Chapter

Microbial Biofilms

Princy Choudhary, Sangeeta Singh and Vishnu Agarwal

Abstract

Biofilms are the aggregation of microbial cells, which are associated with the surface in almost an irreversible manner. It exists in variety of forms like dental plaque, pond scum, or the slimy build up in sink. Biofilm formation involves sequence of steps like conditioning, attachment, metabolism, and detachment. Biofilm consists of water channels, EPS (Exopolysaccharide), and eDNA (Environmental DNA), which plays an important role in nutrient circulation, its development, and structure stabilization. Resistance of planktonic bacteria against antimicrobial agents gets increased on the formation of biofilm, which may be the presence of diffusive barrier EPS or neutralizing enzyme, cells undergoing starvation, or due to spore formation. There are numerous factors, which affects biofilm formation such as substratum effects, conditioning film on substratum, hydrodynamics, characteristics of the aqueous medium, cell characteristics, and environmental factors. Biofilm can cause industrial, medical, and household damage and is a reason for loss of billions of dollars every year. Development of biofilm on catheters, medical implants, and devices is a major cause of infections and diseases in humans. Examples include Plaque, Native Valve Endocarditis, Otitis media, Prostatitis, Cystic fibrosis, Periodontitis, Osteomyelitis, and many more.

Keywords: biofilm, EPS, microbes, medical implants, resistance

1. Introduction

Biofilms are the aggregation of microbial cells, which are associated with the surface in almost an irreversible manner, i.e. cannot be removed by gently rising [1]. They are attached with a biotic or abiotic surface integrated into the matrix that they have produced [2]. An accustomed biofilm provides favorable conditions for genetic material mobility between the cells and has a defined architecture. It is also reported that these surface-associated microorganisms possess definite phenotype with reference to growth rate and gene transcription [1].

The credit of discovery of microbial biofilm can be given to Van Leeuwenhoek who, with his simple microscope first observed the microorganisms on tooth surface [1].

2. Biofilm formation

A biofilm may be composed of one microbial species or many microbial species found on a variety of living or nonliving surfaces. However, mixed species biofilms

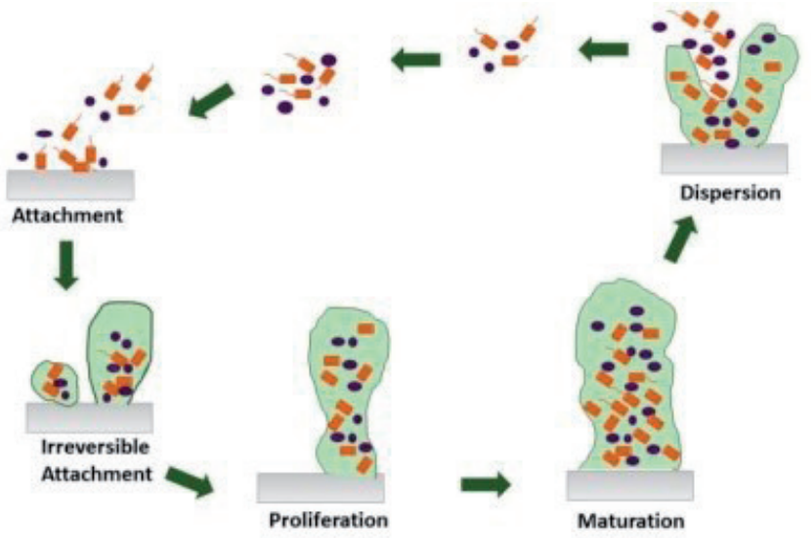

Figure 1.
Stages of biofilm development.

form the majority in most of the environments and single species biofilms host the surface of medical implants and hence being the reason of infections.

The initiation of biofilm formation have some requirements as the bacteria must be capable of attaching itself to and moving on the surface, detecting their cell density and ultimately to form a 3-D mesh of cells enclosed by exo-polysaccharide [3]. There is also an important role of cell membrane proteins, extracellular polysaccharides and signaling molecules [2] (**Figure 1**).

2.1 Biofilm formation steps

Step1. Attachment: Conditioning layer is formed which have a loose collection of carbohydrates and proteins which gets unite with minerals in hard water. It attracts the microbial cells to get attached with the surface.

Step2. Irreversible attachment: As soon as conditioning layer formed, electrical charge accumulates on the surface which attracts the bacteria having opposite charge that result in irreversible attachment of microbial cells. The charges are sufficiently weak that microorganisms could be easily removed by the mild cleanser and sanitizers.

