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City of Bacteria

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Abstract: Especially living tissues, implants, catheters, pacemakers, prosthetic heart valves, composite resins, glass ionomer cements, chronic wounds, contact lenses and ceramic materials, etc. It has now been proven that bacteria on all surfaces live a micro-social lifestyle by using Quorum Sensing System (QS), communicating through *N*-Acyl Homoserine Lactone (AHL) signaling molecules, and forming biofilm layer, which is one of most vital virulence factors. In 1978, with discussions of Robert Koch's hypotheses published in 1884, Bill Costerton warned about magnitude of biofilm damage in chronic infections. In 2012, Father of biofilm Bill Costerton broke new ground in his research on biofilm. The concept of "Cell-to-cell communication", which Smith first brought to agenda in 1905, has brought infectious diseases to huge scientific level with QS system and biofilm, which is most important virulence factor of this system. Within the biofilms, bacteria can easily reproduce and communicate with each other, there's actually a fiber optic system that bacteria communicate with each other at speed of light, QS. Biofilm was defined as a 'City of Microbes' by Watnick and Kolter, (2000). Biofilm is mixture of different microorganisms that are held together and protected by glue-like film. It's a slimy matrix and coating that bacteria, fungi, yeast, mold, mycotoxins, viruses and parasites create in order to stay protected and elude immun system. A dysregulated nervous system can lead to taxed immune system. This layer of glue-like slime creates a protective 'sleeping bag' for bacteria, so they can continue to do what they do best, which is thriving in you, while creating chronic diseases. The immune system fully recognizes bacteria, however, can't get to them due to the protective layer as biofilm, thus many times creating an autoimmune situation or cannot effective. In summary, understanding of biofilm is vital to manage and to eradicate biofilm-related diseases. The current review is, therefore, an effort to encompass the current concepts in biofilm, biofilm architectural and its implications in all living creature health and disease.

Keywords: City of bacteria, Bacteria, Biofilm, Quorum Sensing System (QS), Immune system

Bakteriler Şehri

Öz: Özellikle canlı dokular, implantlar, kateterler, kalp pilleri, protez kalp kapakçıkları, kompozit reçineler, cam iyonomer simanlar, kronik yaralar, kontakt lensler ve seramik malzemeler vb. artık tüm yüzeylerdeki bakterilerin mikro-sosyal bir yaşam tarzı yaşadıkları kanıtlanmıştır. Quorum Sensing System (QS), *N*-Açil Homoserin Lakton (AHL) sinyal molekülleri aracılığıyla iletişim kurarak hayati önemdeki virülens faktörlerinden biri olan biyofilm katmanını oluşturur. Robert Koch'un 1884 yılında yayınlanan varsayımlarının tartışılmasıyla 1978'de Bill Costerton, kronik enfeksiyonlarda biyofilm hasarının büyüklüğü hususunda uyarılarda bulundu. 2012 yılında Biyofilmin babası Costerton'un biyofilm konulu araştırmaları çığır açtı. Smith'in 1905 yılında ilk kez gündeme getirdiği "Bakteri-bakteri iletişimi" kavramı QS sistemiyle, bu sistemin en önemli virülens faktörü olan biyofilm, enfeksiyon hastalıklarını devasa bilimsel bir boyuta geçirmiştir. Biyofilmlerin içinde bakteriler kolaylıkla üreyebiliyor ve birbirleriyle iletişim kurabiliyor, aslında bakterilerin birbirleriyle ışık hızında QS iletişim kurduğu bir fiber optik sistem vardır. Biyofilm, Watnick ve Kolter (2000) tarafından 'Mikroplar Şehri' olarak tanımlanmıştır. Biyofilm, tutkal benzeri bir filmle bir arada tutulan ve korunan farklı mikroorganizmaların bir karışımıdır. Bakterilerin, mantarların, mayaların, küflerin, mikotoksinlerin, virüslerin ve parazitlerin korunmak ve bağışıklık sisteminden kaçmak için oluşturduğu sümüksü bir matris ve kaplamadır. Düzensiz bir sinir sistemi, bağışıklık sisteminin vergilendirilmesine yol açabilir. Bu tutkal benzeri balçık tabakası bakteriler için koruyucu bir 'uyku tulumu' oluşturur, böylece bir yandan kronik hastalıklar yaratırken bir yandan da içinizde gelişmek olan en iyi yaptıkları şeyi yapmaya devam edebilirler. Bağışıklık sistemi bakterileri tam olarak tanır ancak biyofilm gibi koruyucu bir tabaka nedeniyle onlara ulaşamaz ve çoğu zaman otoimmün bir durum yaratır veya etkili olamaz. Özetle, biyofilmin anlaşılması, biyofilmle ilişkili hastalıkların yönetilmesi ve ortadan kaldırılması için hayati öneme sahiptir. Bu nedenle mevcut inceleme, biyofilm, biyofilm mimarisi ve bunun tüm canlıların sağlığı ve hastalıkları üzerindeki etkilerini kapsayan güncel kavramları tartışmaktadır.

