REVIEW ON BACTERIAL BIOFILM AND ASSOCIATED INFECTIONS

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Abstract:-A biofilm is an architectural colony of microorganisms, within a matrix of extracellular polymeric substance that they produce. Biofilm contains microbial cells adherent to one-another and to a static surface (living or non-living). Bacterial biofilms are usually pathogenic in nature and can cause nosocomial infections. The National Institutes of Health (NIH) revealed that among all microbial and chronic infections, 65% and 80%, respectively, are associated with biofilm formation. The process of biofilm formation consists of many steps, starting with attachment to a living or non-living surface that will lead to formation of micro-colony, giving rise to three-dimensional structures and ending up, after maturation, with detachment. During formation of biofilm several species of bacteria communicate with one another, employing quorum sensing. In general, bacterial biofilms show resistance against human immune system, as well as against antibiotics. Health related concerns speak loud due to the biofilm potential to cause diseases, utilizing both device-related and non-device-related infections. In summary, the understanding of bacterial biofilm is important to manage and/or to eradicate biofilm-related diseases. The current review is, therefore, an effort to encompass the current concepts in biofilm formation and its implications in human health and disease.

Key words: *Associated infections Biofilm, Biofilm formation.*

INTRODUCTION

Biofilms matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces represent a significant and incompletely understood mode of growth for bacteria. Biofilm formation appears early in the fossil record is common throughout diverse range of organisms in both the Archaea and Bacteria lineages, including the 'living fossils' in the most deeply dividing branches of the phylogenetic tree. It is evident that biofilm formation is an ancient and integral component of the prokaryotic life cycle, and is a key factor for survival in diverse environments. Recent advances show that biofilms are structurally complex, dynamic systems with attributes of both primordial multicellular organisms and multifaceted ecosystems. Biofilm formation represents a protected mode of growth that allows cells to survive in hostile environments and also disperse to colonize new niches.The implications of these survival and propagative mechanisms in the context of both the natural environment and infectious diseases. [4]

Bacterial infections can occur in almost every part of the human body, which indicates that bacteria have adapted to survive in physiologically distinct anatomical locations. To facilitate this process, an organism must express the proper growth and virulence factors at the appropriate time, endure a potentially harsh surrounding chemical environment, and thwart a host's immune defenses. Several bacterial species use structures referred to as biofilms to combat these hazards.

Biofilms are bacterial communities in which cells are embedded in a matrix of extracellular polymeric compounds attached to a surface. Living in biofilms helps protect bacteria from deleterious conditions and the formation of biofilms appears to be an important factor in the disease cycle of bacterial pathogens in both animals and plants. Bacterial surface components and extracellular compounds primarily flagella, lipopolysaccharides (LPSs), and exopolysaccharides (EPSs), in combination with environmental and quorum-sensing signals, are crucial for autoaggregation and biofilm development in most bacterial species studied to date.[30] In the generally accepted model of biofilm formation, environmental signals trigger the process, and flagella are required for the biofilm community to approach and move across the surface.

The initial steps of attachment are mediated by outer membrane proteins (e.g., calcium-binding proteins), pili, or LPSs. After the formation of microcolonies, the production of quorum-sensing signals is required for the formation of a mature biofilm. [28] EPSs provide the architectural form of biofilms and stabilize their 3-dimensional structure. Biofilms are often permeated by channels that act as a circulatory system, allowing the bacteria to exchange water, nutrients, enzymes, and signals, dispose of potentially toxic metabolites, and display enhanced metabolic cooperativity the dispersal of biofilms allows bacteria to colonize other surfaces or substrates, thus completing a sequential developmental process.

The composition of biofilms varies depending on the system. The major components are typically water and the bacterial cells, followed by the EPSs of the matrix [32] which provides (i) a physical barrier against the diffusion of antibiotics, defense substances, or other important compounds from the host; and (ii) protection against environmental stress factors, such as UV radiation, pH changes, osmotic stress, and desiccation. In *Agrobacterium tumefaciens*, a plant pathogen that persists as surface-associated populations on plants or soil particles, cellulose overproduction resulted in increased biofilm formation on roots. Minor biofilm components

include macromolecules such as proteins, DNA, and various lysis products which affect the overall properties of the biofilm. Bacterial biofilms are widely distributed and play important roles in many environments. The environments occupied by soil bacteria range from rhizospheres rich in nutrients and root exudates to bulk soil deficient in nitrogen, phosphates, water, and other nutrients. The size of bacterial aggregates varies from small to large as a function of the nutrient availability at a given site. A hypothetical model of various 3-dimensional shapes of root-biofilm structures determined by nutrient availability has been presented.