Step3. Proliferation: In this phase, bacteria get attached to the surface as well as with each other by secreting EPS (an extracellular polymeric substance) that entraps the cells within a glue-like matrix.

Step4. Maturation: The biofilm environment consists of the nutrient-rich layer which supports the rapid growth of microorganisms. Complex diffusion channels are present in a mature biofilm to transport nutrients, oxygen and other components required for bacterial growth and removes waste products and dead cells [4, 5].

Step5. Dispersion: It is the process of dispersal of biofilm in which actively growing cells gradually sheds daughter cells [1]. Because as long as fresh nutrients are kept providing, biofilm continues to grow and when they get nutrient deprived, they return to their planktonic mode by detaching themselves from the surface [3]. This process probably happens to allow bacterial cells to get sufficient nutrients [2]. There is also a possibility of the detachment process to be species-specific as *Pseudomonas fluorescence* recolonizes surface after approx. 5 hours, *Vibrio harveyi* after 2 hours and *Vibrio parahaemolyticus* after 4 hours [1].

3. The composition of biofilm

Biofilm is primarily composed of bacterial micro-colonies which are non-randomly distributed in a shaped matrix or glycocalyx [6]. Mostly, these micro-colonies are rod-like or mushroom-shaped or they can have one or more types of bacteria. Based on bacteria type, the composition of micro-colonies contains 10–25% (by volume) of microbial cells and 79–90% (by volume) of the matrix [2, 6]. Extensive bacterial growth assists in the rapid formation of visible layers of microbes accompanied by excretion of EPS in an abundant amount [6]. At bottom of most of the biofilms, a dense layer of microorganism is bound together in polysaccharide matrix with other organic and inorganic components. The successive layer is highly irregular and loose and may extend into surrounding medium [6].

3.1 Water channels

These are present in between the micro-colonies which act as the simple circulatory system for distributing nutrients and receiving harmful metabolites [2].

3.2 EPS

Exopolysaccharide which is produced by the bacteria, are the major component of a biofilm. It constitutes about 50–90% of the total organic matter in a biofilm [6]. It is mainly composed of polysaccharides, some of which may neutral or polyanionic in case of Gram-negative bacteria or cationic as in case of Gram-positive bacteria. The anionic property of polysaccharide is confirmed by the presence of uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked pyruvate. This anionic property plays an important role in the association of divalent cations like calcium and magnesium that have been shown to provide greater binding force in developed biofilm by cross-linking with polymer strands [1]. Along with the polysaccharide (which constitutes 1–2% of EPS), EPS also contains proteins [<1–2% (including enzymes)], DNA (<1%), RNA (<1%) as well as some lipids and humic substances [7].

3.3 eDNA

The microbial genetics and the environment in which bacteria grows are the determining factors for the composition of a biofilm. *Pseudomonas aeruginosa, Streptococcus intermedius*, *Enterococcus faecalis* and *Staphylococcus* are the species in which eDNA was initially observed.

One of the common mechanism by which eDNA is released is Autolysis. Released eDNA plays an important role in the development of the biofilm, biofilm structure stabilization as well as in gene transfer mechanisms. This genetic transfer is responsible for spreading of virulence and antibiotic resistance genes in circulating strains exposed to the selective pressure of medical treatment. *Streptococcus pneumonia* and related *Streptococci* are a good example of this [8].

4. Drug resistance and biofilm

In a biofilm, rendering biofilm becomes ten to thousand times less prone to several antimicrobial agents than the same planktonic culture grown bacterium. As an example, it has been seen that there is an increase of 600-fold concentration in sodium hypochlorite (an oxidizing biocide that is counted in most effective

antibacterial drugs) for killing biofilm cells of *Staphylococcus aureus* as compared with its planktonic form [9]. Moreover, as compared to planktonic form, bacteria in biofilms shows a discrete physiology like reduced metabolic rate and enhanced cell to cell communication which helps in developing resistance to antibiotics or reduce their effects [10]. In the attempt to describe the resistance of biofilms to antibiotics, three assumptions have been made:

1. Slow or partial diffusion of antibiotics into inner layers of biofilm. This is due to EPS matrix which has biofilm entrenched bacteria, act as a diffusive barrier [2].