Anahtar kelimeler: Bakteriler şehri, Bakteri, Biyofilm, Çevreyi Algılama Sistemi (QS), Bağışıklık sistemi

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1. Introduction

Biofilm occurs early in fossil record (~3.25 billion years ago) [1]. The first awareness of biofilm existence in natural environments began in the 1970s [2]. Angst, E.C. 1923 reported the slime formation under ships caused by bacteria. In the early 1933s, the term 'film' was referred to as bacterial attachment, aggregation and proliferation on surfaces, and the term 'sessile' bacteria, which adhere to the surface as opposed to free-swimming 'planktonic' bacteria, was firstly used in marine microbiology. [4] studied the growth and adhesion of bacteria on underwater glass surfaces in seawater, and Scientist Characklis studied microbial slime in industrial water systems in the early 1973s [5]. The first biofilm publication reported using term 'biofilm' was by Mack et al. in 1975. In 1977, first microscopic image of biofilm was published and for first time it was shown that dense slime structure surrounded adherent bacteria. Term biofilm was defined in medical field by Costerton in 1985, and concept of biofilm infections and their importance began 40 years ago, with structure of dental pellicles by Jendresen and Høiby's observation of *Pseudomonas aeruginosa* cells in chronically infected cystic fibrosis cases [6].

Biofilm is a cluster of microorganisms to which cells adhere to each other and/or to the surface on which they are located. These interconnected cells are usually embedded in a self-produced Extracellular Polymeric Substance (EPS). Biofilm EPS is a polymeric complex composed of DNA, proteins, and polysaccharides. Biofilms can form on living or non-living surfaces and represent the dominant aspect of microbial life [1].

If biofilm is break down the immune system senses these bacteria, but cannot reach them, then host will occur autoimmune, MCAS, pain, odd neurological issues, buzzing in brain, weird vibrations. In additional, can see reactivation and repeating cycles of chronic diseases. They Exchange DNA that converge together to from new stronger protective organisms. Biofilm is typically clear, White, nude, jellyfish apperance, slippery, slimy, egg white ‘feel’ and look.

In general, bacterial biofilms show resistance against human immune system, as well as against antibiotics. Health related concerns talk loud due to the biofilm potential to cause diseases, utilizing both device-related and non-device-related infections.

The implications of biofilm architecture, survival and propagative mechanisms of biofilm in the context of both the multidirectional natural environment and infectious diseases are be discussed in this review.

2. Biofilm Structure

Biofilm begins with contact of microorganisms with a surface within their living spaces, and thanks to the various extracellular biopolymers they secrete, they can attach to many different surfaces such as metal, plastic, implants and cell tissue. In many cases, the cost of damage caused by biofilm reaches billions of dollars. Biofilm systems have many negative effects. Antibiotics used to destroy microorganisms do not have sufficient effectiveness on biofilm. In one study, catheter cuffs were coated with silver ions, which are known to have antimicrobial activity, but diffusion of silver ions into environment over time caused coating to lose its antimicrobial activity. The use of antibiotics as coating materials causes microorganisms to become resistant to the antibiotic used and the application loses its effectiveness [7-8].

In medical terms, biofilm formation occurs in 5 steps (Fig. 1). The initial adhesion of the bacteria is be called reversible adhesion and the cells can be easily detached from the surfaces again [9]. The initial weak interactions that develop between bacterial cells and surface are been referred to as reversible adhesion. Various long chain interactions that influence the reversible adhesion process are van der Walls attractive forces, electrostatic forces and hydrophobic interactions. During this stage,

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bacteria show Brownian motion and can easily move with fluid force [10]. Electron transfer between the cell surface and the biofilm layer also plays an important role in bacterial adhesion to inorganic and organic surfaces [11].

Initial adhesion between inanimate surfaces and bacteria generally occurs through nonspecific interactions such as hydrophobic. Adhesion to living surfaces generally occurs through specific molecules. During biofilm development, cell-cell adhesion on inanimate surfaces can be achieved by specific adhesion [12]. Once adhesion occurs, the bacteria goes through a number of adaptation stages. Among these, the production of EPS and the development of resistance to antimicrobial agents are the most important [13]. Following reversible binding of bacteria, bacterial cells proliferate and produce EPS [14].

