Antimicrobial: research, bioknowledge, education chapter on biofilm

Pathak S[1]*, Sushmitha S[1], Ganesan J[1], Murugesan R, Marotta F[2]*, Bissi L[2], Banerjee A[1]

[1]Department of Allied Health Sciences, Chettinad Hospital & Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai-603103, India.
[2]ReGenera Research Group for Aging Intervention, Milano, Italy and San Babila Clinic, Healthy Aging Unit by Genomics and Biotechnology, Milano, Italy
* Corresponding to: fmarchimede@libero.it and Surajit.pathak@gmail.com

This chapter discusses about the formation of biofilms, how the microbial cells adhere to the surfaces, its susceptibility towards antimicrobials, control and eradication methods to step away from it. Biofilm is the accumulation of microbial cells that is irreversibly linked with a surface and normally enclosed in a matrix of polysaccharide substances. These biofilms are generally responsible for chronic bacterial infection, contamination of food and deterioration of water quality. Bacterial appendage is mediated by fimbriae, pilli, EPS and flagella that form a bridge between bacteria and conditioning film. Bacteria within biofilms expand genetically, even during short-term growth. Such genetic changes influence the numerous traits of bacteria growing in biofilms, which can be examined even after these bacteria are removed from biofilm surroundings. Recent genomic and proteomic studies have identified many of the genes and gene products differentially expressed during biofilm formation, disclosing the complexity of this developmental process. Novel strategies are generally necessary because of restriction to treatments like inadequate control supply, disease transfer and acquiescence of issues. The main tool that can be used for the control of biofilms is antibiotics. But the role of antibiotics is questioned since the biofilms are being resistant to it. So, there is a vital need in the near future to develop many alternative antimicrobials to replace antibiotics and thus this chapter gives enough knowledge in antimicrobials which focuses towards research and education.

Keywords: Biofilm, polysaccharide, antimicrobials, deterioration, eradication

1. Formation of Biofilms

Biofilms are comprised of microorganisms which are generally anchored to the surfaces. They are usually embedded in a polymeric matrix for their individual synthesis. The matrix contains nucleic acids, proteins and also polysaccharides which are collectively known as extracellular polymeric substance (EPS) [1]. The matrix will allow the cells to stick to each other in a biofilm and it is the major evolvement of the three-dimensional complex anchored communities. Their function is generally similar to tissues. The water channels will be disseminated throughout the biofilms to interchange all the nutrients, metabolites and excretory or unwanted products (wastes). This biofilm formation is a very complex process which is actually needs a synchronized action of various regulatory proteins. These biofilms can almost form anywhere where the sites include inorganic materials (both natural and man-made), minerals and metals, medical implants and also organic surface like body tissues. The surface of biofilms acts as a support material and also has an energy source (for example: organic carbon source) [2].

The unique characteristic feature of biofilm is that it is continuously permeated with water [3-4]. They account for around 80 percent of the microbial infections in our body. These bacterial biofilms are usually extensive in wet surfaces. They are ample enough to view through the naked eye and it extends to have a protective growth mode so that it continually survives in an antagonistic environment [5-7]. The gene expression pattern varies according to different region of the biofilm. The growth of biofilm is very slow and thus the microbial infections that arise are often deliberate to induce symptoms [8].

It has been recently described that the non-tuberculous mycobacteria comprises of extracellular DNA present in the matrix of biofilm that mainly accord to colonization, endurance and drug tolerance [9-12]. Generally, the microbial cells emancipates antigen and restore the production of antibodies, but those antibodies are not that effective in terminating the bacteria within the biofilms and thus it further cause immune damage to neighboring tissues [13-17]. When the cell density of the bacteria increases in the biofilm, it shows the ability to communicate between each other through cell-to-cell signals. This leads to the extravasation of molecules with less molecular weight capacity which signals when a particular threshold has been reached by cell population [18-20]. This process is considered as quorum sensing which is mainly responsible for the expression of many virulence factors. Under hostile conditions of the environment, it forms endospores where they can survive in temperatures greater than 100°C without being damaged [21]. They are not only occurring on foreign bodies, but also elicit infection on the structure of human or even animal body itself. Some of the microbial infections regarding biofilms include dental implant, gastrointestinal and urogenital tracts, peritoneal membrane and dialysis catheters, cardiac implants, etc. Most of the biomedical systems are susceptible to bacterial contamination and formation of biofilms [22].