2. In the biofilm microenvironment, some microbial cells fall into a state of slow growth or starvation due to nutrient limitation or accumulation of harmful metabolites. These are not vulnerable to many antimicrobial agents [2, 11].

3. The differentiation of a bacterial subpopulation resembles the process of spore formation. It has a distinctive and highly resistance phenotype (a biologically programmed response to bacterial sessile life form) that protects them from antibacterial effects [2].

Presence of neutralizing enzymes also contributes to the antibiotic resistance in the biofilm. These proteinaceous enzymes degrade or inactivate antibiotics by mechanisms like hydrolysis and modification of antimicrobials by different biochemical reactions [7].

Although, intensive and insistent treatment of antibiotic is effective in reducing the biofilm and controlling the exacerbations of chronic biofilm infections but are not able to eliminate biofilm infections it is possibly because the minimal concentration of antibiotic (required to eliminate a mature biofilm) is challenging to reach *in vivo*. Hence, if a bacterial biofilm infection is established, it becomes much difficult to eradicate [12].

Experimental studies suggested that in most of the cases antibiotic treatment alone is not sufficient to eliminate infections of biofilm [12]. In a study, a nanoparticle called ciprofloxacin-loaded poly (lactic-co-glycolic acid), that were functionalized with DNase I, were prepared to observe their antibiofilm activity against *P. aeruginosa* biofilms. It has been found that they release ciprofloxacin in a controlled manner, as well as they effectively target and disassemble the biofilm by degrading the extracellular DNA that stabilizes the EPS [10]. Biofilm combination therapy is usually recommended for treating biofilm infections as this is found to be substantially better than antibiotic monotherapy [12].

5. Factors affecting biofilm formation

A number of factors such as substratum effects, hydrodynamics and various properties of cell surface play an important role in microbial attachment [1].

5.1 Substratum effects

As the surface roughness increases microbial colonization increases because as the roughness increases, surface area increases and shear forces get diminished. And considering extent and rate of attachment, it has been seen that microorganisms get attached to more rapidly to hydrophobic and nonpolar surfaces as Teflon and other plastics rather than to glass and other materials having hydrophilic properties.

5.2 Conditioning films forming on the substratum

When a material surface gets exposed to any aqueous medium, it gets immediately coated with polymers from that surface or become conditioned. The coating or film is found to be organic in nature formed within minutes of exposure. The nature of these films is found to be quite different for surfaces exposed in the human host. As an example, "acquired pellicle," a proteinaceous conditioning film, develops on tooth enamel surface. A pellicle is composed of glycoprotein, lysozymes, phosphoproteins, albumin, lipids and gingival crevice fluid. Oral cavity bacteria get adhered within hours of exposure to this pellicle conditioned surface.

5.3 Hydrodynamics

The hydrodynamic flow layer is the zone of negligible flow which is found at the immediately adjacent to the substratum/liquid interface. The flow velocity of this zone is negligible and its thickness is inversely proportional to the linear velocity. Substantial mixing or turbulence is the main characteristics shown by the region outside the boundary layer. The hydrodynamic boundary layer can considerably affect the interaction between cells and substratum. The velocity characteristic of the liquid governs the association of cells with the submerged surfaces. At, very low linear velocities, the cells must navigate through the hydrodynamic boundary layer, and cell size and cell motility govern its association with the surface. The boundary layer decreases, as the velocity increases and cells will be exposed to progressively larger turbulence and mixing. Therefore, higher linear velocities would be supposed to form a more rapid association with the surface, at least until velocities become high enough to apply abundant shear forces on the attaching cells, that results in detachment of these cells [1].

5.4 Characteristics of the aqueous medium

Characteristics of the aqueous medium such as temperature, pH, nutrient level and ionic strength possibly play an important role in attachment of microbes with the substratum. As an example, it has been found that the attachment of *Pseudomonas fluorescens* to glass surface is affected by an increase in the concentration of several cations (sodium, calcium, lanthanum, ferric iron), perhaps by reducing the repulsive forces between the negatively charged bacterial cells and the glass surfaces.

