resistant to antimicrobial agents andtend to

be significantly less responsive to antibiotics and antimicrobial stressors than planktonic organisms of the same species (9, 34). Antibiotic treatment of bacterialendocarditis was shown to be more successful when serum antibiotic levels were held at least tenfold above the minimal bactericidal concentration (91) but even with 8 weeks of treatment, few patients have been cured by antimicrobial therapy alone (92). Thecombination of rifampicin and a fluoroquinone has proven especially successful in the treatment of various *S. aureus* biofilm infections ranging from infections of orthopaedic prostheses (13) to right-heart endocarditis (93). Replacement or removal of an infected indwelling medical device, combined with systemic antibiotic and/or antifungal therapy, is the most effective treatment in most settings (94). For managing indwelling medical device infections in non-surgery patients, long-term antimicrobial suppressive therapy remains the only option (41). Recent reviews summarize current recommended practices for the treatment of infections of prosthetic joints (94), arterial prostheses (95) , vascular catheters (44), prosthetic heart valves (96), central nervous system shunts (Yogev andBisno in (96), pacemakers and defibrillators (96), endotracheal and tracheotomy tubes (97), and hemodialysis and peritoneal hardware (98), as well as treatment of foreign body infections of the urinary tract (99).

2.3. Treatment of medical implants associated infections with nanoparticles:

The use of nanoparticles is another new approach against biofilm mediated infections. For the treatment of infections, various nanomaterials such as silver nanoparticles, zinc oxide nanoparticles, gold nanoparticles, carbon nanotubes are developed for the direct use as biomedical devices. Nanoparticles bind to bacterial cell walls causing membrane disruption through direct interactions or through free radical production (100). Mammalian cells are able to phagocytose nanoparticles and can subsequently degrade these particles by lysozomal fusion (101) and reduce toxicity and free radical damage. This property allows for the selection of nanoparticles to promotetissue forming cell functions and inhibits bacterial infection. Nanomaterials possessing super paramagnetic properties such as iron oxide nanoparticles can be directed *insitu* using a magnetic field to the site of infection (102). Iron oxide nanoparticles have been used for numerous biomedical applications, such as for the separation of biomolecules from bacteria or delivery of antibiotics and drugs, with simultaneous enhancement of MRI contrast (103-105). A magnetic field can increase the uptake of magnetic nanoparticles into bacterial

biofilms. Using mixed methods of targeting and imaging, super paramagnetic iron oxide nanoparticles (SPION) could further improve the treatment of infections (102). Nanomaterials such as ZnO, titanium dioxide (TiO2), polymers and carbon nanotubes can reduce microbial adhesion, proliferation, and biofilm growth due to their antimicrobial properties. These nanomaterials have an ability to mimic the constituent properties of natural tissues thus nanotechnology is considered as a promising tool in tissue engineering and biomaterials. Medical devices are being designed through the incorporation of carbon nanotubes into sensors to serve as feedback loops to detect bacteria and release antibiotics only when needed (106, 107).

3. Biofilm Models:

Biofilm model systems are essential to gain a better understanding of the mechanisms involved in biofilm formation and resistance. In order to increase the knowledge concerning biofilm biology, biofilm model systems are used for the study of the complex communities under controlled conditions are indispensable (108-110).

3.1. *In vitro* biofilm model systems:

3.1.1. i. Microtiter plate based method:

Several investigators commonly use microtiter plate method as this technique enlightens the biofilm which is either grown in the walls of the microtiter plate or at the bottom or even at the surface of a coupon placed in the wells of the plate. Further, a batch- reactor in a closed system is used which avoids the inflow and outflow during the process (109). It is used to discriminate biofilm-deficient mutants from wild type strains (112) and to monitor the antimicrobial and anti-biofilm effects of different antibiotics, chemicals, plant extracts and disinfectants (113,114). Later, a deep investigation on the effects of modification, coating or impregnation of materials on different biofilm developing stages can be performed using this method (115, 116). Finally, the MTP based systems, were found to differ with its multiple parameters in addition to the composition of growth media, incubating temperatures, humid control, observance of shear stress for O_2 and CO_2 concentrations (117, 118).