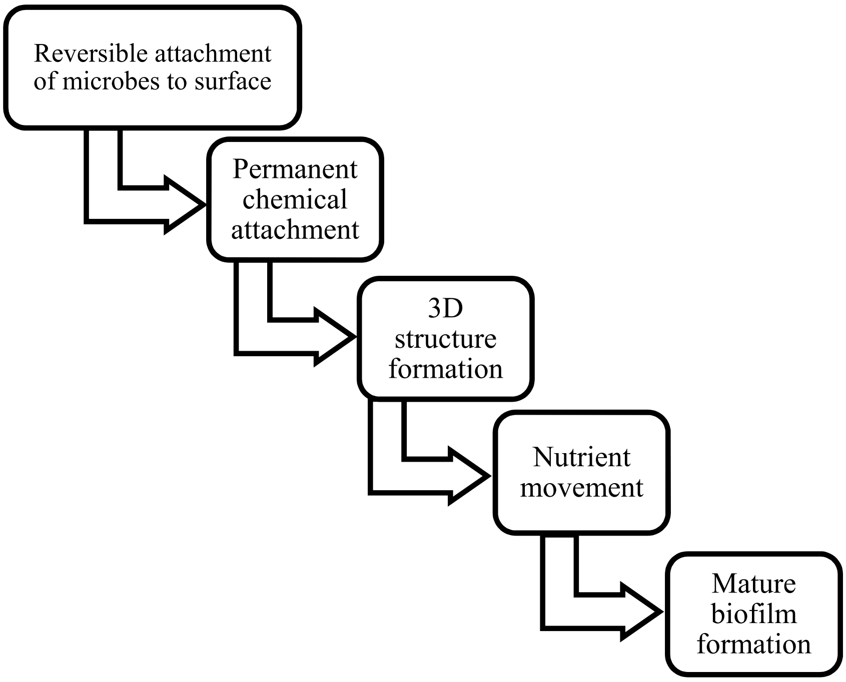

Figure 1: Stages of biofilm formation

2. Properties and unique functions of biofilms

Biofilms are not basically the microbial cells that are stranded at surfaces but they are significantly different in several ways. There is no solitary description regarding how the biofilms generally arise and what type of advantages they dispense to inhabitant microbes. Inclusively, the defensive mechanism seems to be well defined from those that are accountable for the antibiotic resistance in planktonic cells. Substandard antimicrobial penetrance, limitation of oxygen and nutrients, slow growth, etc is the defense mechanisms generated by biofilms for the provocations from outside. The bacteria present in the biofilms generally expand well even though there is less growth. These genetic changes thus impact various other traits of microbes propagating in the biofilms which can be actually noticed even when the bacteria has been removed from the biofilm. The genetic assortment within the biofilm assumes to produce protection under altering environmental conditions [23-24].

3. Adhesion of microbes

The formation of biofilms and adhesion of microbes are considered to have impact in controlling of biofilm related infections. These bacteria promptly habituate to the extracellular conditions by forming communities like biofilms. The microbes that are hiding in crevices, those that are adhered and those microbes which are responsible for forming biofilms may elude out of disinfection protocols and they remain as a source of recontamination of all the food products during the processing time. The adherence of microbes and biofilm formation may also cause corrosion and less heat transfer [25-27].

4. Mechanism of adhesion

4.1 Bacterial adhesion to surfaces

There is an organized sequence in attaching of microbes to the surfaces by accumulation of particular adhesive proteins which binds to the surface in reversible manner. This consecutive accumulation of cells further develops strong binding by cells cohesion and binding proteins. The extracellular enzymes are primarily used to hydrolyze cell adhesion molecules. The adhesion of bacteria is directly linked to adsorption of proteins [28].