The impact of bacterial biofilms on various aspects of our day-to-day lives has led to an increased number of biofilm- related studies in the past decade. Biofilms are differentiated groups of sessile microorganisms (e.g. bacteria and fungi) arranged as aggregated structures called microcolonies with distinct community properties. Biofilms constitute a unique mode of growth that allows survival in hostile environments. In particular, biofilms exhibit increased resistance to chemical disinfection, antimicrobial therapy, and human immune responses. Despite tremendous research efforts, our current understanding of the physiology and complexity of biofilm communities is still inadequate, especially as it is based mostly on studies of mono-species biofilms (i.e. population of cells). However, interspecies dynamics within mixed biofilms, such as communication and/or competition for nutrients and physical resources, represent those of a community, rather than a single-species population. This distinction is important, as it constitutes a layer of complexity that critically influences the phenotypes of the entire community within the biofilm.

Objectives

- To review bacterial biofilm and associated infections
- To summarize how bacteria biofilm form and associated infections
- To high light bacterial biofilm formation and steps

LITERATURE REVIEW

Biofilm Defination

A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material. Noncellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix. Biofilm-associated organisms also differ from their planktonic (freely suspended) counterparts with respect to the genes that are transcribed. Biofilms may form on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems. The variable nature of biofilms can be illustrated from scanning electron micrographs of biofilms from an industrial water system and a medical device, respectively the water system biofilm is highly complex, containing corrosion products, clay material, fresh water diatoms, and filamentous bacteria. The biofilm on the medical device, on the other hand, appears to be composed of a single, coccoid organism and the associated extracellular polymeric substance (EPS) matrix.[4]

A core definition of a biofilm accommodating the diversity of BAI is needed. A biofilm is often defined as ‘an aggregate of microbial cells adherent to a living or nonliving surface, embedded within a matrix of EPS of

microbial origin.' Biofilm EPS is an amalgam of extracellular macromolecules including nucleic acids, proteins, polysaccharides, and lipids [9] Within the biofilm, microbial cells are physiologically distinct from planktonic or single, free-floating cells of the same organism; however, at present, this crucial distinction is not a simple determination that can be evaluated by the tests and examinations usually employed in medical diagnostic work-ups. Classically, bacteria exhibit recalcitrance to antibiotics when they are in biofilms. Pseudomonas aeruginosa exhibits higher tolerance to tobramycin and colistin when it is surface-attached in vitro, [1] compared with when it is planktonic.

Microbial Biofilm Composition

Biofilm is an organized aggregate of microorganisms living within an extracellular polymeric matrix that they produce and irreversibly attached to fetish or living surface which will not remove unless rinse quickly.

Formation of extracellular polymeric substances (EPS) occurs in the attachment stage of a biofilm to the surface. Whether a microbial biofilm will form on an inanimate or solid surface or not is a consequence of the formation of an exopolysaccharide matrix, which provides strength to the interaction of the microorganisms in the biofilm usually thickness of EPS matrix is 0.2e1.0mm, however the size of the biofilm does not exceed 10e30 nm (Sleytr UB1997) typically 5e35% of the biofilm volume is constituted by the microorganisms while the remaining volume is extracellular matrix. This extracellular matrix is partially or mostly composed of proteins (Sun D *et al.*, 2005) Some important nutrients and minerals are trapped from the surrounding environment through the scavenging system, created by the extracellular matrix. Different types of components are present in extracellular polymeric substances protein in majority (>2%); other constituents, such as polysaccharides (1e2%); DNA molecules (<1%), RNA (<1%); ions (bound and free), and finally 97% of water. The flow of essential nutrients inside a biofilm is attributed to the water content. [19]

The microbial cells within biofilms are arranged in way with significant different physiology and physical properties. Bacterial biofilms are normally beyond the access of antibiotics and human immune system. Microorganisms that produce biofilm have enhanced potential to bear and neutralize antimicrobial agents and result in prolonged treatment. Biofilm forming bacteria switch on some genes that activate the expression of stress genes which in turn switch to resistant phenotypes due certain changes e.g. cell density, nutritional or temperature, cell density, pH and osmolarity. [10] When the biofilm water channels are compared with system of circulations showed that biofilms are considered primitive multi-cellular organisms.[12]

Table 1. Biofilm chemical composition [114].

