# Adhesion of Biofilm forming Staphylococcus epidermidis strains on Intraocular Lenses – An Update

**S. Ahmad**, **H. Ashraf** and **S.M. Akram**

Institute of Ophthalmology, Aligarh Muslim University, Aligarh, 202002, India

This mini review contains a brief update on the pathogenicity of *Staphylococcus epidermidis* in ocular infections and it's capability of biofilm formation and adhesion on the materials used in intraocular lens implants in cataract surgery patients. The review also gives an update on the genetic and molecular aspects in the understanding of biofilm formation by *S. epidermidis* strains on medical implants. A better understanding is needed to evaluate therapeutic strategies against *S. epidermidis* population.

**Keywords:** biofilm; intraocular lens

## 1. Pathogenicity of *Staphylococcus epidermidis* in ocular infections

Whereas previously only regarded as an innocuous commensal microorganism in the human eye, *Staphylococcus epidermidis* is nowadays seen as an important opportunistic pathogen. It is now the most frequent cause of nosocomial infections, at a rate about as high as that due to its more virulent cousin *Staphylococcus aureus* [1]. In particular, *S. epidermidis* represents the most common source of infections on indwelling medical devices. This likely stems from the fact that *S. epidermidis* is a permanent and ubiquitous colonizer of human eye, and the resulting high probability of device contamination during insertion [2].

While *S. epidermidis* infections only rarely develop into life-threatening diseases, their frequency and the fact that they are extremely difficult to treat represent a serious burden for the public health system. Treatment is complicated by specific antibiotic resistance genes and the formation of biofilms, multicellular agglomerations that have intrinsic resistance to antibiotics and mechanisms of host defense [3]. Furthermore, recent investigation has identified specific molecular determinants facilitating *S. epidermidis* immune evasion and ability to cause chronic disease. Interestingly, many of these determinants are believed to have original functions in the non-infectious lifestyle of this microorganism, emphasizing the accidental nature of *S. epidermidis* infections.

A better understanding of *S. epidermidis* physiology not only during infection, but also in its commensal status is urgently needed to evaluate therapeutic strategies against *S. epidermidis* population.

## 2. Biofilms- An introduction

Initial microbial adhesive interactions are divided into adhesion to substratum surfaces, coaggregation between microbial pairs and co-adhesion between sessile and planktonic microorganisms of different strains or species. The physico-chemical mechanisms underlying the adhesive interactions could be understood with the use of the parallel plate flow chamber along with other methods as discussed by Bos *et al.* [4]. For each of the three microbial adhesive interactions distinguished, there is a role of Lifshitz-van der Waals, acid-base and electrostatic interactions.

An understanding of the relationships among species in the biofilm city is essential to the appreciation of the benefits of biofilm-associated living, as mentioned by Watnick *et al.* [5]. The multispecies bacterial biofilm is like a city where bacteria settle selectively, limit settlements of new bacteria, store energy in exopolysaccharide, and transfer genetic material horizontally all for the good of the many. A genetic and biochemical understanding of the interactions between species in a biofilm, complex though they may be, is critical to the understanding of how the biofilm city functions and survives. In multiple-species biofilms many different types of soluble biofilm-specific signals would be discovered whose influence on dissimilar bacterial neighbours would be sometimes helpful and sometimes detrimental or even fatal. When conditions in the biofilm change, such interactions may determine which cells survive, which perish, and which move on.

The process of surface adhesion and biofilm development is a survival strategy employed by virtually all bacteria and refined over millions of years. This process is designed to anchor microorganisms in a nutritionally advantageous environment and to permit their escape to greener pastures when essential growth factors have been exhausted. Bacterial attachment to a surface can be divided into several distinct phases, including primary and reversible adhesion, secondary and irreversible adhesion, and biofilm formation. Each of these phases is ultimately controlled by the expression of one or more gene products. Ultrastructurally, the mature bacterial biofilm resembles an underwater coral reef containing pyramidal or mushroom-shaped microcolonies of organisms embedded within an extracellular glycocalyx, with channels and cavities to allow the exchange of nutrients and waste. The biofilm protects its inhabitants from predators, dehydration, biocides, and other environmental extremes while regulating population growth and

diversity through primitive cell signals. From a physiological standpoint, surface-bound bacteria behave quite differently from their planktonic counterparts, as reported by Dunne [6].