The adhesion to the surfaces depends on various biological, microbiological, physical and chemical parameters. Interestingly, the main parameter is the surface topography which is actually broadly discussed. The microbes which are encased in the biofilms are impervious to the immunological defense mechanisms of the body. When they come in contact with a solid surface, they instigate the expression of bacterial enzyme which generally assists in colonization and protection. There are various parameters used to distinguish the material surface based on the two-dimensional features such as roughness average, maximum peak to valley height in sample length and average maximum profiler height. Nowadays, atomic force microscopy and scanning electron microscopy are most probably used to give out three-dimensional visuals regarding the surface topography [29].

4.2 Immune responses for biofilm formation

Treatment of biofilms is becoming very tough with the antimicrobials and the antibiotic resistance is getting increased upto thousand fold when it is grown in planktonic conditions. The immune responses are pointed towards the antigens that are present on the outer side of the biofilm. Salivary proteins, antibodies, etc does not able to perforate into the biofilm and thus the cells remain to be concealed inside from antibodies. Due to the presence of biofilms, it will regulate the synthesis of cytokines and intrude the generation of antibodies. It is becoming very difficult for the phagocytes to inundate the bacteria that are grown within the matrix which has been attached to the surface. This in turn makes the phagocytes to release many cytokines before leading to inflammation and destroying of neighboring tissues. Biofilms assists in genetic diversity and sustain the increased cell density needed for the genetic exchange. Most of the bacteria have plasmids where they can be transferred horizontally to other species by conjugation method. The expanded knowledge and proofs of the phenotype evolves majorly from *in vitro* studies in increased numbers and thus it is considered to be a helpful tool for biofilm dispersal [30-31].

5. Development of Biofilms on medical devices

The development of biofilms on medical devices has risen from the studies in which the devices were either scrutinized upon unfasten from patients or were demonstrated in animal systems. The scanning electron microscope (SEM) is used to display that central venous catheters detached from patients were usually colonized by biofilms [32-34].

Many investigators used culture media and also other techniques to separate, culture and then determine the biofilm related microbes from the medical devices [35].

Table 1: Biofilm development on medical devices

Endotracheal tubes
Prosthetic joints
Pacemakers
Voice prostheses
Central venous catheters
Central venous catheters
Intrauterine devices
Peritoneal dialysis catheters
Urinary catheters
Mechanical heart valves
Contact lenses
Central venous catheter needleless connectors

To remove biofilm mass from devices, artificial hip prostheses is investigated using sonication procedure. These biofilm aggregates are visualized using confocal laser scanning microscope. Even echocardiography technique is used to visualize in prosthetic heart valves. The type of organism that forms biofilms includes a large number of known pathogens [36-37].

6. Factors responsible for Biofilm formation

Biofilms are developed in a broad range of surfaces which includes living tissues, medical devices, water system, etc. The biofilm formation on water system is very complex because it comprises of corrosion products, clay, filamentous bacteria and water diatoms.

Various factors which are responsible as follows:

6.1 Effect of substratum

The microbial colonization seems to be increase as the roughness of the surface increases. Generally, maximum adherence depends on the increased surface free energy. For example: stainless steel and glass. These surfaces tend to display greater bacterial adherence when compare to hydrophobic surfaces like Teflon, etc.

6.2 Hydrodynamic condition

The biofilms are investigated under different conditions such as laminar as well as turbulent flow. Biofilms developed under laminar flow remains to have patch type and they comprise of rough cell mass unfasten by interstitial voids. The formation under turbulent flow is also found to be patchy but they are elongated streamers type that tends to move to and fro in bulk fluid.

6.3 Horizontal method of gene transfer

This method is very significant in evolution of natural microbial communities. The genetic elements are the one that allows for gene transfer between microbes. The elements include transposons, bacteriophages, plasmids, etc. The microbes present express varying phenotypic characters because there are various genes that are transcribed in biofilm linked phases of life cycle.

6.4 Quorum sensing

In cell adherence, cell to cell signaling plays a major role and the communication between microbes can be mediated by microbial products that have the ability to diffuse from one cell to other cell. The generation of quorum sensing molecules is known as acyl-homoserine lactone. In addition to the above factors mentioned the cell factors such as fimbriae, flagella, cell surface hydrophilicity, etc influence the adherence of microbial cells.

