Nishita Jain et al. / Journal of Pharmacy Research 2016,10(1),50-53

Review Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Biofilm Formation on Contact Lenses by Bacterial Pathogens

Nishita Jain, Preeti Bhosale, Vidya Tale*

Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth Deemed University, Pune-411046,Maharastra, India

Received on:05-10-2015; Revised on: 22-12-2015; Accepted on: 06-01-2016

ABSTRACT

Biofilm is an assemblage of microbial cells that is irreversibly associated with the surface and usually enclosed in a matrix of polysaccharide material. The process of any contact lens related keratitis usually starts when opportunistic pathogens binds to the cell surface. Lens material characteristics influence bacterial adhesion to lens surface. Various factors affect biofilm formation on contact lens such as substratum effect, conditioning film, hydrodynamics, quorum sensing etc. The effect of bacterial adhesion on lens varies amongst individuals. This is due to difference between the errors, responses of bacterial strain and the ability of certain tear film protein when bound to the cell surface to kill certain types of bacteria. Prevention of biofilm formation is a necessary step in the successful prophylaxis of infections. Polymer surface modification is one approach to inhibit bacterial adhesion.

KEYWORDS: Biofilm, Contact lenses, Bacterial pathogens

INTRODUCTION

A biofilm is a well organized, cooperating community of micro organisms and is usually enclosed in a matrix of polysaacharide material. It is composed primarily of microbial cells and extra cellular polymeric substances (EPS). A biofilm is developed when microbial cells attach to the surface. They require intracellular signaling and a new set of genes are synthesized. Multiple steps are involved in biofilm formation, such as formation of conditioning layer, bacterial adhesion, bacterial growth and biofilm expansion. Bacterial adhesion is mediated by flagella, pilli, frimbriae and EPS that act to form a bridge between bacteria and the conditioning film. Biofilms can exist on all types of surfaces such as metal, glass, plastic, wood, soil particles, tissues, medical implant materials and food products [1].

The use of plastic corrective contact lenses (CL) worn directly over the cornea was developed to improve vision and to overcome the inconvenience of wearing conventional spectacles. The use of contact lenses (CL) had increased remarkably because of its optical, occupational and cosmetic advantages. Common bacteria flora found on eyes included *Staphylococcus epidermidis, Staphylococcus aureus, Enterobacter* and *Pseudomonas sp* and hence also observed on soft contact lenses[2]. Approximately 100 million people use contact lenses as an alternative to spectacles (1.6%). As the contact lens market continues to grow, public health issues associated with use of contact lens have become increasing important. The most devastating complication of using contact lens is infectious keratitis and may result in permanent vision loss from corneal scarring or perforation. In spite of advances in lens design and materials, infection by pathogenic bacteria is a vision-threatening problem. Insufficient knowledge, improper handling and use of lens are the important factors for such infections [3].

***Corresponding author.**
Vidya Tale
Rajiv Gandhi Institute of IT and Biotechnology,
Bharati Vidyapeeth Deemed University,
Pune-411046,Maharastra, India

FORMATION OF BIOFILM

Biofilm comprises primarily of micro colonies of different species of microbial cell (+15% by volume) and of matrix material (+85%). EPS primarily consists of polysaccharides but may vary in physical and chemical properties. Polysaccharides are neutral or polyanionic. The presence of uronic acid confers anionic properties. Backbone of EPS contains 1,3- or 1,4- ß linked hexose residues. Different organism produces varying amount of EPS and it increases with the age of biofilm. EPS may associate with macro molecules (DNA, lipid, protein etc), divalent cations and metal ions. Nutrient status of growth medium affects EPS production that is excess carbon and reduced amount of nitrogen, potassium and phosphate. The technique used to directly observe biofilm without disrupting the community is confocal scanning laser microscope (CSLM). CSLM allows visualization of fully hydrated sample and also reveals the elaborate three dimensional structure of biofilm.

ROLE OF CONTACT LENS MATERIAL IN BIOFILM FORMATION

Ever since the introduction of soft contact lenses, thorough research

has been conducted to investigate and improve soft lens materials. Silicone hydrogel lenses were introduced in 1999 due to the low oxygen transimmibility of hydrogel lenses. Lens material characteristics influence bacterial adhesion to lens surface. The following are the distinguishing lens material characteristics and their effect on bacterial adhesion [4].