## 3. Adherence of *S. epidermidis* strains to various materials used in intraocular lenses (IOLs)

The role of colonisation in the pathogenesis of endophthalmitis has been speculated by Griffiths *et al.* [7]. It was demonstrated, with an *in vitro* model, that *Staphylococcus epidermidis* is able to colonise intraocular lenses. Adherent organisms were quantitated by light microscopy, scanning electron microscopy, and viable counting. Bacterial adherence was associated with production of a polysaccharide glycocalyx. Organisms which were attached to the lenses were resistant to apparently bactericidal concentrations of antibiotics, as determined by conventional testing.

Pinna *et al.* showed that adherence of *S. epidermidis* to IOLs plays a role in the pathogenesis of some forms of endophthalmitis after cataract surgery [8]. A clinically relevant ocular isolate of *Staphylococcus epidermidis* was investigated for adherence onto polymethylmethacrylate (PMMA) and Acrysof (Alcon Surgical, Fort Worth, TX) IOLs. Results suggested that *in vitro* adherence of *S. epidermidis* to IOLs is influenced by IOL materials. After 3 minutes incubation, Acrysof IOLs appeared to be more permissive to *S. epidermidis* adherence than PMMA IOLs. The difference was statistically significant ($P < 0.05$). However, at 90 minutes, Acrysof IOLs had a lower viable bacterial count than did the PMMA IOLs. Bacterial adherence appeared to be greater in areas with surface irregularities.

Similarly, an *in vitro* adherence of slime-producing and non-slime-producing *Staphylococcus epidermidis* to different IOLs was compared by Garcia-Saenz *et al.* to study the organism's contribution to postoperative endophthalmitis [9]. Slime-negative strains of *S epidermidis* adhered to all IOLs but at a lower level than slime-positive strains. The most adherent lenses were acrylic with the positive strain and PMMA with the negative strain. The least adherent IOLs were PMMA with the positive strain and hydrogel with the negative strain. There were no significant differences between rigid and foldable lenses. Polypropylene was significantly more adherent than PMMA to the slime-positive strain. The acrylic and the heparin-surface-modified (HSM) PMMA IOLs were significantly more adherent to the positive strain.

Pinna *et al.* also reported that adherence appears to be greater when the bacterial DNA contained the *ica* locus [10]. For this the adherence of two clinically relevant ocular isolates of *Staphylococcus epidermidis* to ACRYSOF IOLs (Alcon Surgical, Fort Worth, Texas) was investigated and whether the strains under study carried the intercellular adhesion (*ica*) locus was determined, which encodes production of *S. epidermidis* antigens mediating adherence to biomaterials. Direct counting of adherent bacteria in scanning electron microscopy photographs revealed that the total amount of adhered bacteria per area of IOL optic after 3, 30, and 90 minutes of incubation in bacterial suspension was 1306/mm$^2$, 3389/mm$^2$, and 7195/mm$^2$ (*S. epidermidis 1*) and 778/mm$^2$, 1056/mm$^2$, and 3861/mm$^2$ (*S. epidermidis 2*). Differences at 30 and 90 minutes were statistically significant ($P = 0.01$ and 0.02, respectively). Polymerase chain reaction amplification revealed that *S. epidermidis 1* contained the *ica* locus, whereas *S. epidermidis 2* was *ica* negative. Different ocular isolates of *S. epidermidis* may differ significantly with regard to adherence to ACRYSOF IOLs depending upon the presence of *ica* locus.

In another study by Kodjikian *et al.*, the *in vivo* behavior of the ability of the *Staphylococcus epidermidis* strain (American Type Culture Collection [ATCC] 14990) to attach to 120 IOLs made of five different biomaterials: fluorine PMMA, heparinized PMMA, silicone, hydrophobic acrylic, and hydrogel [11]. The pig was chosen as an animal model of endophthalmitis, after a bibliographical analysis and a personal study of its aqueous humor composition. The extent of bacterial binding (expressed as bound bacteria per area unit) was found to range in increasing order from hydrogel, to fluorine PMMA, to hydrophobic acrylic, to heparinized PMMA, to silicone polymer. Comparison of pairs of materials showed statistically significant differences, except between hydrogel and fluorine PMMA. Bacterial adhesion to the implant surface seemed to depend on the hydrophobicity or hydrophilicity of the biomaterial.