In addition to the MTP-based assay, the encapsulation of inert paramagnetic beads is included in a medium during the formation of biofilm and it is available as 'Biofilm Ring Test'. To collect the non-encapsulated beads, a magnet is used in to a single spot which is later measured through particular image algorithms. This is generally used to study the kinetics of biofilm formation of *Listeria monocytogenes, E.coli, Staphylococcuscarnosus*and *Staphylococcus xylosus,* (119) to measure the biofilm matrix mechanismson *Leuconostocmesenteroides* biofilm formation (120). It can also be used to assess the antibiotics effects on *Pseudomonas aeruginosa* biofilms (121), to relate the formation of biofilm amongst *Campylobacter coli* and *Campylobacter jejuni (122)*.

3.1.2. ii. Flow Displacement Method:

Compared to the conventional MTP -based method, an open system which is used for the growth of the medium with nutrients is added and the waste-products are removed. The flow displacement system is sub-classified into 'Continuous flow stirred tank reactor' or the 'plug flow reactor' methodology. During its major comparison, the continuous flow reactor has a perfect mixing and made identical for the rate at which the growth medium and is considered as the 'feed rate'. In this method, when the dilution rate is higher than the doubling time of the microorganisms in the reactor, planktonic cells are eroded out of the reactor and only the sessile cells attached to a surface will remain and have the ability to multiply. But in plug flow reactor, the influent moves as a single 'plug' in the axial direction through diffusion (Heersink in (109) (109)).

3.1.3. iii. Modified Robbins Method [MRD]:

Modified Robbins method uses a device which is used to immediately produce and forms the biofilm in the fluid. This can be constructed using a stainless steel or plastics containing a number of separate ports in a linear array through a channel of rectangular cross-section (123, 124). Later, this device is filled with a suspension of microorganisms and is reversed over to enhance the adhesion of planktonic cells to thediscs. As soon as the devices are filled with

suspensions, the tubing at the inlet and outlet side is fixed off and the remaining suspension in the tubing at the inlet is flushed out through the bypass. Next to this phase, the devices are exploded leading to loosening of clamp and continuous flow of the growth medium on the disc from the pump. Thus biofilm is formed on the discs (124). In addition, its application has the potential of antibiotic lock therapy for biofilm removal from colonized surfaces (125-127).

3.1.4. iv. CDC Biofilm Reactor:

A glass vessel containing polyethylene lid supporting eight removable polypropylene rods are used in CDC biofilm reactor. In this reactor, each rod can grasp three removable coupons on which biofilm formation occurs and the coupon is perpendicular to the rotating baffle (128, 129). A magnetic stirrer is placed in the center of the device providing a continuous flow of nutrients by means of a peristaltic pump over the colonized surfaces (130). This device is recognized as a perfect tool to grow

Pseudomonas aeruginosa biofilms with high shear and continuous flow. Later, 24 similarbiofilms can be designed instantaneously as the setup allows for the easy removal of discs during the experiment (131, 132). Currently this CDC reactor was used to investigate the activity of high dose vancomycin, moxiflaxacin in combination with other antibiotics against *Staphylococcus aureus* biofilms (133). Also, its application includes with the testing of materials coated with antimicrobial compounds and the simulation of deposition of urinary catheters (134, 135).

3.1.5. v. Cell-culture and Micro fluidic based models:

Biotic surfaces are also an important source for the formation of biofilms compared to abiotic surfaces, in which human cell lines are made to mimic the *in vivo* situation. A major form of mucosal biofilm is formed by *Candida albicans*, by inoculating it in reconstituted human epithelium(RHE), forming a structure on the top of the epithelial layer, representing that they can serve as *in vitro* biofilm model systems (136). Immortalized human microvascular endothelial cells were coated on glass slidesembedded in a parallel plate flow chamber which is perfused

with a *C. albicans* suspension. Many models were used to study the interaction of human cells with bacterial biofilms. Commonly used are *Pseudomonas aeruginosa* biofilms on airway epithelial cells (137), *Streptococcus gallolyticus* biofilms grown on endothelial cells (138), biofilms of entero-hemorrhagic *E.coli* on HeLa cells (139, 140) and *Stenotrophomonasmaltophilia*biofilms formed on cystic fibrosis from bronchial cells (141). The cell-culturebased models not only allow screening microbiological biofilm formation, but also used to assess the damage occurred upon the human cells by this process.