Several authors have observed that adhesion of *P. aeruginosa* to lenses composed of nonionic polymers is high as compared to those with ionic polymers [5, 6]. Initial adhesion of *S. aureus* was higher to an ionic hydrogel compared to a non-ionic hydrogel [7]. Soft contact lens materials are classified by Federal Drug Administration as being of high water content (more than 50%) or low water content (less than 50%). Results of research into bacterial adhesion based on water content have been remarkably consistent with multiple studies concluding that bacterial adhesion increases inversely to the water content. Two strains of *S. epidermidis* showed increased adhesion to low water content hydogel lenses. Other scientists found the same effect for strains of *P. aeruginosa* although few reported no strict correlation between water content and adherence. However, two studies could not establish any relation between water content and adhesion. Silicone hydrogel lenses are all of relatively low water content. Polymer composition and surface hydrophobicity can mask the effects of water content. Hydrophobicity is a crucial contact lens surface property. Hydrophobic surfaces attract greater numbers of bacteria than hydrophilic surfaces. Bacteria attach with higher affinity to low-energy hydrophobic surfaces than to high energy hydrophilic surfaces. Hydrophobicity of silicone hydrogels such as Lotrafilcon A, Balafilcon A, or Lotrafilcon B has been reported to be higher as compared to hydrogel lenses such as Etafilcon A. *In vitro P.aeruginosa, S.aureus*, or *S. epidermidis* adhere in greater numbers to the hydrophobic silicone hydrogel lenses. It is probable that hydrophobic bacteria prefer adhering to hydrophobic lenses whereas hydrophilic bacteria adhere well to hydrophilic lenses. Atomic force microscopy can be used to determine lens topography and roughness. Comfilcon A and Omafilcon A lens materials had relatively smooth surfaces compared to Senofilcon A, Nelfilcon A and Ocufilcon B. *S. epidermidis* showed stronger adhesion to the materials with higher surface roughness. Similarly with *S. epidermidis,* when adhering to surfaces with similar hydrophobicity but differing surface roughness was examined, a higher surface roughness resulted in an increase in bacterial adhesion. There are no reports investigating a link between adhesion of *P. aeruginosa* and lens surface roughness. The hydrophobicity of silicone hydrogel lenses show reduces after wear. Worn hydrogel and silicone hydrogel lenses usually exhibit higher degrees of roughness. Therefore, these changes in surface characteristics of lenses during/ after wear may influence bacterial adhesion. The presence of sorbed protein can increase adhesion of *S. epidermidis*.

In vitro Pseudomonas adhesion is highly correlated with the number of large (more than 150 µm) focal deposits on the lens after wear [8]. In contrast, though enzymatic cleaning is recommended for protein deposition, it does not appear to considerably reduce the adhesion of *P. aeruginosa* to lens surfaces [9]. Bacteria were found to be adhered in lower numbers and less tenaciously to worn lenses, except *S. aureus* 835 adhering in higher numbers to worn balafilcon A lenses. However, Etafilcon A increases with length of contact lenses wear reducing the viable *Staphylococcus* adhering to lense [10,11]. Fascinatingly, *P. aeruginosa strain* 6294 adhere to a greater extent to the unworn Etafilcon A lenses than to one month continuously worn lenses. *In vitro* adhesion of *P. aeruginosa* significantly varies when lenses are worn by different individuals. These differences in the effect of lens wear on adhesion may be related to difference in the bacterial strain. The presence of specific adsorbed tear proteins on worn contact lenses affects bacterial adhesion. Adhesion of *P. aeruginosa* GSU#3 to contact lenses was enhanced when the lens contained adsorbed mucin, IgA, BSA, lysozyme, and lactoferrin. Adhesion of *P. aeruginosa* strain RT-1, a corneal ulcer isolate increased when albumin was adsorbed to a lens surface. However, the presence of lactoferrin increased the total numbers of *P. aeruginosa* adhering to lenses but reduced their viability, killing the attached bacteria. Lenses coated with secretary phospholipaseA2 show reduced adherence of *S. aureus* [12]. The ability of the tear film lipids cholesterol or phospholipids to modulate bacterial adhesion has been measured [13]. Bacterial adhesion is not affected due to lipid adhering to lenses. Generally, it is the lens surface hydrophobicity and roughness, as well as polymer characteristics such as water content and iconicity that appear to modulate bacterial adhesion. The effect of lens wear is not constant and may be affected by the individual who has worn a lens and the types of proteins deposited on the surface.