In support of the above, another study by Schauersberger *et al.* showed that hydrophilic IOLs can help reduce the rate of postoperative endophthalmitis as a result of their surface properties [12]. Four types of rigid IOLs (hydrophobic PMMA and hydrophilic HSM PMMA) and 5 types of foldable intraocular lenses (hydrophobic silicone, hydrophobic acrylic, and hydrophilic acrylic) were used in the experiment to determine the *in vitro* bacterial adherence to IOLs routinely used at 1 center in Austria. Under standardized conditions, the IOLs were contaminated with 2 strains of *Staphylococcus epidermidis*. A sonication method and impression method were used for quantification. The mean bacterial density per lens type (colony forming units/mm$^2$) was compared. With both germs and both quantification methods, more bacteria was found on hydrophobic lenses than on IOLs with hydrophilic surfaces ($P = .001$). The Alcon AcrySof® and Askin UV80F® IOLs had the greatest and the Corneal Acrygel® and Bausch & Lomb Hydroview® IOLs the least affinity to these microorganisms.

Another similar study was carried out by Kodjikian *et al.* to analyze and compare the adherence of *Staphylococcus epidermidis* to IOLs made of five different biomaterials (native or heparinized polymethylmethacrylate, silicone, hydrophilic acrylic, or hydrogel) and to detail the different steps and mechanisms of bacterial adhesion to a polymer [13]. Bacterial adhesion was statistically weakest on hydrogel and then on hydrophilic acrylic polymer. Adhesion depended on the hydrophobicity or hydrophilicity of the biomaterials. Slight differences were found between

 

bioluminescence and scanning electron microscopy (SEM), and these differences were explained. Furthermore, SEM observations highlighted two different patterns of bacterial adhesion (isolated bacteria and clusters of bacteria), assuming that hydrophobic IOLs (silicone and PMMA) probably facilitate bacterial colonization and biofilm production. Attachment mechanisms may be different in each case, depending on the polymer material and the infecting organism, because there are various types of behavior among *S. epidermidis* strains. Hydrophilic polymer surfaces (hydrogel and probably hydrophilic acrylic) seem to be useful in avoiding the development of bacterial colonies and hence in preventing endophthalmitis. Fewer bacteria were attached, demonstrating inhibition or delay in bacterial colonization.

Adherence is known to be greater when the bacterial DNA contain the *ica* locus, as reported by Kodjikian *et al.* [14]. Full knowledge of the pathogenesis of bacterial adhesion is necessary to gain a better understanding of IOL infection and endophthalmitis. For this, it was determined whether the *Staphylococcus epidermidis* strain carried the intercellular adhesion (*ica*) locus, which encoded production of adhesins mediating adherence to biomaterials and with scanning electron microscopy, the morphologic features of this coagulase-negative *Staphylococcus* strain that adhered to IOLs were studied. Polymerase chain reaction amplification revealed that *S. epidermidis* N890074 contained the *ica* locus. The bacteria appeared to be anchored to the surface of the lenses by several different means—particularly by leglike appendages and a slime layer—which probably came into play step by step.

Bailiff *et al.* described the binding of bacteria to IOLs as a two-phase process including an initial, instantaneous, and reversible phase followed by a time-dependant and irreversible molecular and cellular phase [15]. The binding of bacteria is affected by many factors including environmental factors such as medium composition, presence of proteins and flow conditions, the bacterial cell surface characteristics, and the material's surface properties. A better understanding of these mechanisms would make it possible to reduce the bacterial adhesion process and thus could help decrease the incidence of postoperative endophthalmitis.

## 4. Understanding biofilm formation by Staphylococcus species on medical implants – genetic and molecular aspects

Slime production is not a generally recognized feature of *Staphylococcus epidermidis*. In an outbreak of *S. epidermidis* intravascular catheter associated sepsis, it was noted that 63% of clinically implicated strains grew as a slimy film coating the culture tube walls when propagated in tryptic soy broth. Only 37% of randomly collected blood culture contaminants and skin isolates demonstrated a similar phenomenon ($p < 0.05$). When grown *in vitro*, slime producers accumulated on the surface of intravascular catheters as macrocolonies, whereas non-slime, producers did not. Transmission and scanning electron micrographs showed slime producers to be encased in an adhesive layer on the catheter surface, whereas non-producers were not encased. These results of the work done by Christensen *et al.* suggest that slime-mediated adherence may be a critical factor in the pathogenesis of *S.epidermidis* infections of medical devices [16]. Clinical studies performed by Christensen *et al.* have also found an association between slime production and strains of coagulase-negative staphylococci that infect indwelling medical devices [17]. They concluded that phenotypic expression of slime production is subject to both *in vitro* and *in vivo* variation, with regard to their adherence characteristics and 50% infective dose and could play a role in the pathogenesis of foreign body infection.