Fabrication of these devices for the formation of biofilm requires photolithography, which is a process of transferring a pattern from a mask into a thin layer of photosensitive polymer and later onto the surface of a substrate (142). In microfluidic devices, the sizes of the channels used are model-dependent and it is in the range of 50-500Qm wide and 30-250Qm deep and its length also varies from 5 to 40mm. However, a 'well plate microfluidic' device is used to allow high-throughput evaluation of biofilms in a microfluidic device (143-145). This device consists of micro-channels combined into a MTP. Pneumatic pressure pushes fresh medium through the micro-channel, from an inlet containing fresh medium to an outlet containing spent medium.

3.2. In-vivo Biofilm models:

3.2.1. i. *Caenorhabditiselegans and Sub-cutaneous foreign body infection models:*

*C.elegans*systems were also used to emphasis on the virulence for the effect ofparticular chemical compounds on the survival of the worms. A study by Darby et al., 2002 (146), on *C.elegans* with *Yerstiniapestis* showed that, *Y.pestis* biofilm formation in the head and mouth region prevented *C. elegans* feeding. By evaluating a transposon-insertion mutant bank, it also revealed that *Y. pestis* genes are involved in the formation of a polysaccharide matrix which is required for the biofilm formation. Also different microorganisms were tested in the *C. elegans* system like, *S. epidermidis* and *S. aureus* (147) and *Xenorhabdusnematophilia* (148). The *C. elegans* model is also used to recognize a host gene which is needed for the bacterial adhesion.

These subcutaneous models were developed in hamsters, mice, rabbits, guinea pigs and rats. A foreign particle is inserted into the subcutaneous pockets and a biofilm is allowed to mature on the implant. Mostly, this process is carried on *S. aureus* and *S.epidermidis,* but other microorganisms like *E.coli, P. aeruginosa and C. albicans*werealso reported. Thus the inflammatory response associated with surgery may prevent biofilm formation in less virulent organisms like *C. albicans* and for these microorganisms, it is recommended with the use of immunosuppressive drugs. In addition to this, there is also a chance for the materials to contaminate either in pre-implantation or in post-implantation (52, 149, 150). Hence this subcutaneous model is compatible to investigate the effect of substrates on the biofilm formation (151-153). Based on subcutaneous implantation, perforated cylinders made of Teflon, has glass beads or other materials to enhance the surface area for biofilm formation were used as a tissue cage (154). This model has an advantage that the microbial cells are recovered from the fluid inside the tissue cage without the need for the explanation (155). This model is used to study the immune responses from the host (156) *in vivo* gene expression (157), the efficacy of specific antimicrobial agents (158) and to regulate the role of genes in establishing bio-film associated infections (159, 160).

3.2.2. ii. Respiratory and Urinary Tract infection systems:

In respiratory tract models, *P. aeruginosa* cells were improved from sputum of CF [cystic Fibrosis] patients which is in the form of biofilm structure with colonies encapsulated in a matrix material, it contains *P. aeruginosa* quorum sensing molecules in the ratio found in *in vitro* grown biofilms. This method confirms the hypothetical condition of the respiratory tract infections were related to biofilms (9). Bacteria enclosed in agar beads were used in laboratory animals which resulted in proliferation and chronic infection with histological damage parallel to that observed in patients with cystic fibrosis or chronic obstructive pulmonary diseases. Infections are categorized by reliable numbers of organisms with a steady-state immunological response were also added in agar-bead based models (Sokol in (161)). The majority of investigations involving microorganisms for respiratory tract infections were carried with *P.aeruginosa and Burkholderiacepacia* organisms (8, 162-164). To mimic the infections detected in diffuse panbronchiolitis (165), a model of severe *P. aeruginosa* respiratory tract infection model has