FACTORS AFFECTING BIOFILM FORMATION

Different factors affecting the biofilm formation are as follows:

1) Substratum effect

Increase in surface roughness increases microbial colonization. Maximum attachment depends on wettability of surface or high surface free energy. Stainless steel and glass have high surface free energies and are hydrophilic. These surfaces show greater bacterial contamination than hydrophobic surfaces.

2) Conditioning film

Solid surfaces become unconditional or coated with polymers when

exposed in an aqueous medium. The rate and effect of microbial attachment is affected by the chemical modification of surfaces. For example- organic soil adheres to the oxide, producing a conditioned substratum to which bacteria adhere. A number of host produce conditioning films such as blood, saliva, tears, urine, intravascular fluid and respiratory secretion influence the attachment of bacteria to bio materials[14].

3) Hydrodynamics

Biofilms have also being examined under various hydrodynamic conditions such as turbulent and laminar flow. In flow conditions, biofilm response is altered. Biofilms are found to be patchy with rough cell aggregates when grown under laminar flow. Biofilms grown under turbulent flow cells are also patchy but are elongated streamers that move to and fro in the bulk fluid[15]. Cell size and cell motility also affect association of cells with the surface.

4) Characteristics of aqueous medium

pH, nutrient levels, ionic strength, temperature etc. play an important role in the rate of microbial attachment to surfaces. Bacterial attachment and biofilm formation is also affected by environmental conditions. Increase in concentration of certain cations such as sodium, calcium, lanthanum, ferric ions affect the attachment of *P. fluorescence* by reducing the repulsive forces between cell and the glass surface.

5) Horizontal Gene Transfer

Horizontal gene transfer is important for the evolution and genetic diversity of natural microbial communities. Evolution results in the attainment of new genetic traits via horizontal gene transfer [16]. Horizontal gene transfer between bacteria is mediated by mobile genetic elements. These elements can be bacteriophages, transposons or conjugative plasmids. Bacteria in biofilms express different phenotypic characters from planktonic counterparts. This is due to different genes transcribed in the planktonic and biofilm associated phases of bacterial life cycle.

6) Quorum Sensing

Cell to cell signaling has recently been demonstrated to play a role in cell attachment. Bacterial products that are able to diffuse away from one cell and enter into another cell are used to carry out intracellular communication between bacteria. [17] Production of quorum sensing molecules is called as acyl- homoserine lactone (acyl- HSL). *P. aeruginosa* is responsible for defining separation between bacterial pillars in the three dimensional structures of biofilms. The role of biofilms in multispecies greatly differs from single species. Signals can be classified as actively or passively transmitted bacterial products that alter the state of neighbouring microbes. These might include acyl- HSL, bacterial metabolites, secreted proteins, genetic material such as DNA, RNA etc. Other factors, such as cell surface hydrophilicity, flagella, presence of fimbriae and production of EPS 1 influence the rate and extent of attachment of microbial cell.

7) Biofilm examination and measurement

Various methods such as light, fluorescent, differential interference contrast (DIC), transmission electron (TE), scanning electron (SE), atomic force (AF), confocal laser scanning microscopy (CLSM) are used to study and analyze the structure of bioflms. The nature of extracellular fibres in biofilm was studied using TEM and polysaccharide stains like Ruthenium Red. Biofilms on medical devices and in human infections have been examined and characterized using electron microscopy. Microstructure and metabolism of biofilm was observed using Fluorescent *in situ* hybridization and 16-23S rRNA hybridization with CLSM. The FISH method was used to confirm decrease in the mobility of cell as biofilm ages.

PREVENTION OF BIOFILM FORMATION

Biofilms are simply a survival mechanism of bacteria. An individual bacterium comes together as a whole in order to become stronger. Prevention of biofilm formation is a necessary step in the successful prophylaxis of infections. Polymer surface modification is one approach to inhibit bacterial adhesion. Polymer modification was investigated by glow discharge treatment in order to study the influence of the modified surface on bacterial adherence.[18]Surface roughness, surface charge density and contact angles of the modified polymers were determined and related to the adherence of *Staphylococcus epidermidis* KH6. A correlation between the free enthalpy of adhesion (estimated from contact angle measurements) and adherence was observed although no influence of surface roughness and charge density on bacterial adherence was noticed. Independent of the nature of the polymer surface, there still exits minimum bacterial adherence. Modified polymers with negative surface charge allow minimum bacterial adherence. Polymers could be improved further by the ionic bonding of silver ions to the surface. Bacterial colonization can be prevented using these antimicrobial polymers. It is suggested that modification of polymers and subsequent surface coupling of antimicrobials might be an effective approach for the prevention of bacterial biofilm formation [19].