5.5 Properties of the cells

The rate and extent of adherence of microbes depends on the properties of cells like cell surface hydrophobicity, as hydrophobic interactions tend to increase with an increasing nonpolar nature of one or both involved surfaces and adhesion increases with increase in hydrophobicity, presence of fimbriae and flagella as fimbriae contribute to cell surface hydrophobicity probably by overcoming the initial electrostatic repulsion barrier that exists between the cell and substratum and production of EPS. EPS might be hydrophobic, although mostly they are both hydrophilic and hydrophobic. Numerous bacterial EPS have the backbone of 1,3- or 1,4-β-linked hexose residues and tend to be less deformable, more rigid and inadequately soluble or insoluble in specific cases although other EPS molecules may be water soluble. Researches also showed that different organisms produce different amounts of EPS and the amount of EPS increases with age of the biofilm. Antimicrobial resistance properties in the biofilm are possibly mediated by the EPS

by impeding the mass transport of antibiotics through the biofilm, which might be by binding directly to these agents [1]. EPS formation is an essential part of biofilm formation as studies on *Staphylococcus epidermidis* have shown that if genes responsible for the synthesis of EPS matrix are inactivated then bacteria lose the ability to form biofilm [2].

5.6 Environmental factors

Different environmental factors affect the biofilm formation; listed below:

5.6.1 Availability of certain nutrients

It has been shown by studies on *Listeria monocytogenes* that an optimum level of phosphate is very important for biofilm formation and gets stimulated by the presence of carbohydrates mannose and trehalose.

5.6.2 Presence of oxygen

Presence of oxygen regulates Biofilm formation in *Escherichia coli*. In the absence of sufficient oxygen supply biofilm does not form as bacteria could not adhere to the substrate surface.

5.6.3 Environmental pH

Environmental pH effects were observed by studying on *Vibrio cholerae*. Optimal pH for multiplication of *V. cholerae* is 8.2 and below pH 7 i.e., in acidic environment the bacteria lose their ability to form biofilm as they lose mobility.

On the other hand, bacteria like *S. epidermidis* and *E. coli* do not need an alkaline environment for multiplying hence they easily form a biofilm on urethral catheters where urine pH is acidic.

5.6.4 Temperature

When temperature was kept high, *L. monocytogenes* did not form biofilm as the bacteria wasn't able to adhere itself to the substrate surface [2].

6. Diseases due to biofilm

Besides infecting the industrial pipelines, waste water channels, oral cavity, ventilators, catheters, and medical implants, they are a major cause of human diseases [11]. Infections and diseases in humans are mostly due to development of biofilm on or within indwelling implants or devices such as contact lenses, bio prosthetic and mechanical heart valves, pacemakers, intra-arterial and intravenous catheters, central venous catheters, peritoneal dialysis catheters, urinary catheters, joint prosthesis, voice prosthesis, penile prosthesis, ureteral stents, biliary stents, endotracheal tubes, nephrostomy tubes, intrauterine contraceptive devices (IUDs) [13, 14]. A biofilm may be composed of gram-positive or gram-negative microorganisms which may arise from the skin of a patient, health worker, tap water or any other environmental source [5].

Biofilm growth usually was seen in the lungs of cystic fibrosis patients causing chronic bronchopneumonia, in the middle ear in patients with chronic and secretory otitis media, in chronic rhino sinusitis, in chronic osteomyelitis and in chronic

Gram-positive microorganisms	**Site of infections and diseases**
Acidogenic gram-positive cocci (e.g. *Streptococcus*)	Dental caries
Gram-positive cocci (e.g. *Staphylococci*)	Musculoskeletal infections
Group A *Streptococci*	Necrotizing fasciitis
Viridans Group *Streptococci*	Native valve endocarditis
S. epidermidis and *S. aureus*	Sutures, exit sites and arteriovenous shunts
S. epidermidis, E. faecalis	Urinary catheter cystitis
S. epidermidis, S. aureus, Corynebacterium species, *Micrococcus* species, *Enterococcus* species, *Candida albicans,* Group B *Streptococci*	IUDs
C. albicans, S. epidermidis	Hickman catheter
S. epidermidis, S. aureus, E. faecalis, C. albicans	Central venous catheter
Viridans Streptococci, Enterococci	Mechanical heart valves
Hemolytic Streptococci, Enterococci	Orthopedic devices
S. epidermidis, S. aureus,	Penile prosthesis
Gram-negative microorganisms	**The site of infections and diseases**
Nontypable strains of *Haemophilus influenzae*	Otitis media
E. coli (enteric bacteria)	Biliary tract infection, bacterial prostatitis
P. aeruginosa and Burkholderia cepacia	Cystic fibrosis pneumonia
Pseudomonas pseudomallei	Melioidosis nosocomial infections
Klebsiella pneumoniae, Proteus mirabilis	Urinary catheter cystitis
K. pneumoniae, P. aeruginosa	Central venous catheter
Proteus mirabilis, Bacteroides species, *P. aeruginosa, E. coli*	Orthopedic devices

Table 1.
Different infections and involved microorganisms [11].

wounds [15]. Infections and then diseases occur because of these two reasons: (a) Implantation of any medical device cause tissue damage which attracts platelets and fibrin accumulation at the site of the attachment. The damaged tissue aids in colonizing the microorganisms [13]. (b) Drug resistance and inflammation in host might get stimulated by biofilm formation which results in sustained infections [16] (**Table 1**).