Jones *et al.* in the year 1992 reported that slime production and adherence had a limited role in the differentiation between clinically significant and contaminant strains isolated from blood cultures; however, the absence of slime and adherence in isolates of *S. epidermidis* suggested a lack of pathogenicity [18]. A study was conducted in which 251strains of coagulase-negative staphylococci (CNS) isolated from patients in hospital and the community were investigated for slime production and adherence as indicators of pathogenicity. *Staphylococcus epidermidis* formed 68.5% (126) of the isolates of CNS from blood and central venous catheter (CVC) tips, of which 46.0% (58) were slime-positive and adherent. Clinically significant infections were associated with 55.2% (32) of the slime-positive adherent strains isolated and 11.1% (four) of slime-negative non-adherent strains of *S. epidermidis*. For other species of CNS isolated from blood and CVC tips 74.1% (43) were slime negative non-adherent and 18.6% (eight) of these were considered clinically significant isolates while none of the slime positive adherent strains were associated with a clinically significant infection. Slime production and adherence were not characteristic properties of CNS causing community-acquired urinary tract infection or colonizing the nasal mucosa.

Several methods have been introduced to assess the mass of adherent bacteria and the slimy matrix in which they are embedded. Some methods measure total biofilm, others measure the organisms or the slime alone. *In vitro*, the type of medium, the atmosphere during incubation, and the nature of the solid surface, affect the quantity of biofilm that is formed. In most studies on the chemistry of the slime, the material used was formed on complex media solidified with agar. Contamination by ingredients of the media or by agar may not always have been recognised. Recent work with chemically defined medium (liquid or solidified with silica gel) shows that the slime is a mixture of about 80% (w/w) teichoic acid and 20% protein, as discussed by Hussain *et al.* [19]. Growth as a biofilm may protect the staphylococci from antibiotics. At present, the greatest success in preventing infection has come from improved surgical techniques during the insertion of implants.

In another study done by Muller *et al.*, clinical isolates of coagulase-negative staphylococci were analyzed for elaboration of the capsular polysaccharide/adhesion (PS/A) and extracellular biofilm or slime [20]. Of the 151 analyzed, 103 (68%) produced PS/A and 69 (46%) made extracellular slime; 87% of the slime-producing isolates made PS/A. Among isolates from all clinical infections examined except peritonitis, PS/A-positive isolates bound significantly ($P < .001$) more colony-forming units after 15 min to 1.5-cm segments of silicone-elastomer catheter than did PS/A-negative isolates. Slime-positive isolates were not more adherent than slime-negative isolates, because 42% of the PS/A-positive isolates were slime-negative. Thus, PS/A expression is common among clinical isolates of coagulase-negative staphylococci, accounting for most slime-positive and a proportion of slime-negative isolates.

According to O'Gara *et al.* epidemiological data that address whether invasive *S.epidermidis* strains can be traced to commensal organisms or an endemic occurrence of distinct strains with enhanced virulence, have important implications for the implementation of appropriate infection control measures [21]. An extracellular polysaccharide adhesin represents a key virulence determinant in *S. epidermidis* and is required for biofilm formation. Production of this adhesin, which is encoded by the *ica* operon, is subject to phase variable regulation (ON <---> OFF switching).

Cerca *et al.* showed that high levels of initial adherence do not necessarily lead to thick biofilm formation [22]. These two aspects of the pathogenesis of medical device related-infection needs to be evaluated independently to ascertain the contribution of each to the virulence of *S. epidermidis* causing device-related infections. In this study, the initial adhesion to different materials (acrylic and glass) of 9 clinical isolates of *S. epidermidis,* along with biofilm positive and biofilm negative control strains, was assayed using physico-chemical interactions to analyze the basis for bacterial adherence to the substratum. X-ray photo electron spectroscopy (XPS) analysis of the cell surface elemental composition was also performed in an attempt to find a relationship between chemical composition and adhesion capabilities. Biofilm formation on the two surfaces was evaluated by dry weight measurements. Human erythrocytes were used to evaluate the ability of *S. epidermidis* strains to cause hemagglutination, an indicator of the production of a poly- *N* -acetyl glucosamine cell surface polysaccharide also involved in biofilm formation. The clinical isolates exhibited different cell wall physico-chemical properties, resulting in differing abilities to adhere to surfaces. Adhesion to hydrophobic substrata for all strains occurred to a greater extent than that to hydrophilic surfaces. Bacterial cell hydrophobicity seemed to have little or no influence on adhesion. X-ray photoelectron spectroscopy analysis showed a high ratio of oxygen/carbon for all strains, which is a common characteristic of *S. epidermidis* species. No relevant relationship was found between XPS data and adhesion values. All strains forming biofilms were able to agglutinate erythrocytes. However, no direct relationship was found between the amount of biofilm formed and the initial adhesion extent.