CONCLUSION

The microbes in the biofilm are resistant to a few antimicrobial drugs, hence it is very essential to avoid biofilm formation rather than treatment. *P. aeruginosa* can adhere to contact lenses very efficiently and hence is the most predominant microorganism which causes contact lens-associated Microbial Keratitis.

REFERENCES

1. Kokare CR., Chakraborty S, Khopade AN, Mahadik KR. Biofilm: Importance and applications. Indian Journal of Biotechnology. (2009); 8:159-168.
2. Michael Osita Emina, Faustina Kemdinum Idu. Bacteria and parasites in contact lenses of asymptomatic wearers in Nigeria. *Department of Optometry, University of Benin, Benin city, Nigeria* (2011).
3. Abbas Obaid Farhan Al-Janabi , Sarab Fawzi Al-Ani , Sarah Imad. Biofilms Formation on Contact Lenses: Clinical and Bacteriological Study. Diyala Journal of Medicine (2013); 5, 2:11-18
4. Debarun Dutta, Nerida Cole, Mark Willcox. Factors influencing bacterial adhesion to contact lenses. Molecular Vision (2012); 18:14-21.
5. Miller MJ, Ahearn DG. Adherence of *Pseudomonas aeruginosa* to hydrophilic contact lenses and other substrata. Journal of Clinical Microbiology, (1987); 1392-1397.
6. Stapleton FDJ, Matheson M, Woodward E. Bacterial adherence and glycocalyx formation on unworn hydrogel lenses. J Brit Contact Lens Assoc., (1993); 16:113-6.
7. Arciola CR, Maltarello MC, Cenni E, Pizzoferrato A. Disposable contact lenses and bacterial adhesion. In vitro comparison between ionic/high-water-content and non-ionic/ low-water-content lenses. Biomaterials (1995); 16:685-90.
8. Butrus SI, Klotz SA. Contact lens surface deposits increase the adhesion of *Pseudomonas aeruginosa*. Curr Eye Res (1990) 9:717-24.
9. Boles SF, Refojo MF, Leong FL. Attachment of *Pseudomonas* to human-worn, disposable etafilcon A contact lenses. Cornea (1992); 11:47-52.
10. Vermeltfoort PB, Rustema-Abbing M, de Vries J, Bruinsma GM, Busscher HJ, van der Linden ML, Hooymans JM, van der Mei HC. Influence of day and night wear on surface properties of silicone hydrogel contact lenses and bacterial adhesion. Cornea (2006); 25:516-23.
11. Hume EB, Cole N, Parmar A, Tan ME, Aliwarga Y, Schubert T, Holden BA, Willcox MD. Secretory phospholipase A2 deposition on contact lenses and its effect on bacterial adhesion. Invest Ophthalmol Vis Sci (2004); 45:3161-4.
12. Babaei Omali N, Zhu H, Zhao Z, Ozkan J, Xu B, Borazjani R, Willcox MD. Effect of cholesterol deposition on bacterial adhesion to contact lenses. Optom Vis Sci (2011); 88:950-8.
13. Donlan R M, Bioflims: Microbial life on surfaces, Emerg Infect Dis, (2002); 8:881-890.
14. Allison P G, Ruiz B, Sanjose & Gillbert P, Extracellular products as mediators of the formation and detachment of Pseudomonas fluoroscens bioflim, FEMS Microbial Lett, (1998);167: 179-184.
15. Davey EM & Otoole AG, Microbial bioflim: From ecology to molecular genetics, Microbial Mol Biol, (2000);64: 847-867.
16. Koonin EV, Horizontal gene transfer in prokaryotes: Quantification and Qualification, Annu Rev Microbial, (2001);55: 709-742.
17. Watnick P & Kolter R, Bioflim, city of microbes, J Bacteriol, (2000);182: 2675-2679.
18. Ronald L. Barnes and D. Kevin Caskey, Using Ozone in the Prevention of Bacterial Biofilm Formation and Scaling. Water Conditioning and Purification Magazine (2002).
19. Jansen B, Kohnen W. Prevention of biofilm formation by polymer modification. Journal of Industrial Microbiology (1995);15, 4: 391-396.

Source of support: Nil, Conflict of interest: None Declared