7. Biofilm on some common medical devices

7.1 Central venous catheter biofilms

Commonly found organisms on catheter biofilm are *S. epidermidis, S. aureus, K. pneumoniae, C. albicans, P. aeruginosa,* and *E. faecalis.* These might get emerged from patient's skin microflora, exogenous microflora from health-care personnel, or infected infusates. It has been reported that inner lumen of long-term catheters (30 days) and an external surface of short-term catheters (<10 days) has more biofilm formation. Microbial growth may depend on the nature of fluid delivered through a central venous catheter, as it has been seen that gram-negative microorganisms grow well in the intravenous fluid than gram-positive organisms [17].

Many studies have been done to control or avoid biofilm formation in these devices. Few remarkable results are:

- It has been found in a research that microbial colonies of the left arterial catheter can be eliminated by addition of sodium metabisulfite to the dextrose-heparin flush.

- Less colonization was seen on catheters coated with minocycline and rifampin than those coated with chlorhexidine and silver sulfadiazine [5].

7.2 Mechanical heart valve biofilms

Microorganisms like *S. epidermidis*, *S. aureus, Streptococcus* species, Gram-negative bacilli, diphtheroids, *Enterococci and Candida* species develop biofilm on the components of mechanical heart valves and surrounded heart tissues, which lead to a condition called prosthetic valve endocarditis. Also, it more often develops on the tissue surrounding the prosthesis or on the sewing cuff fabric that attaches a device to the tissue than on the valve itself. The source of the microorganism somehow tells its identity as, if it gets originate from an invasive process like dental work then it possibly belongs to *Streptococcus* species or it also might get originated during surgery (early endocarditis, mainly due to *S. epidermidis*) or from an indwelling medical device.

To prevent initial attachment of the microbes, anti-microbial agents are provided during valve replacement or any invasive process like dental work. It has also been found out that less inflammation was caused when silver coated sewing cuff of St. Jude mechanical heart valve was implanted than an uncoated one [5, 17].

7.3 Urinary catheter biofilms

Organisms which develop biofilm on these devices are *S. epidermidis*, *E. faecalis*, *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *K. pneumonia* and other Gram-negative organisms [17]. These catheters are tubular latex or silicone devices that are inserted via urethra into the bladder. It may be of an open system in which catheter drains into an open collection center or close system in which it vacates into a securely fastened bag. In open system, catheter gets quickly contaminated and chances of UTI (Urinary Tract Infection) are much more than in closed system. The chances of microbes to develop biofilm and hence causing UTI is more as long as the catheter remains on its place as it has been found out that approximately 10 to 50% of the patients undergoing short-term catheterization (up to 7 days) and around all the patients undergoing long-term catheterization (>30 days) gets infected with UTI [5].

It has been shown in studies that hydrophobicity of both organism and surface is responsible factors for microbial attachment on the catheter as a wide range of microbial colonies are found to be attached on the catheter's surface which displays both hydrophobic and hydrophilic regions [17]. Bacterial attachment is also enhanced by an increase in urinary pH and ionic strength by divalent cations (Mg and Ca). Urease is produced by some of the organisms of this biofilm which is responsible for hydrolyzing the urea to ammonium hydroxide. As a result, pH at the biofilm-urine interface gets higher, which causes precipitation of minerals such as struvite and hydroxyapatite. These biofilms having mineral components form encrustations which can completely block the catheter's inner lumen [5].

Several approaches have been done to control biofilm formation on urinary catheters like the use of antimicrobial ointments and lubricants, bladder instillation,

antimicrobial agents in collection bags, impregnation of catheters by silver oxides like antimicrobial agents or systemic antibiotics. Also, biofilm of many Gram-negative microorganisms can be reduced by exposing to mandelic acid in combination with lactic acid [17].