In recent years, an increase in ocular pathologies related to soft contact lens has been observed. The most common infectious agents were Staphylococcus spp. Some strains produce an extracellular polysaccharidic slime that can cause severe infections. Polysaccharide synthesis is under genetic control and involves a specific intercellular adhesion (*ica*) locus, in particular, *ica*A and *ica*D genes. Conjunctival swabs from 97 patients with presumably bacterial bilateral conjunctivitis, wearers of soft contact lenses were examined. The ability of staphylococci to produce slime was related to the presence of *ica*A and *ica*D genes. It was found that 74.1% of the *S. epidermidis* strains and 61.1% of the *S. aureus* strains isolated were slime producers and showed *ica*A and *ica*D genes. Both *S. epidermidis* and *S. aureus* slime-producing strains exhibited more surface hydrophobicity than non-producing slime strains. The Pulsed Field Gel Electrophoresis (PFGE) patterns overlapped *in S. epidermidis* strains with high hydrophobicity. The similar PFGE patterns were not related to biofilm production. In this study by Catalanotti *et al.* there was scarce matching among the Staphylococcus spp. studied, slime production, surface hydrophobicity and antibiotic susceptibility [23].

The organism, *S. epidermidis* displays a high diversity of antibiotic resistance profiles and biofilm formation capacity, as shown by Juárez-Verdayes *et al.* [24]. The infection frequency associated to bacterial conjunctivitis, corneal ulcers (CU), and endophthalmitis was studied along a five years period. The isolation and identification of microorganisms were performed by culture-based methods and biochemical test respectively. Also, a nested polymerase chain raction (PCR) to detect gram-negative and gram-positive bacteria in the clinical samples was assayed. Nested PCR was a more efficient method than culture to detect bacteria in the samples. The most frequently isolated species was *Staphylococcus epidermidis*, a bacterium commonly considered as a human saprophyte. The *S. epidermidis* strains from conjunctivitis, CU, and endophthalmitis exhibited 46, 33.9, and 34.1% of oxacillin-resistance respectively. A total of 28% of intermediate-vancomycin resistance (MIC = 8-16 microg/ml) was observed among *S. epidermidis* strain collection. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis of the multi-resistance profile data of intermediate vancomycin-resistant *S. epidermidis* strains showed a high phenotypic diversity and no relationship between each group and their clinical origin. The biofilm formation capacity was broadly distributed (66%), particularly among intermediate vancomycin resistant strains (> 75%). These phenotypic traits could explain the high isolation frequency of *S. epidermidis* from ocular infections and oblige to review the saprophytic status of these bacteria.

Recent progress in elucidating the role of the *ica*ADBC-encoded polysaccharide intercellular adhesin (PIA) or polymeric N-acetyl-glucosamine (PNAG) in staphylococcal biofilm development has in turn contributed significantly to our understanding of the pathogenesis of device-related infections. Nevertheless, our understanding of how the *ica* locus and PIA/PNAG biosynthesis are regulated is far from complete and many questions remain. Moreover, beyond *ica*,

 

evidence is now emerging for the existence of *ica*-independent biofilm mechanisms in both *Staphylococcus aureus* and *Staphylococcus epidermidis*. Teichoic acids, which are a major carbohydrate component of the *S. epidermidis* biofilm matrix and the major cell wall autolysin, play an important role in the primary attachment phase of biofilm development, whereas the cell surface biofilm-associated protein and accumulation-associated protein are capable of mediating intercellular accumulation. According to O'Gara *et al.* these findings raise the exciting prospect that other surface proteins, which typically function as antigenic determinants or in binding to extracellular matrix proteins, may also act as biofilm adhesins[25].