6.5 Features of Aqueous medium

The main basic features include pH, temperature, ionic strength, level of nutrients, etc. These characteristics play a major role in attachment of microbes to the surfaces. The adherence and biofilm development in aqueous medium are affected by various seasons [38-39].

7. Effect of Biofilms on deterioration of water quality

During the storage of water, the deterioration of the quality of water is the main problem which is generally experienced by the water suppliers. The final quality of water can be determined only by the distribution system. When the treated water is flowed through the distribution system, it normally gets affected by various adverse conditions. The main chance of deterioration is by the entry of microbes from outer source into the distribution network through open reservoirs and as well as breakage because of the advanced pipeline construction which may perturb the already existing system. There is increase in the number of microbes because it starts regrowing inside the system and thus results in formation of biofilms. The material gives the ability to supply nutrients to the microbes on which the biofilm is formed. The biofilm formation depends on multiple factors like temperature, disinfectant type, nature of material, etc.

When the disinfectants are used in suitable concentrations, then it turns out to be effective in detaching the microbes. This also strengthens the formation of biodegradable substances which can be used as an energy source for assisting in biofilm formation. Once the microbe's become resistant towards disinfectant, they begin to exist even in very higher concentrations. Resistance is developed by microbes due to the procurement of the gene. Examples of disinfectants include chlorine, hydrogen peroxide, ozone, etc. Hydrogen peroxide is the most potent controller of formation of biofilms throughout the distribution system rather than the chlorine. Chlorine is used only at the final stage to ensure for the protection and followed by monochloramine to fortify the determined concentration of disinfectant for the distribution system [40-41].

8. Susceptibility testing towards anti-microbial agents

There are different protocols for assessing the antimicrobial agents against biofilms. The method would be acceptable if it is of easy use, inexpensive, reproducible and gives out rapid results. The Calgary biofilm device which has been developed by Ceri et al. is used to test the antimicrobial agents against the biofilms of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The grown biofilms on pegs are tested by means of a 96 well plate apparatus [42].

This device was found to be advantageous, reproducible and inexpensive. The similar way of devices has to be developed to assess the efficacy of antimicrobial agents against the formation of biofilms [43].

9. New approaches to control the formation of biofilms

During the formation of biofilms, the pathogenicity and virulence state of the microorganisms begin to frequently display an enhanced effect. Thus, this led to the emergence of finding new approach to control the formation of biofilms. Most of the microbes begin to occupy in the biofilm region, where it causes further problems in delineating new anti-microbial agents. So, there is a need for a different approach to cope up with the microbial infections in the biofilm. Due to the increased resistance of microbes to the inhibitors, an increased concentration of antibiotics are needed which may tend to critical damage to the environment and may cause nosocomial infections [44-45].

Considering public health concerns and economical loss linked to the formation of biofilms, there elevates a need for forming biofilm resistant systems. Generally, the biofilm formation can be controlled by altering the surface by anti-bacterial compounds which in turn reduce or halt the formation. The anti-microbial substances including antiseptics, antibiotics, enzymes, etc are used to graft on different materials where this surface exhibit anti-microbial potential.

9.1 Increasing the competence of anti-microbials

Inclusion compounds have been developed in order to reduce the increased toxicity level caused by the antiseptic agents. These inclusion compounds are advantageous interms of efficacy, increased long term activity and less antimicrobial concentrations. Cyclodextrin is the important compound that generates a hydrophobic cavity which surrounds the drug or lead molecule. These cyclodextrins (CD) are the water soluble oligosaccharides which are formed by 6, 7 and 8 glucopyranose units. They are also non-hygroscopic and non-reducing agent. These compounds are often termed to be a truncated cone, which dispenses to the cyclodextrins a hydrophilic exterior part and the hydrophobic cavity that permits to form supramolecular complexes which is stabilized by vanderwaal's forces, hydrophobic interactions and hydrogen bonds. The cavity would accommodate say another drug for forming the inclusion compounds through host-guest interactions. This inclusion complex increases the guest's stability against the dehydration. These CD's shows a better curative efficacy of deprived water-soluble drugs, increased physical and chemical stability and secure the guest agents from degrading in the gastrointestinal tract. By understanding the properties of antimicrobial surfaces at nano level would help in developing new compounds [46].