7.4 Contact lenses biofilms

Microbes get readily attached to the surface of both type of contact lenses i.e. soft contact lenses and hard contact lenses (differentiated according to the material used, design, wear schedule and frequency of disposal). Nature of substrate, water content, polymer composition, electrolyte concentration and type of bacterial strains governs the degree of adherence of microbes to the lenses. The storage case of a lens has been implicated as the primary source of contamination [5].

Staphylococcus, *Serratia* and *Pseudomonas* are some most common bacterial species obtained in contact lenses. *Staphylococci* are found affiliated with contact lens induced peripheral ulcer, blepharitis and conjunctivitis while *Serratia* and *Pseudomonas* species known to contribute in corneal inflammation and infection [18].

7.5 Intrauterine devices

The tail part of IUDs which is made up of a plastic microfilament surrounded by nylon sheath is possibly the primary source of infection. Microorganisms that contaminate IUDs are *Lactobacillus plantarum*, *S. epidermidis*, *C. albicans*, *S. aureus*, species of *Corynebacterium*, *Enterococcus* species [5].

8. Some common biofilm infections

8.1 Dental biofilms

Dental biofilms, commonly known as plaque are the most studied biofilm in human. It involves hundreds of species of bacteria. Some significant microbes include *Porphyromonas gingivalis, Bacteroides forsythus*, *Actinobacillus actinomycetemcomitans*, *Treponema denticola*, and a number of *Streptococci* including *Streptococcus mutans* [11].

After a good oral wash or dental cleaning, the tooth enamel acquires a coating called as pellicle which is composed of various proteins and glycoproteins of host origin. Then with the help of adhesion molecules and pilli, first *Streptococci* then *Actinomycetes* colonizes the teeth surface. Bacterial cells start interacting with each other on the pellicle and a number of *Streptococci* and related organisms starts synthesizing insoluble glucan via glucan binding protein. After few successive colonization with few more organisms, demineralization of tooth enamel starts (which leads to caries) by the acids which are produced by fermentation of the dietary sucrose and other carbohydrates [11].

8.2 Native valve endocarditis (NVE)

This condition arises due to the interaction between bacteria, vascular endothelium and generally of mitral, aortic, tricuspid and pulmonary valves of the heart. The organisms responsible for these conditions are species of *Streptococcus*, *Staphylococcus*, *Pneumococci*, *Candida*, *Aspergillus,* and some Gram-negative bacteria, which get access to the blood stream via the oropharynx, gastrointestinal, and urinary tract. When the intact endothelium gets damaged, microbes

adhered to it and as a result nonbacterial endocarditis (NBTE) develops at the site of injury and thrombus (accumulation of platelets, fibrin, and red blood cells) formed [13]. Fibronectin which has been found as a thrombotic lesion of the heart valve can simultaneously bind to fibrin, collagen, human cell and bacteria. Fibronectin receptors are found in many bacterial species like *Staphylococcus and Streptococcus* [5].

Many antibiotic therapies are suggested depending on the organisms involved as Penicillin is recommended for normal treatment of *Streptococcal endocarditis* and for synergistic killing gentamycin may be supplemented. Fluconazole can successfully terminate the effect of *Candida endocarditis* [5].

8.3 Otitis media

It is a condition of chronic ear infection caused due to inflammation of mucoperiosteal lining [5]. In the middle ear cavity, fluid gets accumulated which ultimately affects speech development and learning capability of the patient. However, its complete etiology is still under research [7]. Various organisms responsible for otitis media include *S. pneumoniae, H. influenzae, Moraxella catarrhalis, S. epidermidis, P. aeruginosa,* etc. As due to limited penetration of antibiotic, its low concentration is present in middle ear fluid, hence strong antibiotics like amoxicillin, cefaclor, erythromycin, and clarithromycin are needed for combating otitis media [5].

8.4 Chronic bacterial prostatitis

Prostatitis is the inflammation of the prostate gland which possibly occurs due to the microorganisms that have ascended from the urethra or by the reflux of infected urine into prostatic ducts which vacates into the posterior urethra. Once the microbe gets entered in the prostatic duct, they start multiplying rapidly and can form sporadic micro-colonies and biofilms which gets adhered to the epithelial cells of the system of ducts. Microbes responsible for this infection are *E. coli, P. aeruginosa*, species of *Klebsiella*, *Proteus, Serratia*, *Bacteroides,* etc. [5].