The population dynamics of *S. epidermidis* revealed that there are significant genetic variations that can be detected through fluorescence-amplified fragment length polymorphism (FAFLP) between ocular disease causing isolates and the commensal population, as shown by Duggirala *et al.* [26]. For this, the potential phenotypic and genetic differences among the *Staphylococcus epidermidis* isolates obtained from control subjects (lower conjunctival sac; *n*-14) with those from patients with keratitis (corneal scrapings; *n*- 18) or endophthalmitis (vitreous; *n*- 24) were investigated. Biofilm-forming capacity was detected by PCR for the *ica*AB gene and phenotyping by microtiter plate assay and congo red agar plate. Genotyping was performed by using FAFLP and *in silico* analysis of the FAFLP profiles. Biofilm phenotyping (congo red agar/microtiter plate) differentiated disease-causing strains from control subjects. PCR assays (*mec*A, *ica*AB) were not useful in differentiating disease-causing strains from that of control subjects. The biofilm-forming capability appeared more critical in the pathogenesis of keratitis than in that of endophthalmitis. Cluster analysis of FAFLP data generated 11 clusters comprising 4 major clusters (I, II, III, and V) and 7 minor ones. FAFLP analysis clearly showed clustering of most of the commensal isolates in cluster I, separate from keratitis and endophthalmitis isolates. *In silico* analysis mapped signature bands to genes such as *ebh, tag*D, *pts*I, and *sep*A, which might have a significant role in transforming less virulent populations of *S. epidermidis* to more virulent ones.

A series of surface proteins mediate initial attachment to host matrix proteins, which is followed by the expression of a cationic glucosamine-based exopolysaccharide that aggregates the bacterial cells, and may function as alternative aggregating substances. Furthermore, surfactant peptides have now been recognized as key factors involved in generating the 3-dimensional structure of a staphylococcal biofilm by cell-cell disruptive forces, which eventually may lead to the detachment of entire cell clusters. Transcriptional profiling experiments have defined the specific physiology of staphylococcal biofilms and demonstrated that biofilm resistance to antimicrobials is due to gene-regulated processes. Finally, novel animal models of staphylococcal biofilm-associated infection have given an important information on which factors define biofilm formation *in vivo*. These recent advances constitute an important basis for the development of anti-staphylococcal drugs and vaccines, as reported by Otto [27].

Since bacteria embedded in biofilms are far more difficult to eradicate than planktonic infections, it would be useful to know whether certain *Staphylococcus aureus* lineages are especially involved in strong biofilm formation. For this reason, *in vitro* biofilm formation of 228 clinical *S. aureus* isolates of distinct clonal lineages was investigated. At 0.1% glucose, more than 60% of the *S. aureus* strains associated with multilocus sequence typing (MLST) clonal complex (CC)8 produced large amounts of biomass, compared to 0-7% for various other clonal lineages. Additionally, *S. aureus* bloodstream isolates associated with MLST CC8 and CC7 had similar biofilm forming capacities as their commensal counterparts. Furthermore, strong biofilm formation could not be attributed to a specific accessory gene regulator (*agr*) genotype strictly associated with clonal lineages, as suggested previously. The adherence to polystyrene surfaces under physiologic glucose concentration (0.1%) was dependent on the clonal lineage. Strains associated with MLST CC8 were markedly more often classified as strong biofilm former at glucose concentrations of 0%, 0.1% and 0.25%. This study by Croes *et al.* (2009) reveals that the MLST CC8 associated genetic background was a predisposing factor for strong biofilm formation *in vitro*, under all tested glucose concentrations [28].

At present, polysaccharide intercellular adhesin (PIA) is the best studied factor involved in *S.epidermidis* biofilm accumulation. PIA is a glycan of beta-1,6-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues of which 15 % are non-N-acetylated. PIA-producing *S. epidermidis* are widespread in clinical strain collections and PIA synthesis has been shown to be essential for *S. epidermidis* virulence. According to Rohde *et al.* (2010), PIA homologues have been identified in many other staphylococcal species, including the major human pathogen *Staphylococcus aureus*, and also Gram-negative human pathogens, suggesting that it might represent a more general pathogenicity principle in biofilm-related infections [29].

The surface properties of bacteria play an important role on adhesion to the biomaterial surface. In this study done by Sudağidan *et al.* (2010), the surface properties of *Staphylococcus epidermidis* strains isolated from clinically used polymeric biomaterial surfaces were investigated on the basis of zeta potential, hydrophobicity and surface topography [30]. A total of 10 *S.epidermidis* strains isolated from intravenous catheters (n = 5), endotracheal tubes (n = 3) and central venous catheters (n = 2) which were used in the patients of pulmonary Intensive Care Unit, Ege University Medical Faculty Hospital, were included to the study. Seven of those isolates were biofilm producers, inhabiting biofilm genes, 2 were non-biofilm producers, however, inhabiting biofilm genes, and 1 was non-biofilm producer, inhabiting no biofilm genes. Zeta potential analyses have been performed in 3 different buffers and at different pH values, in order to simulate *in vivo* environment of the biomaterials. Hydrophobicities of the strains were examined by bacterial adhesion to hydrocarbon (BATH) test and the surface topography of biofilms and slime layers were visualized by atomic force microscopy (AFM) and scanning electron microscopy (SEM) methods. It was found that all strains have negative zeta