9.2 Modification of physical and chemical surfaces

The surface is most important for biofilm formation where the physicochemical factors regulate in the initial attachment of the microbes to the surface. The general concept in biofilms is that it gets colonized in the surface which is hydrophobic and in contrast it is subjected to the hydrophilic surfaces. All the materials are forced to the microbial contaminations when they are exposed to air and humidity. To solve these problems, creation of coatings which are of antimicrobials were followed. The non-fouling nature of those created coatings helps in preventing or reducing the attachment of microbes further to the surfaces. In a study of titanium surfaces, they found that the roughness in the surface increases the attachment or adherence of bacteria. The parameters such as surface charge, zeta potential and surface energy were measured and they concluded that the topography was found to be the most important and effective factor on adherence of bacteria. Previously, the copper-nickel alloys were used in the marine environment because of the increased corrosion. The usage of copper-nickel bilayer and multifilms on the surface helps in preventing the adhesion of microbes. Silver dressings are utilized nowadays for chronic wound infections because of the antibacterial properties, even though more studies on chronic wound management for long-term is needed [47-49].

9.3 Treatment of biofilms by Bacteriophages

Bacteriophages shortly termed as phages, is a virus which infects bacteria. It has outer capsid region enclosed with genetic material. For the entry into a host cell, the bacteriophage assembles on the bacteria's receptors which are present on the surface of the bacteria. Phages are considered as anti-bacterial agents, but still western countries presume that they are not scientifically viable. Scientists are working on the ability of bacteriophage to infect the microbe present in the biofilm, which is a polysaccharide lyase enzyme that has an ability of degrading polysaccharides in the biofilm.

Pseudomonas sp. was the first to be infected by bacteriophage by utilizing polysaccharide lyase enzyme where inturn it degrades the polysaccharide in the biofilm.

9.4 Prevention of biofilms by antibodies

From the studies of Hermans on antibody technology showed that, the antibodies are coated on the medical devices to cure particular microbial infection. The coated antibodies are inserted or placed into the humans. These antibodies are then used to treat naturally occurring biofilms during the infection stage [50].

9.5 Bacterial amyloid disruption

Bacteria generally contain amyloid fibers on their cell surface. More bacterial amyloids tend to formation of biofilms on the surface. *Escherichia coli* and species of *Enterobacteriaceae* generated an amyloid fiber termed as Curli. This fiber promotes formation of biofilms in E.coli surfaces whereas the analog FN075 arrests the genesis of the amyloid fiber and turns out to be a anti biofilm on these compounds [51].

10. Conclusion

From the public health concern, the role of biofilms in resistance to antimicrobial drugs is of much importance. The microbes that reside in the biofilms are resistant towards the antimicrobial agents which normally produce a great threat to the pharmaceutical industries. Thus, preventing the formation of biofilms is a better way than executing the treatment strategy. Nowadays, the major tool for controlling biofilms is antibiotics. But this substance has been questioned due to the resistance of known biofilms. There is an essential need for antimicrobials to substitute the antibiotics for treating the entire bacterial diseases. Choosing an effective control agent is very critical due to the microbial contamination. So finding the potent source of contamination can dispense the required disinfection measures. A better understanding of the biofilm formation and its major role in infection should actually improvise the clinical decision making and dispense for future research on control strategies [52].