8.5 Cystic fibrosis

Cystic Fibrosis is a chronic bacterial infection of intrapulmonary airways with *P. aeruginosa* [19]. Its consequences include thickening of mucus in many body systems which results in impaired mucociliary clearance of microorganisms and chronic infection in lungs. The infection gets punctuated by acute aggravation of disease and inflammation which will lead to lung failure and premature death [20]. According to the genetic etiology, one out of more than 1500 potential mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene results in its malfunction, as a result sodium absorption is inhibited through epithelial sodium channel. And due to hyper-absorption of water, airway surface liquid gets depleted, mucociliary clearance get depleted and inhaled bacteria are allowed to remain within airway [20, 21].

Microscopic studies of sputum samples and lung tissue section have shown the presence of biofilm or micro-colonies in the airways. These biofilms are able to grow larger than 100 μm in diameter [22]. Some common cystic fibrosis pathogens include *S. aureus, H. influenzae* and ultimately predominant one *P. aeruginosa* [20, 21]. *P. aeruginosa* has some adaptive mechanisms which make it survive and persist for several decades in CF patient's respiratory tract. Biofilm adaptation of *P. aeruginosa* makes it resistant to antibiotic therapy and inflammatory defense

mechanism. This also makes it survive in different conditions like whether it is aerobic respiratory zone or the conductive zone of the lungs which have anaerobic sputum or the paranasal sinuses where mucus too has a lower concentration of oxygen [15].

Early antimicrobial treatment (i.e. during early colonization period of microbes) for preventing chronic infection of *P. aeruginosa* may give a possibility for successful treatment of cystic fibrosis, as this chronic infection may postpone for several years by giving an early treatment with ciprofloxacin and colistin [5, 22].

8.6 Periodontitis

Periodontitis is the infection of supporting tissues of teeth, gums (gingiva) and periodontal tissues (gingiva, alveolar bone, and periodontal ligament). Its chronic form may lead to exfoliation of teeth. The primary site of periodontitis is sub gingival crevice which is the channel between the tooth root and the gum. Organisms responsible for this infection are *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Eubacterium timidum*, *E. brachy*, *Pseudomonas anerobicus,* and predominate one *P. gingivalis.* They can easily colonize the surface of the oral cavity which helps them in invading mucosal cells, altering calcium flux in epithelial cells and in releasing toxins. As a result, plaque (a climax biofilm community) is formed within 2–3 weeks. Calculus or tartar is the mineralized plaque which acts as a resistance against the antimicrobial activity of saliva in protecting tooth enamel, as a consequence of which dental carries and periodontal diseases occurs [5].

Dental plaque or biofilm cannot be eliminated, only their pathogenic nature can be minimized by minimizing the bioburden and effectively maintain a normal oral flora via oral hygiene methods [6, 23].

8.7 Osteomyelitis

Osteomyelitis is an inflammatory bone disorder characterized by infection in bone/bone marrow which leads to necrosis and bone destruction [24, 25]. When complex multi-resistant biofilm has established, treatment of osteomyelitis becomes more challenging. Due to increased bacterial resistance to antibiotics in biofilm mode, they cause persistent infections. It has been found that in more than 50% osteomyelitis cases, causative organisms are *S. aureus* and *S. epidermidis* [24].

Although, endoprostheses which are found to be an increasingly common source of infection, surgically implanted devices or other implants like orthopedic internal fixation devices also represents a remarkable risk factor for the development of osteomyelitis. Stainless steel, titanium, titanium alloys are most commonly used materials in implants in which stainless steel is found to be associated with greater infection rate as compared to titanium. A possible reason of this is might be that soft tissues get firmly adhered to a titanium-implant surface while a fibrous capsule is formed enclosing a liquid filled space around the steel implants. This unvascularized space is less accessible to host defense mechanisms where bacteria can multiply and freely spread. Studies showed that *S. aureus* and *S. epidermidis* adhesion to the surface can be reduced by the use of coatings based on human proteins such as albumin or human serum. Coatings of poly(1-lysine)-grafted-poly(ethylene glycol) (PLL-g-PEG) when extensively studied for use in biomedical applications, it has been found to be highly effective in reducing the absorption of blood serum, blood plasma and single proteins like fibrinogen and albumin. Fibroblast and osteoblast cell adhesion get remarkably reduced by spreading of metal oxide surface coated with PLL-g-PEG in comparison to uncoated surfaces [25].

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