S. No	Components	Percentage of matrix
1	Microbial cells	2-5%
2	DNA and RNA	<1-2%
3	Polysaccharides	1-2%
4	Proteins	<1-2% (including enzymes)
5	Water	Up to 97%

STEPS IN BIOFILM FORMATION

Genetic studies tell us about the formation of biofilm that it occurs in many steps. It requires special type of signaling, known as quorum sensing, between the microorganism cells. Also, it requires transcription of different set of genes compared to those of planktonic forms of the same microbial organisms.**[8]**

In addition, there are channels in the biofilm that separate the micro colonies. Mechanical stability of a biofilm is attributed to the viscoelastic features of the EPS matrix. Formation of biofilm is complex but according to different researchers it occurs in few common steps: initial contact/attachment to the surface, followed by micro-colony formation, maturation and formation of the architecture of the biofilm, and finally detachment/dispersion of the biofilm. Microbial biofilm develops through five consecutive stages such as initial reversible attachment, irreversible attachment, maturation stage I, maturation stage II and dispersion. In the first stage, planktonic microbial cells adhere to the surface either by physical forces or by bacterial appendages such as Pilli or flagella. [21]

Different factors like surface functionality, temperature and pressure can modulate the bacterial adhesion greatly. Attachment of a microbial cell to a surface is known as adhesion, whereas the attachment among microbial cells is termed as cohesion. Physical forces related to bacterial adhesion to surfaces include the van der Waals forces, steric interactions and electrostatic (double layer) interactions. [11]

Biofilm formation is a highly complex process, in which microorganism cells transform from planktonic to sessile mode of growth. [24] It has also been suggested that biofilm formation is dependent on the expression of specific genes that guide the establishment of biofilm. [29] The process of biofilm formation occurs through a series of events leading to adaptation under diverse nutritional and environmental conditions. This is a multi-step process in which the microorganisms undergo certain changes after adhering to a surface (Figure 1). Microorganisms which form biofilms are shown to elicit specific mechanisms. Biofilm formation has following important steps (a) attachment initially to a surface (b) formation of micro-colony (c) three dimensional structure formation (d) biofilm formation, maturation and detachment (dispersal).[4]

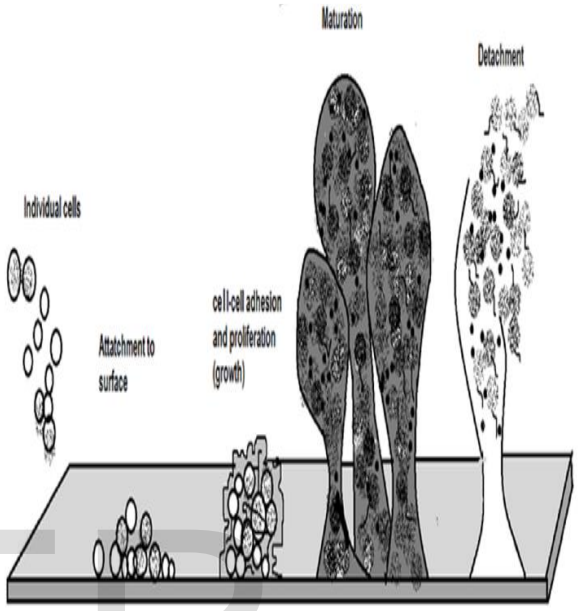

Figure 1. The biofilm life cycle in three steps: attachment, growth of colonies (micro-colony formation and formation of three dimensional structures) and detachment in clumps.