been developed. In this model a plastic intravenous catheter is coated with *P. aeruginosa* and is inserted in the trachea through the mouth (165-167), in which the infection is controlled to the lungs. Similarly, a pulmonary infection model without syntheticencapsulation is developed. Animals are infected intratracheally with a small amount of planktonic culture of an alginate-producing *P.aeruginosa* strain (168).

In surgical and non-surgical urinary tract methods, a first system was designed with zinc which was implanted in the rat's bladder, preceded by transvesical inoculation with *Proteus mirabilis*. It is used to validate the significance of biofilm and matrix formation to develop the urinary tract models (169). In this condition, a catheterized rabbit model was advanced to study catheter-associated urinary tract infections, which is used to study the effect of various antibiotics on *E. coli* biofilms developing on these catheters and on adjacent tissues (169-172). Models were designed in rabbits to study the effect of coating urinary stents with RNAIII-inhibiting peptide [RIP] against *S. aureus* infections (173). *In vivo* models have been used to investigate the ability of other coated catheters in avoiding urinary tract infections (174, 175).

3.2.3. iii. ENT infection models:

Several models have been established to study the biofilm-associated infections in ear, nose and throat. The traditional design of Chinchilla model made a definite establishment of a relation between otitis medium and biofilm, by allowing direct visualization of the biofilm on the middle-ear mucosa following transbullar injection with *Haemophilus influenza (176, 177).* Later, this system was used to determine the efficacyof *S. pneumoniae* to form nasopharyngeal and middle-ear mucosal biofilms with transbullar inoculation.

4. Conclusion:

Implant associated microbial infection poses a serious menace and remains a major cause for the failure of biomaterial implants. Various bioactive coatings with the polymers have the potential to reduce and eliminate the microbial adhesion of biofilm in the prosthetic biomaterial devices. Biofilm model systems are essential and it provides the enhanced understanding in the

mechanism of biofilm formation. These systems can be used for the investigation and comparison of various biofilm control treatments and biomaterial device design modifications. Further research efforts on novel control strategies of biofilm and various innovative surface modifying approaches for the prevention of bacterial infection in medical implants should be enhanced and investigated in order to provide a unique shield against biofilm infection.

4.1. Prospects of Future works:

As the surface and material topography of biomaterials have an important effect on microbial colonization, an extensive research on effect of various surface compositions and topography on the formation of biofilm is required to combat this problem. In addition, the efficient control of biofilm associated infections in medical implant will require an intensive effort to develop therapeutic agents that target the morphology of the biofilm and interrupts the cell signaling. Thus the key for the success of antibiofilm approaches may hinge upon a more complete understanding of the biofilm phenotype and surface modification in the medical implant along with the therapeutics which acts as quorum quenchers/ inhibits and prevents the formation of bacterial adhesion and its related infections.