During initial adhesion, there is a bulk flow of organic and inorganic molecules. These molecules are transported across the surface by diffusion or turbulent flow [14]. In turbulent flow, small turbulent turns that may occur suddenly can drag small particles contained in bacterial cells in a direction parallel to the surface, and these small particles can neutralize the Gibbs energy barrier required by the bacteria communicating with a surface, and this Gibbs energy barrier; In fact, van der Waals interactions include all common attractive forces and electrostatic interactions [15]. It has been determined that the adhesion abilities of microorganisms are higher in the logarithmic growth phase, and it has been reported that this is due to the increased hydrophobic properties of the cell wall in the growth phase [16].

Irreversible adhesion of cells is the next important stage in biofilm development. Repulsive forces often prevent bacterial cells from making direct contact with the surface, but this contact occurs through cell surface extensions of bacteria such as flagella, fimbriae, pili and EPS fibrils [17]. For transition from reversible adhesion to irreversible adhesion, various short-chain interactions are required, including dipole-dipole interactions, hydrogen, ionic and covalent bonds, and hydrophobic interactions. Thanks to the interactions between these polymeric fibrils, a bridge is be formed between bacterial cells and surface, resulting in irreversible relationship with surface [14]. Irreversibly adherent bacterial cells develop and divide with the use of nutrients offered in mature biofilm layers and are been surrounded by fluid. This causes microcolonies structures and the surface covered with cells expands to form an integrated layer. During this process, adherent cells also produce EPS, and EPS stabilizes colony against fixation of surface cells and environmental movements [18]. Here, because the bacteria produce exopolysaccharides, the attached microorganisms have hard time breaking away from the surfaces, and as a result, they cling tightly to the surface when complete biofilm matrix is formed [9].

The third and fourth stages, biofilm maturation, result in formation of complex structure containing water channels. And this complex structure is affected by biological factors such as hydrodynamic properties and cell-cell signals, growth rate of bacteria, EPS production, and bacterial motility [19]. Maturing biofilms can separate from biofilms and disperse in order for survive [20-21]. Bacterial proliferation never stops in the mature biofilm layer. Just as a single cell can be detached, a cluster of cells with a diameter of 500 μM can also be detached from the surface [22].

Fragmentation is different process and is periodic dispersal of sizeable fraction of biomass from biofilm. It may be due to flow dynamics, shear effect of liquid volume, presence of certain chemicals in liquid environment, changes in surface properties of bacteria or layer [21]. The released bacteria can been transported to new regions and the biofilm process begins again [10]. It has be reported that the number of cells detached from biofilm disintegration varies between 10 and 300 cells, depending on the bacterial species [23].

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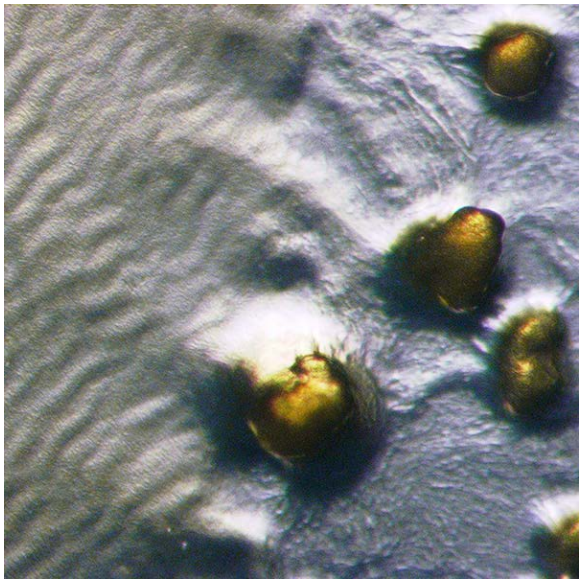

Figure 1. A colony of Biofilm [24].

3. Role of Infections

Pathogens can enter the human body through trauma, medical treatments, dental procedures, or other methods. Biofilms are harmful when they come into touch with exposed wounds. It is be estimated that about 65% of all bacterial infections are associated with bacterial biofilms. These include both, device- and non-device-associated infections [25].

Most research on bacterial pathogenesis has focused on acute infections, but these diseases are now being supplemented by new category, chronic infections, caused by bacteria growing within biofilms. Biofilm infections, such as chronic wounds and fibrinous peritonitis infections, affect millions of fish in world every year and cause many deaths. However, in cases where bacteria manage to form biofilm within their host, the infection often proves incurable and can become chronic. The key to chronic biofilm infections is extreme resistance to antibiotics and evasion of host defenses. Bacteria have similar lifestyle (biofilm) in both living environments, but struggle for survival and dominance is different [26].

Bacterial contamination on implants and prosthetic medical instruments causing infections can be life-threatening, chronic infections and mortal [27]. A mature biofilm layer is sticky formation that can been seen even with naked eye. In the presence of biofilm, it is impossible to cure the infection with antibiotic treatment alone [28].