Initial Contact/Attachment to the Surface

Planktonic cells adhere to a surface by physical force or bacterial appendages like Pilli or flagella. The nature of the surface, temperature and pressure can modulate the adhesion greatly. Related physical forces are van der Waals forces, steric interactions and electrostatic interactions.

In this step of biofilm formation, microbial cells attach to the surface through their appendages like pilli and flagella and may also get attached through other physical forces like van der Waal's forces, electrostatic interactions etc. Other factors are also greatly affecting the bacterial adhesion to a surface. Adhesion the attachment of microbial cells to a surface, and cohesion the

interaction/attachment within the cells, occur in biofilm formation. [11]

Biofilms form when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them to a variety of materials including metals, plastics, soil particles, medical implant materials and, most significantly, human or animal tissue. The first bacterial colonists to adhere to a surface initially do so by inducing weak, reversible bonds called van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion molecules, proteins on their surfaces that bind other cells in a process called cell adhesionectly to earlier colonists.

The fimbriae, pilli and flagella give strength to the interaction between bacteria and the surface of attachment. The hydrophobicity of the surface may also play a role in strengthening the attachment of microbes, because it reduces the force of repulsion between the bacteria and the surface. [33]

The attachment step could be further categorized as a two-stage process: initial reversible attachment and irreversible attachment. [27] The irreversible attached biofilm can tolerate stronger physical or chemical shear forces (Sutherland I 2001) in the initial attachment, flagella and type IV pili-mediated motilities are important. Flagella are critical for initial interactions between cells and surface. Type IV pili-mediated twitching motilities enable attached cells to aggregate and form microcolonies O'Toole and Kolter demonstrated that *P. aeruginosa* flagella-deficient mutants could not land onto surfaces and type IV pili-deficient mutants could not form microcolonies. [23]

Micro-Colony Formation

After an attachment of microorganisms to a biotic or an abiotic surface occurs and this attachment becomes stable, a process of multiplication and division of microbial cells starts, initiated through particular chemical signaling within the EPS. This process then leads to the formation of micro-colonies. [4]

Micro-colony formation takes place after bacteria adhered to the physical surface/biological tissue and this binding then becomes stable which results in formation of micro-colony. Multiplication of bacteria in the biofilm starts as a result of chemical signals. The genetic mechanism of exopolysaccharide production is activated when intensity of the signal cross certain threshold.[20] So by this way using such chemical signal, the bacterial cell divisions take place within the embedded exopolysaccharide matrix, which finally result in micro-colony formation.[21]

Biofilms generally grow on surfaces and, following a period of attachment, cells within the biofilm form microcolonies that protrude away from the attachment plane. The micro colonies grow away from surfaces to distance themselves from competing cells and gain better access to nutrients in a manner analogous to tree growth in a forest as plants compete for the same resource (light), the only way to outcompete their neighbors is to grow higher and wider. Confocal imaging is a microscopy technique that achieves spatial resolutions and contrast levels much higher than are possible with regular epifluorescence microscopy. By rejecting most of the out-of-plane light using a pinhole in the conjugate plane of the detector, it is possible to reconstruct 3D volumes by recording planar sections of a material.[16]

Maturation and Architecture

After micro-colony formation stage of biofilm, expression of certain biofilm related genes take place. These gene products are needed for the EPS which is the main

structure material of biofilm. It is reported that bacterial attachment by itself can trigger formation of extracellular matrix. Matrix formation is followed by water-filled channels formation for transport of nutrients within the biofilm. Researcher have proposed that these water channels are like a circulatory systems, distributing different nutrients to and removing waste materials from the communities in the micro-colonies of the biofilm.[26]

Tolker-Nielsen and Molin noted that every microbial biofilm community is unique although some structural attributes can generally be considered universal. The term biofilm is in some ways a misnomer, since biofilms are not a continuous monolayer surface deposit. Rather, biofilms are very heterogeneous, containing micro colonies of bacterial cells encased in an EPS matrix and separated from other micro colonies by interstitial voids (water channels).[17]

A biofilm of P.aeruginosa,*Klebsiella pneumoniae*, and *Flavobacterium* spp that has developed on a steel surface in a laboratory potable water system. This image clearly depicts the water channels and heterogeneity characteristic of a mature biofilm. Liquid flow occurs in these water channels, allowing diffusion of nutrients, oxygen, and even antimicrobial agents. This concept of heterogeneity is descriptive not only for mixed culture biofilms (such as might be found in environmental biofilms) but also for pure culture biofilms common on medical devices and those associated with infectious diseases.