References

[1] Boles, BR, Thoendel M, Singh PK. Self-generated diversity produces “insurance effects” in biofilm communities. *Proc. Natl. Acad. Sci.* 2004; 101: 16630-16635.
[2] Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* 2002; 56: 187-210.
[3] O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. *Annu Rev Microbiol*. 2000; 32:49-79.
[4] Teixeira K, Bueno A, Cortés ME. Processos Físico-Químicos no Biofilme Dentário Relacionados àProdução da Cárie. *Química Nova na Escola.* 2010; 32: 145-50.
[5] Costerton JW, Stewart PS, Greenberg EP. Bacterial Biofilms: A Common Cause of Persistent Infections. *Science.* 1999; 284: 1318-22.
[6] Kolter R, Losick R. One for all and all for one. *Science.*1998; 280: 226-7.
[7] Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S. Gene Expression in Pseudomonas Aeruginosa Biofilms. *Nature*. 2001; 413: 860-64.
[8] Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial Biofilms. *Annu Rev Microbiol*. 1995; 49: 711-45.
[9] Flemming HC, Wingender J. The biofilm matrix. *Nat Rev Microbiol.* 2010; 8: 623-633.
[10] Zambrano MM, Kolter R. Mycobacterial biofilms: a greasy way to hold it together. *Cell*. 2005; 123:762–764.
[11] Ojha A, Anand M, Bhatt A, Kremer L, Jacobs WR, Hatfull GF. GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. *Cell.* 2005; 123:861-873.
[12] Ojha AK, Baughn AD, Sambandan D, Hsu T, Trivelli X, Guerardel Y, Alahari A, Kremer L, Jacobs WR, Hatfull GF. Growth of Mycobacterium tuberculosis biofilms containing free mycolic acids and harbouring drug-tolerant bacteria. *Mol Microbiol.* 2008; 69:164-174.
[13] Freeman R, Geier H, Weigel KM, Do J, Ford TE, Cangelosi GA. Roles for cell wall glycopeptidolipid in surface adherence and planktonic dispersal of Mycobacterium avium. *Appl Environ Microbiol.* 2006; 72: 7554-7558.
[14] Nessar R, Reyrat JM, Davidson LB, Byrd TF. Deletion of the mmpL4b gene in the Mycobacterium abscessus glycopeptidolipid biosynthetic pathway results in loss of surface colonization capability, but enhanced ability to replicate in human macrophages and stimulate their innate immune response. *Microbiology*. 2011; 157:1187-1195.
[15] Recht J, Kolter R. Glycopeptidolipid acetylation affects sliding motility and biofilm formation in Mycobacterium smegmatis. *J Bacteriol.* 2001; 183: 5718-5724.
[16] Yamazaki Y, Danelishvili L, Wu M, Macnab M, Bermudez LE. Mycobacterium avium genes associated with the ability to form a biofilm. *Appl Environ Microbiol.* 2006; 72: 819-825.
[17] Chen JM, German GJ, Alexander DC, Ren H, Tan T, Liu J. Roles of Lsr2 in colony morphology and biofilm formation of Mycobacterium smegmatis. *J Bacteriol.* 2006; 188: 633-641.