potential values (surface charge) in all buffers and pH values. In hydrophobicity analysis, the highest value (86%) was determined for non-biofilm forming *S. epidermidis* strain YT-169b (endotracheal tube isolate) and the lowest hydrophobicity (2.5%) was determined for biofilm forming *S. epidermidis* strain YT-212 (central venous catheter isolate). SEM analysis showed that bacteria highly adhered to rough surfaces on biomaterial surfaces and the produced slime layers covered the surface of bacteria.

The multitude of biomolecular and regulatory factors involved in staphylococcal adhesion and biofilm formation owe much to their ability to colonize surfaces, allowing the biofilm form to become the preferential bacterial phenotype. Judging by total number, biomass and variety of environments colonized, bacteria can be categorized as the most successful life form on earth. This is due to the ability of bacteria and other microorganisms to respond phenotypically via biomolecular processes to the stresses of their surrounding environment. Laverty *et al.* (2013) reported that there are specific pathways involved in the adhesion of the Gram-positive bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* with reference to the role of specific cell surface adhesins, the *ica* operon, accumulation-associated proteins and quorum-sensing systems and their significance in medical device-related infection [31].

## References


[1] CDC. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control. 2004; 32:470–485.

[2] Uçkay I, Pittet D, Vaudaux P, Sax H, Lew D, Waldvogel F. Foreign body infections due to *Staphylococcus epidermidis*. Ann Med. 2009; 41:109–119.

[3] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284:1318–1322.

[4] Bos R, van der Mei, H. C. and Busscher H. J. Physico-chemistry of initial microbial adhesive interactions – its mechanisms and methods for study. FEMS Microbiology Reviews. 1999; 23:179–230.

[5] Watnick P, Kolter R. Biofilm, City of Microbes. J Bacteriol. 2000 May; 182(10):2675–2679.

[6] Dunne WM Jr. Bacterial Adhesion: Seen Any Good Biofilms Lately? Clin Microbiol Rev. 2002 April; 15(2):155–166.

[7] Griffiths PG, Elliot TS, McTaggart L. Adherence of *Staphylococcus epidermidis* to intraocular lenses. British Journal of Ophthalmology. 1989; 73:402-406.

[8] Pinna A, Zanetti S, Sechi LA, Usai D, Falchi MP, Carta F. *In vitro* adherence of *Staphylococcus epidermidis* to polymethyl methacrylate and acrysof intraocular lenses. Ophthalmology. 2000 June; 107(6):1042-1046.

[9] García-Sáenz MC, Arias-Puente A, Fresnadillo-Martinez MJ, Matilla-Rodriguez A. *In vitro* adhesion of *Staphylococcus epidermidis* to intraocular lenses. Journal of Cataract & Refractive Surgery. 2000 Nov; 26(11):1673-1679.

[10] Pinna A, Sechi LA, Zanetti S, Delogu D, Carta F. *In vitro* adherence of *staphylococcus epidermidis* to polymethyl methacrylate and acrysof intraocular lenses: A scanning electron microscopy and molecular biology study. Ophthalmology. 2000 Dec; 107(12):2162-2166.

[11] Kodjikian L, Burillon C, Chanloy C, Bostvironnois V, Pellon G, Mari E, Freney J, Roger T. *In Vivo* Study of Bacterial Adhesion to Five Types of Intraocular Lenses. Invest Ophthalmol Vis Sci. 2002; 43:3717–3721.

[12] Schauersberger J, Amon M, Aichinger D, Georgopoulos A. Bacterial adhesion to rigid and foldable posterior chamber intraocular lenses. Journal of Cataract & Refractive Surgery. 2003 Feb; 29(2):361-366.

[13] Kodjikian L, Burillon C, Roques C, Pellon G, Freney J, Renaud FN. Bacterial Adherence of *Staphylococcus epidermidis* to Intraocular Lenses: A Bioluminescence and Scanning Electron Microscopy Study. Invest Ophthalmol Vis Sci. 2003; 44:4388–4394.