Cell-to-cell communication is an important process, during which the required microbial cell density is attained. This leads to the secretion of signaling molecules, known as auto inducers. These auto inducers facilitate quorum sensing.[8]

Once the first layer of the biofilm is established, cells of the same species or other species are recruited to the biofilm from the bulk fluid. Biofilm grows from a thin layer to a ‘mushroom’ or ‘tower’ shape structure. In a thick biofilm (>100 layers), bacteria are arranged according to their metabolism and aero tolerance. For example, anaerobic bacteria prefer to live in deeper layers to avoid exposure to oxygen. Bacteria within biofilm communities ‘talk’ to each other and take specialized functions. As the biofilm matures, more biofilm scaffolds, such as proteins, DNA, polysaccharides, etc. are secreted into the biofilm by the entrapped bacteria.[13]

Detachment /Dispersion of Biofilm

After biofilm formation, the researchers have often noticed that bacteria leave the biofilms itself on regular basis. By doing this the bacteria can undergo rapid multiplication and dispersal. Detachment of planktonic bacterial cells from the biofilm is a programmed detachment, having a natural pattern.[4]

In this phase, microbial cells within the biofilm perform quick multiplication and dispersion in order to convert fromsessile into motile form. Detachment then occurs in a natural pattern.[4]

Researchers often note that, once biofilms are established, planktonic bacteria may periodically leave the biofilm on their own. When they do, they can rapidly multiply and disperse. There is a natural pattern of programmed detachment of planktonic cells from biofilms. This means that biofilms can act as what Costerton refers to as “niduses” of acute_infection. Because the bacteria in a biofilm are protected by a matrix, the host immune system is less likely to mount a response to their presence.[21]

Dispersal of cells from the biofilm colony is an essential stage of the biofilm life cycle. Dispersal enables biofilms to spread and colonize new surfaces. Enzymes that degrade the biofilm extracellular_matrix, such as dispersin B and deoxyribonuclease, may play a role in biofilm

dispersal. Biofilm matrix degrading enzymes may be useful as anti-biofilm agents recent evidence has shown that a fatty acid messenger, *cis*-2-decenoic acid, is capable of inducing dispersion and inhibiting growth of biofilm colonies. Secreted by *Pseudomonas aeruginosa*, this compound induces cyclo hetero morphic cells in several species of bacteria and the yeast *Candida albicans* . Nitric oxide has also been shown to trigger the dispersal of biofilms of several bacteria species at sub-toxic concentrations. Nitric oxide has the potential for the treatment of patients that suffer from chronic infections caused by biofilms.[18]

Cells may detach individually from biofilms as a result of cell growth and division within the biofilms, or cell aggregates or clusters may detach or be sloughed from the biofilm. Though detachment has not been well characterized for medical device biofilms, some aspects of the process can be considered universal for all biofilms. Laboratory studies have shown that an increase in shear stress, as would occur during changes in direction or rate of flow, will result in an increase in the rate of cell erosion from the biofilm.[3]

After biofilm maturation the dispersal step, which is also critical for the biofilm life cycle, follows. Biofilms disperse because of myriads of factors, such as lack of nutrients, intense competition, outgrown population, etc. Dispersal could occur in the whole biofilm or just a part of it. Release of planktonic bacteria promotes the initiation of new biofilms at other sites.

Biofilm dispersal can be the result of several cues, such as alterations in nutrient availability, oxygen fluctuations and increase of toxic products, or other stress-inducing conditions (Rowe et al. 2010) increase in extracellular iron induces biofilm dispersal (Rowe *et al.*, 2010), whereas P. aeruginosa biofilms disperse in response to increased amounts of various carbon and nitrogen sources. Several sensory systems monitor the levels of small molecules, as a proxy to environmental changes, and alter gene expression accordingly, promoting dispersal.[14]