[18] Ma L, Jackson KD, Landry RM, Parsek MR, Wozniak DJ. Analysis of Pseudomonas aeruginosa conditional Psl variants reveals roles for the Psl polysaccharide in adhesion and maintaining biofilm structure post attachment. *J Bacteriol*. 2006; 188: 8213-21.
[19] Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science.* 1998; 280: 295-8.
[20] Schauder S, Bassler BL. The Languages of Bacteria. *Genes Dev.* 2001; 15: 1468-80.
[21] Fuqua WC, Winans SC, Greenberg EP. Quorum Sensing In Bacteria: The Luxr-Luxi family of cell density-responsive transcriptional regulators. *J Bacteriol.* 1994; 176: 269-75.
[22] Steinhauer K. Antimicrobial efficacy and systematic use of disinfectants. Current research, technology and education topics in Applied Microbiology and Microbial Biotechnology. 2010; 369 - 76.
[23] Furukawa S, Kuchma SL, O'Toole GA. Keeping their options open: acute versus persistent infections. *J Bacteriol.* 2006; 188: 1211-1217.
[24] Gonzalez Barrios AF, Zuo R, Hashimoto Y, Yang L, Bentley WE, Wood TK. Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, B3022). *J Bacteriol.* 2006; 188: 305-316.
[25] Marriott NG, Gravani RB. Principles of food sanitation. Fifth edition. New York, *Springer*. 2006.
[26] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial Biofilms: From the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004; 2: 95-108.
[27] Criado MT, Suarez B, Ferreiros CM. The Importance of bacterial adhesion in the dairy-Industry. *Food Technology.* 1994; 48: 123-6.
[28] Lewin R. Microbial adhesion is a sticky problem. *Science.*1984; 224: 375-7.
[29] Costerton JW, Lappin-Scott HM. Behavior of bacteria in biofilms. *ASM News*. 1989; 55 650-4.
[30] Pavithra D, Doble M. Biofilm formation, bacterial adhesion and host response on polymeric implants-Issues and Prevention. *Biomed Mater.* 2008; 3: 1-13.
[31] Heukelekian H, Heller A. Relation between food concentration and surface for bacterial growth. *J Bacteriol.* 1940; 40: 547-58.
[32] Raad I, Costerton W, Sabharwal U. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. *J Infect Dis.* 1993; 168: 400-7.
[33] Anaissie E, Samonis G, Kontoyiannis D. Role of catheter colonization and infrequent hematogenous seeding in catheter-related infections. *Eur J Clin Microbiol Infect Dis.* 1995; 14: 134-7.
[34] Ganderton L, Chawla J, Winters C. Scanning electron microscopy of bacterial biofilms on indwelling bladder catheters. *Eur J Clin Microbiol Infect Dis*. 1992; 11: 789-96.
[35] Wolf AS, Kreiger D. Bacterial colonization of intrauterine devices (IUDs). Arch Gynecol 1986; 239: 31-7.
[36] Everaert EPJM, Van de Belt-Gritter B, Van der Mei HC. In vitro and in vivo microbial adhesion and growth on argon plasma-treated silicone rubber voice prostheses. *J Mat Science: Mat in Med.* 1998; 9: 147-57.
[37] Saidi IS, Biedlingmaier JF, Whelan P. In vivo resistance to bacterial biofilm formation on tympanostomy tubes as a function of tube material. *Otolaryngol Head Neck Surg.* 1999; 120: 621-7.
[38] Donlan RM. Biofilms: Microbial life on surfaces. *Emerg Infect Dis*. 2002; 8: 881-890.
[39] Davey EM, Otoole AG. Microbial biofilm: From ecology to molecular genetics, *Microbiol Mol Biol*. 2000; 64: 847-867.
[40] Overman PR. Biofilm: A new view of plaque. *J Cont Dent Prac.* 2000; 1: 2-7.
[41] Bishop PL. Biofilm structure and kinetics. *Water Sci Technol.* 1987; 36: 287-294.
[42] Donlan RM. Role of biofilms in antimicrobial resistance. *ASAIO J*. 2000; 46: S47–52.
[43] Ceri H, Olson ME, Stremick C. The Calgary biofilm device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol*. 1999; 37: 1771–6.
[44] Cortes ME, Pedroso MA, Sinisterra R. BR PI0405347 (A) - Processo De Preparação De Géis Mucoadesivos Para Prevenção De Cárie, Usos E Produtos Derivados.
[45] Yue IC, Poff J, Cortes ME, Sinisterra RD, Faris CB, Hildgen P. A novel polymeric Chlorhexidine delivery device for the treatment of periodontal disease. *Biomaterials.* 2004; 25: 3743-50.
[46] Cheng G, Xite H, Zhang Z, Chen S, Jiang S. A switchable biocompatible polymer surface with self-sterilizing and non-fouling Capabilities. *Angewandte Chemie-International Edition.* 2008; 47: 8831-4.
[47] Truong VK, Rundell S, Lapovok R, Estrin Y, Wang JY, Berndt CC. Effect of ultrafine-grained titanium surfaces on adhesion of bacteria. *Appl Microbiol Biotechnol*. 2009; 83: 925-37.
[48] Gabashvili IS, Khan SA, Hayes SJ, Serwer P. Polymorphism of bacteriophage T7. *Journal of Molecular Biology.* 1997; 273: 658-67.
[49] Burton G, Raul R, Elizabeth K. Basic phage biology. *Bacteriophages: CRC Press*; 2004.
[50] Jonas K, Edwards AN, Simm R, Romeo T, Romling U, Melefors O. The RNA binding protein Csra controls cyclic di-GMP metabolism by directly regulating the expression of GGDEF proteins. *Molecular Microbiology.* 2008; 70: 236-57.
[51] Leshner AI. Innovation needs novel thinking. *Science.* 2011; 332: 1009-1009.
[52] Rodney M. Donlan Biofilm Formation: A Clinically Relevant Microbiological Process. *Clinical Infectious Diseases.* 2001; 33:1387–92.