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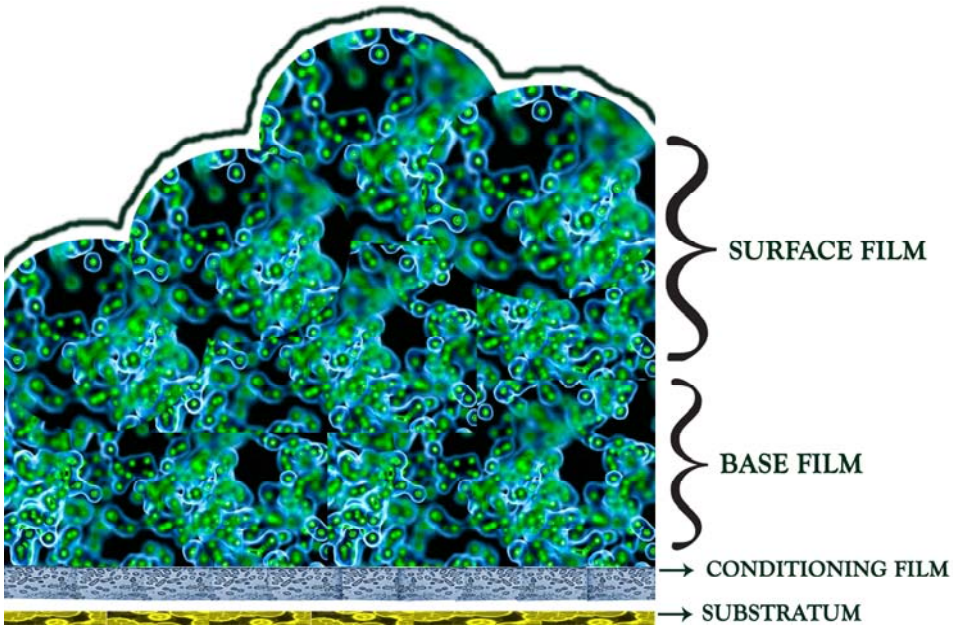