INFECTIONS ASSOCIATED WITH BIOFILM

It is estimated that about 65% of all bacterial infections are associated with bacterial biofilms. These include both, device and non-device-associated infections. Data for device related infections have been estimated for several devices, such as: 2% for breast implants; 2% for joint prostheses; 4% for mechanical heart valves; 10% for ventricular shunts; 4% for pacemakers and defibrillator, and about 40% for ventricular-assisted devices.[5]

Native valve endocarditis (NVE) is an inflammation caused by interaction of bacteria with the vascular endothelium and pulmonic valves of the heart. This is usually the result of Streptococci, Staphylococci, gram negative bacteria, and/or fungal infections [15] in this condition microbial cells gain access to the heart and blood through the gastrointestinal tract, urinary tract and/or through the oropharynx. As the intact valve endothelium gets damaged by the microorganisms that attach to it, even after the bacteria have been cleared by the immune system a non-bacterial thrombotic endocarditis (NBTE) develops at the injury location, as a result a thrombus formation occurs, a condition where platelets, red blood cells and fibrin are aggregated.

The notion that some infections are specifically mediated by bacteria in biofilms and distinct from those due to single-celled planktonic bacteria was first advanced by J.W. Costerton.[4] Similarly, Niels Høiby had observed that the aggregation of P. aeruginosa in the sputum of chronically infected CF patients was relevant to CF-associated lung infection compared with single-celled

bacteria in 1984, Costerton formally outlined the hypothesis that organisms like P. aeruginosa could behave similarly in human infections to the way they behaved in the environment. He further suggested that 'glycocalyx-enclosed biofilms of P. aeruginosa or other bacteria have been identified in experimental or clinical infections arising from contaminated prostheses and in chronic refractory infections, such as endocarditis, osteomyelitis, and P. aeruginosa pneumonia associated with cystic fibrosis. Clinicians may be more familiar with foreign body (implant) infections because of microbial attachment to a nonliving surface distinguished from biofilms associated with host tissues, or 'native tissue infections'.

BAI present significant challenges to current clinical practice guidelines because of the inherent difficulty in determining whether the infection is biofilm-related or is due to an acute infection with planktonic microorganisms. Therefore, functional, clinically relevant criteria would help to: (1) better distinguish BAI from acute planktonic infections, (2) obtain appropriate clinical samples, and (3) provide focus for the development of routine clinical tests. Criteria for biofilm infections have been previously proposed and modified based on the initial Parsek–Singh criteria.

Device –Related Biofilm Infections

In biofilms causing intravascular device-related bloodstream infection, however, methods have been developed that do not necessarily require device removal. These methods are based on qualitative or quantitative blood cultures through the device and paired quantitative blood cultures both through the device and percutaneously, with the number of bacteria greater in device-drawn cultures compared with peripherally drawn cultures, and the time to positive culture during continuous monitoring of growth, faster.[22]

Contact lenses are categorized as soft and hard contact lenses. Microorganisms can adhere to both types of lenses. Their classification is based on construction materials, frequency of disposal, wear schedule and design. The type of microorganisms which are attached to contact lenses are mainly E. coli, P. aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, species of Candida, Serratia and Proteus, etc., but most importantly, the degree of adherence to the lenses depends on the water content, substrate nature, electrolyte concentration, type of bacterial strain involved and lastly the composition of the polymer. Under scanning electron microscopy biofilm has been observed on contact lenses of a patient diagnosed with keratitis, produced by P. aeruginosa. Biofilms can also form more frequently on contact lenses that are usually kept in lens storage cases. The lens storage cases, therefore, have been declared as a source of lens contamination.

Biofilms usually occur on or within indwelling medical devices such as contact lenses, central venous catheters, mechanical heart valves, peritoneal dialysis catheters, prosthetic joints, pacemakers, urinary catheters and voice prostheses. Biofilms may be composed of only a single or of different types of microbial species. This depends on the devices and their duration of action.[7]

Biofilms of various medical devices have been studied extensively over the last 20 years, though much of the published research used very basic tools, such as viable culture techniques and scanning electron microscopy, to characterize the microbial diversity and visualize the biofilms. For certain devices, such as urinary catheters and contact lenses, research has also elucidated the susceptibility of various materials to bacterial adhesion and biofilm formation.[4]