[14] Kodjikian L, Burillon C, Lina G, Roques C, Pellon G, Freney J, Renaud FN. Biofilm Formation on Intraocular Lenses by a Clinical Strain Encoding the *ica* Locus: A Scanning Electron Microscopy Study. Invest Ophthalmol Vis Sci. 2003; 44:4382–4387.

[15] Baillif S, Hartmann D, Freney J, Kodjikian L. Intraocular lens and bacterial adhesion: influence of the environmental factors, the characteristics of the bacteria, and the target material surface. J Fr Ophtalmol. 2010 Mar; 33(3):210-21.

[16] Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of Slime-Producing Strains of *Staphylococcus epidermidis* to Smooth Surfaces. Infection And Immunity. 1982 July; 37(1):318-326.

[17] Christensen GD, Baddour LM, Simpson WA. Phenotypic Variation of *Staphylococcus epidermidis* Slime Production *In Vitro* and *In Vivo* Infection And Immunity. 1987 Dec.; 55(12):2870-2877.

[18] Jones JW, Scott RJ, Morgan J, Pether JV. A study of coagulase-negative staphylococci with reference to slime production, adherence, antibiotic resistance patterns and clinical significance. J Hosp Infect. 1992 Nov; 22(3):217-27.

[19] Hussain M, Wilcox MH, White PJ. The slime of coagulase-negative staphylococci: biochemistry and relation to adherence. FEMS Microbiol Rev. 1993 Apr; 10(3-4):191-207.

[20] Muller E, Takeda S, Shiro H, Goldmann D, Pier GB. Occurrence of capsular polysaccharide/adhesin among clinical isolates of coagulase-negative staphylococci. J Infect Dis. 1993 Nov; 168(5):1211-8.

[21] O'Gara JP, Humphreys H. *Staphylococcus epidermidis* biofilms: importance and implications. J Med Microbiol. 2001 Jul; 50(7):582-7.

[22] Cerca N, Pier GB, Vilanova M, Oliveira R, Azeredo J. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis.* Res Microbiol. 2005 May; 156(4): 506–514.


 

[23] Catalanotti P, Lanza M, Dei Prete A, Lucido M, Catania MR, Gallè F, Boggia D, Perfetto B, Rossano F. Slime-producing *Staphylococcus epidermidis* and *S. aureus* in acute bacterial conjunctivitis in soft contact lens wearers. New Microbiol. 2005 Oct; 28(4):345-54.
[24] Juárez-Verdayes MA, Reyes-López MA, Cancino-Díaz ME, Muñoz-Salas S, Rodríguez-Martínez S, de la Serna FJ, Hernández-Rodríguez CH, Cancino-Díaz JC. Isolation, vancomycin resistance and biofilm production of *Staphylococcus epidermidis* from patients with conjunctivitis, corneal ulcers, and endophthalmitis. Rev Latinoam Microbiol. 2006 Jul-Dec; 48(3-4):238-46.
[25] O'Gara JP. *ica* and beyond: biofilm mechanisms and regulation in and *Staphylococcus aureus*. FEMS Microbiol Lett. 2007 May; 270(2):179-88.
[26] Duggirala A, Kenchappa P, Sharma S, Peeters JK, Ahmed N, Garg P, Das T, Hasnain SE. High-Resolution Genome Profiling Differentiated *Staphylococcus epidermidis* Isolated from Patients with Ocular Infections and Normal Individuals. Invest Ophthalmol Vis Sci. 2007; 48:3239–3245.
[27] Otto M. Staphylococcal Biofilms**.** Curr Top Microbiol Immunol. 2008; 322: 207–228.
[28] Croes S, Deurenberg RH, Boumans ML, Beisser PS, Neef C, Stobberingh EE**.** *Staphylococcus aureus* biofilm formation at the physiologic glucose concentration depends on the *S. aureus* lineage. BMC Microbiology. 2009; 9:229.
[29] Rohde H, Frankenberger S, Zähringer U, Mack D. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to *Staphylococcus epidermidis* biofilm formation and pathogenesis of biomaterial-associated infections**.** Eur J Cell Biol. 2010 Jan; 89(1):103-11.
[30] Sudağidan M, Erdem I, Cavuşoğlu C, Ciftçloğlu M. Investigation of the surface properties of *Staphylococcus epidermidis* strains isolated from biomaterials. Mikrobiyol Bul. 2010 Jan; 44(1):93-103.
[31] Laverty G, Gorman SP, Gilmore BF. Biomolecular mechanisms of staphylococcal biofilm formation. Future Microbiol. 2013 Apr; 8(4):509-24.

 