Fig 1: Layers of Biofilm

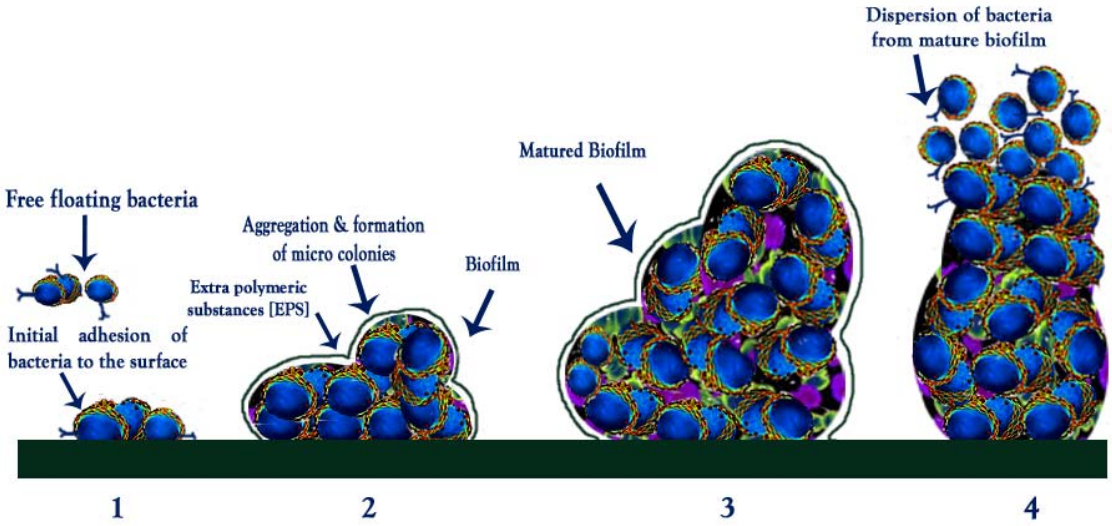

Fig 2: Schematic representation of biofilm formation

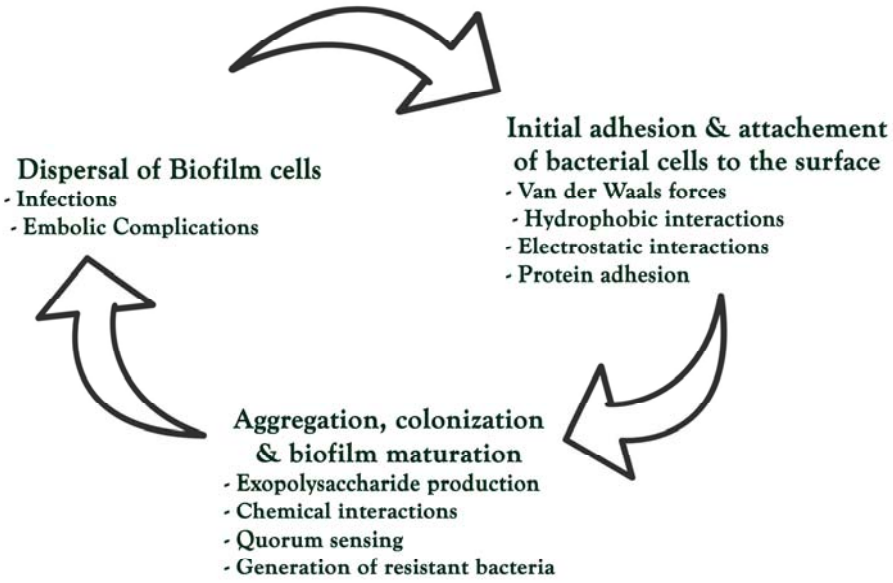

Fig 3: Steps involved in biofilm formation and the factors responsible for the biofilm

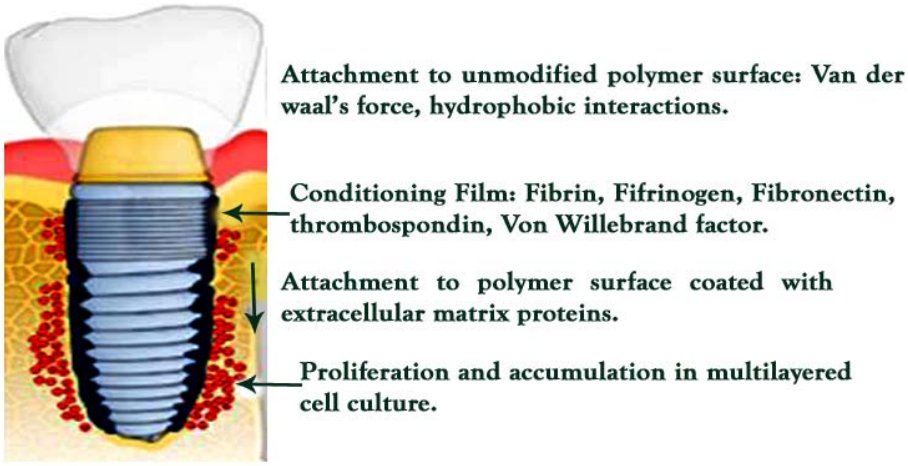

Fig 4: Biofilm formation in dental implant

Table 1: Biofilm producing microorganism in medical implants

Medical implants	Biofilm producing microorganism	References
Artificial voice prostheses	*Candida albicans, Streptococcus mitis, Streptococcus salivarius, Rothia dentrocariosa, Candida tropicalis, Streptococcus sobrinus,Staphylococcusepidermidis,Stomatococcus mucilaginous*	Bryers, 2008 Rodrigues et al., 2007
Artificial hip prosthesis	Coagulase-negative *Staphylococci,* β-hemolytic *Streptococci, enterococci, Proteus mirabilis, Bacterioides* species, *Staphylococcus aureus,Streptococcus,Escherichia coli, Pseudomonas aeruginosa*	Darouiche, 2001
Replacement joints	*S. aureus* and *S. epidermidis*	Bryers, 2008
Prosthetic heart valves	*Streptococcus viridans, coagulase-negative Staphylococci, enterococci, Staphylococcus aureus*	Rodrigues et al., 2007
Cardiac pace makers	*S.aureus*	Darouiche, 2001
CSF shunts	*S.aureus,S.epidermidis,Enterococcus*	Darouiche, 2001
Endotracheal tubes	*S. aureus, S. epidermidis, C. albicans,P.aeruginosa*	Darouiche, 2001
Urinary catheters	*S. epidermidis, K. pneumoniae, E. faecalis, Proteus mirabilis*	Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007
Peritoneal dialysis catheters	*Streptococci, Staphylococci*	Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007
Central venous catheters	*S. epidermidis*, *S. aureus*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans*	Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007