Device-related bacteremia is thought to be due primarily to erosion or sloughing of biofilm cells because of mechanical shear when flushing the catheter, which detaches microbial cells from and results in cells or cell aggregates entering the bloodstream and leading to the signs and symptoms of blood stream infection. Indwelling catheters are frequently colonized with biofilm shortly after insertion and Kim et al. linked biofilm on a central venous catheter (CVC) to an outbreak of Alcaligenes xylosoxidans bloodstream infection Many others, including have noted that catheter colonization does not necessarily directly correlate with infection as measured by positive blood cultures.Many bacterial infections are biofilm related, for example, chronic lung, wound and ear infections. Bioflms are also able to colonize on medical devices such as catheters and implants. According to the NIH more than 80% of all microbial infections are biofilm related.[6]

Non-Device Related Biofilm Infections

Periodontitis is an infection of the gums. In this infection damaging of soft tissues, as well as that of bones supporting the teeth occurs. Normally, it is caused by poor oral hygiene. Tooth-loss is also possible P. aerobicus and Fusobacterium nucleatum are among the causative agents of periodontitis. These microbes also have the ability to form biofilms on a variety of surfaces; including mucosal surfaces in the oral cavity. Microbial colonization of teeth surfaces may permit them to invade mucosal cells and alter the flow of calcium in the epithelial cells, as well as to release toxins. A plaque can then develop within 2-3 weeks. The plaque may mineralize with calcium and phosphate ions, forming the so called tartar or calculus.[25]

CONCLUSIONS

In conclusion, biofilm formation on indwelling medical devices greatly affects surgical and instrumental procedures and public health as well. It also has implications in non device- related human-health complications. There is a need for an in-depth research to optimize measures for its prevention. Good hygienic conditions and practices are very necessary to avoid biofilm formation. With the passage of time, and with the advent of new technologies, progress has been made to remove and control biofilm-associated infections. However, new anti-biofilm strategies are necessary to handle biofilm associated chronic infections.

Biofilm represents a specific life form of microorganisms which provides not only efficient protection from negative outside influence, but also physically and chemically suitable micro-environment necessary for growth and survival. The fact that biofilm is the cause of many chronic diseases infections of catheters and other biomaterials used in medicine, makes the research on biofilm extremely important for medicine. It is estimated that 65% of all bacterial infections are caused by biofilm. Contemporary interdisciplinary research, based on genetic analyses, microscopic observations and studies of gene expression, has resulted in advanced knowledge of molecular and genetic basis of biofilm development and survival. It has also contributed to an increasing number of strategies for biofilm prevention and control. Biofilm formation can be prevented by signalling molecules that block the attachment of bacterial cells to substrate surface and by chemical reactions that prevent synthesis of polymers in extracellular matrix. Substances that block communication between bacteria can prevent biofilm formation or stimulate its dispersion. Biofilm dispersion can be induced by enzymes that break down polymers in extracellular matrix. To develop new treatments for biofilm destruction, it is extremely important to carry on

research on mechanisms that lead to increased biofilm resistance to antimicrobial agents.

REFERENCES

[1] Alhede M, Kragh KN, Qvortrup K et al. (2011) Phenotypes of non-attached Pseudomonas aeruginosa aggregates resembles surface attached biofilm. PLoS ONE 6: e27943.

[2] Branda, S.S.; Vik, S.; Friedman, L.; Kolter, R. Biofilms: The matrix revisited. *Trends Microbiol.* **2005**, *13*, 20–26.

[3] Characklis, W. G., G. A. McFeters, and K. C. Marshall**.** 1990. Physiological ecology in biofilm systems, p. 341–394. *In* W. G. Characklis and K. C. Marshall (ed.), Biofilms. John Wiley and Sons, New York, N.Y.

[4] Costerton J, et al. Bacterial biofilms: a common cause of persistent infections. Sci. 1999; 284: 1318-1322.

[5] Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med 2004; 350:1422e9.

[6] Davies D. Understanding biofilm resistance to antibacterial agents. *Nat. Rev. Drug Discov.* 2(2), 114–122 (2003).

[7] Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002; 15:167e93.

[8] Federle MJ, Bassler BL. Interspecies communication in bacteria. J Clin Invest 2002;112:1291—9

[9] Flemming HC & Wingender J (2010) the biofilm matrix. Nat Rev Microbiol 8: 623–633.

[10] Fux CA, Costerton JW, Stewart PS, Stoodley P Survival strategies of infectious biofilms *Trends Microbiol* 13(1), 34–40 (2005).

[11] Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18(9):1049–1056.

[12] Gilbert, P.; Das, J.; Foley, I. Biofilms susceptibility to antimicrobials. *Adv. Dent. Res.* **1997**, *11*, 160–167.

[13] Heilmann C, Hussain M, Peters G, Götz F. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. *Mol. Microbiol.* 24(5), 1013–1024 (1997).

*[14] Kaplan JB, Ragunath C, Velliyagounder K, Fine DH, Ramasubbu N (July 2010). "Enzymatic detachment of Staphylococcus epidermidis biofilms". Antimicrobial Agents and Chemotherapy. **48** (7): 2633–6. doi:10.1128/AAC.48.7.2633-2636.2004. PMC 434209. PMID 15215120.*

[15] Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: importance and applications. Indian J Biotechnol 2009; 8:159e68.

[16] Lawrence J R, Korber D R, Hoyle B D, Costerton J W and Caldwell D E 1991 J. Bacteriol. 173 6558–67

[17] Lewandowski Z. Structure and function of biofilms. In: Evans LV, editor. Biofilms: recent advances in their study and control. Amsterdam: Harwood Academic Publishers; 2000. p. 1–17.

*[18] López, Daniel; Vlamakis, Hera; Kolter, Roberto (2010). "Biofilms". Cold Spring Harbor Perspectives in Biology. **2** (7): a000398. doi:10.1101/cshperspect.a000398. ISSN 1943-0264. PMC 2890205. PMID 20519345*

[19] Lu TK and Collins JJ Dispersing biofilms with engineered enzymatic bacteriophage PNAS 2007; 104:11197-11202..

[20] Maric S, Vranes J (2007) Characteristics and significance of microbial biofilm formation Period Bilogor 109:115–121.

[21] Mckenney D, Hubner J, Muller E, Wang Y, Goldmann DA, Pier GB. The ica locus of Staphylococcus epidermidis encodes production of the capsular polysaccharide/adhesin. Infect Immun 1998; 66:4711e20.

[22] Mermel LA, Allon M, Craven DE, Flynn P, O'Grady NPO, Raad II, Rijnders BJA, Sherertz RJ & Warren DK (2009)

[23] O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development *Mol Microbiol* 30 (2), 295–304 (1998).

[24] Okada M, et al. Structure of the Bacillus subtilis quorum-sensing peptide pheromone ComX. Nat Chem Biol.2005; 1: 23-24.

[25] Overman PR. Biofilm: a new view of plaque. J Contemp Dent Pract 2000; 1:18e29.

[26] Parsek MR and Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol 2003; 57: 677-701.

[27] Renner LD,Weibel DB Physicochemical regulation of biofilm formation *MRS Bull* 36(5), 347–355 (2011).

[28] Rinaudi L.V; Giordano, W An integrated view of biofilm formation in rhizobia *FEMS Microbiol Lett.* **2010**, *304*, 1–11.

[29] Sauer FG, et al. Fiber assembly by the chaperone-usher pathway. Biochim Biophys Acta. 2004; 1694: 259-267.

[30] Schembri, M.A; Christianesen, G.; Klemm,P.Fim H-mediated autoaggregation of *E. coli*. *Mol. Microbiol.* **2001**, *41*, 1419–1430.

[31] Stoodley P, Boyle JD, Dodds I, Lappin-Scott HM. Consensus model of biofilm structure. In: Wimpenny JWT, Gilbert PS, Lappin-Scott HM, Jones M, editors. Biofilms: community interactions and control. Cardiff, UK: Bioline; 1997. p. 1–9.

[32] Sutherland I. Biofilm exopolysaccharides: a strong and sticky framework *Microbiology* 147(1), 3–9 (2001).

[33] Tribedi P, Sil AK Cell surface hydrophobicity: a key component in the degradation of polyethylene succinate by Pseudomonas sp. AKS2. J Appl Microbiol 2014; 116:295e303.