Contact lenses	*P. aeruginosa* and Gram-positive cocci	Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007
Dental implants	Acidogenic Gram-positive cocci (e.g. *Streptococcus*),Gram-negative anaerobic oral bacteria	Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007
Implanted prosthetic devices for erectile dysfunction	*S. aureus* and *S. epidermidis*	Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007
Intrauterine contraceptive devices	*Micrococcus* sp.,*Enterococcus* sp., *Candida albicans*, Group B *Streptococci*	Rodrigues et al., 2007
Orthopaedic implants	*Hemolytic streptococci*, *Enterococci*, *P. mirabilis*, *Bacteroides*sp.,*P.aeruginosa, E. coli.*	Rodrigues et al., 2007
Breast implants	*S. aureus, Enterococcusand S. epidermidis*	Bryers, 2008

Table 2: Novel anti biofilm approaches (Maureen et al., 2008)

Approaches	Mechanism of action	Target	Reference
QS interference RNA III-inhibiting peptide (RIP)	QS interruption	RNAIII synthesis	Cirioni et al 2003; Balaban et al, 2005.
Impairing adhesion Biosurfactants,including RC14 biosurfactant 'surlactin'. Furanone compounds Diterpenoids (salvipisone and aethiopinone)	Anti-adhesive activity; interference with initial bacterial attachment. Reducing adhesion and colonization. Destabilising biofilm matrix allowing detachment +/ altering bacterial cell surface hydrophobicity.	Microbial Adhesion Gene encoding adhesion and slime production Biofilm matrix +/ bacterial cell surface	Rodrigues et al 2006. Baveja et al .,2004; Hume et al.,2004. Kuz´ma et al., 2007; Walencka et al., 2007
Targeting slime formation N-acetyl-D glucosamine-1-phosphate acetyl transferase (GlmU) inhibitors(N-substituted maleimides)**.** N-acetylcysteine (NAC) Bacteriophage therapy; phage K & Bacteriophage 456	Inhibiting bacterial cell wall synthesis and PIA formation Reducing production of extracellular polysaccharide matrix and promoting disruption of mature biofilm Lytic activity on biofilm cells	PIA biosynthetic enzymes; GlmU enzyme Extracellular polymeric matrix Biofilm exopolysaccharide and biofilm cells	Burton et al., 2006 Pe´rez-Giraldo et al 1997;Aslam et al 2007 Curtin &Donlan 2006; Cerca et al 2007
Immunotherapy FN binding receptor monoclonal antibodies (MAbs). Anti-PIA antibodies Surface binding protein/Fbe antibodies Anti-Aap domain B	Blocking adhesion Inhibition of PIA formation. Blocking adhesion Inhibiting accumulation and intercellular adhesion	FN binding receptor PIA Fbe Aap	Bryers& Ratner 2004. McKenney et al., 2000. Pei et al 1999; Pei & Flock 2001; Rennermalm et al., 2004. Rohde et al., 2005;

antiserum Aap antibodies			Sun et al.,2005; Rohde et al., 2007.
Enzymatic removal Oxido reductases & Polysaccharide hydrolyzing enzymes Lysostaphin(staphylolytic endopeptidase)	Enzymatic removal and disinfection of biofilm Disruption of biofilm matrix and killing ofreleased bacteria	Biofilm matrix Peptidoglycan pentaglycine Interpeptidecross-bridges of Staphylococcal cell wall.	Johansen et al., 1997. Wu et al., 2003.
Dispersin B (DspB) Serratiopeptidase	Enzymatic degradation of cell Bound exopolysaccharide adhesin, an essential component of the biofilm polymeric matrix. Induces biofilm degradation via Proteolytic activity,also enhances antibiotic activity	β-1,N-acetyl-D-glucosamine. Biofilm slime matrix	Kaplan et al., 2004; Donelli et al.,2007. Selan et al., 1993; Mecikoglu et al., 2006.
Immunomodulation Interferon γ	Reversal of Macrophage deactivation in the vicinity of implanted Biomaterial	Macrophages	Boelens et al .,2000a, b.