Biofilm infections is their resistance mechanism against antimicrobial agents. EPS, which forms basic structure of biofilm matrix and forms outer layer of biofilm, prevents antimicrobial agents from diffusion. Microbial agents that cannot overcome the EPS barrier cannot reach the microorganism cells within biofilm structure and cannot show their effects. In studies in literature; It has been shown that microorganisms in biofilm form are 1000 times more resistant to antimicrobial agents than free cells. Microorganisms within biofilm structure also cause emergence of multidrug resistance by transferring resistance genes they have developed [29].

Antibiotic resistance is complex in biofilms. Most importantly, biofilm-specific features such as differential expression of multiple gene networks, extracellular matrix, and metabolic heterogeneity of subpopulations within a biofilm colony contribute greatly to antibiotic resistance [30].

Bacteria that form biofilms can transfer plasmids carrying resistance genes to each other by conjugation. As a result, resistance mechanisms develop against various types of antibiotics, and there

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is an increase in the incidence of cases in which antibiotics are inadequate even when used multiple times. In its report published in 2013, The United States Centers for Disease Control and Prevention (CDC) states that 23,000 of the more than 2 million bacterial infections occurring in the USA resulted in death due to antibiotic resistance [31].

The European Center or Disease Prevention and Control (ECDC) explains that 25,000 of the bacterial infections occurring in Europe result in death due to antibiotic resistance. Bacteria are among that cause high rates of infection with the biofilm form they form [32-33].

4. Biofilm scenario and biofilm popular spaces

Although researchers such as Henrici and Zobell studied presence of surface-attached bacteria almost 70 years ago, meaning of biofilm communities is only now being understood. Moreover, perspective on microbiology has also changed as bacteria began to be viewed from perspective of multicellular act. Considering that there are countless bacteria that can communicate and countless types of polysaccharides that can be produced, number of different types of biofilms that can be achieved is approaching infinity. For this reason, biofilms formed by single species are very rarely in nature, and biofilms formed by more than one organism are more common [12].

The three major components required for biofilm are microorganisms, solid surface and liquid flow. Oral cavity contains all three components and is susceptible to biofilm formation in short time. It provides conducive environment. Because bacterial community embedded in extracellular polysaccharide matrix that adheres to surfaces such as teeth and surrounding tissues, root canals, implant components, restorative and prosthetic materials is called oral biofilm. Oral biofilm; It is considered clinically important microbiological process because it causes primary and secondary caries, failure of endodontic treatments, periodontal diseases and implant loss due to effect of cariogenic bacteria, depending on surface on which it occurs in mouth.

The National Institutes of Health (NIH) reported that among all microbial infections, 65% and 80%, respectively, are associated with biofilm [25-28].

[34] showed that the adhesion of *Streptococcus spp.* was reversible less than a hundred times in less than 10 minutes. [35] reported that irreversible adhesion of cells to a cleaned surface occurred within 30 min after exposure to a fresh suspension containing *A. flavithermus* B12-Cm cells. [36] found that *"Streptococcus thermophilus* and *Bacillus cereus* cultures reversibly adhered to stainless steel in less than 60 seconds. [12] described that exopolysaccharides and/or specific ligands lock bacteria onto surfaces and form complexes with the surface through the production of pili or fimbriae. After this stage, very strong physical or chemical forces such as scraping, brushing or chemical cleaners are required to remove bacteria from surfaces [10-37].

5. Discussion

Biofilms widely spanning from natural surroundings like rivers and oceans to human-made constructs like pipelines, medical instruments, and even dental plaque in your mouth. They exhibit capacity to flourish in challenging environments, including depths of deep-sea hydrothermal vents and highly acidic hot springs.

Biofilm structures act as shields, permitting the bacteria to cluster together and from a sac-like biofilm. These biofilms serve as defensive effect, making it difficult for the immune system to challeng infections effectively.

Biofilm architecture create challenging situation for immune system by making it harder to combat infections effectively. While immune system recognizes presence of biofilms as foreign entities, it struggles to reach and eliminate them because of their protective structure.

By using in experimental phase drugs and some disruptors, it becomes possible to combat infections that hide within biofilms more effectively. These natural compounds can break down the protective structure of biofilms, rendering them more vulnerable to the immune system.

The difficulty in antibiotic penetration into matrix is quite striking. In *P. aeruginosa* infection, ciprofloxacin reaches the infection site in 40 seconds if there is no biofilm, while it reaches the infection site in 21 minutes if there is biofilm [28].

When oral biofilm is be evaluated, dental plaque is defined as complex communities of microorganisms attached to tooth surface, and with this features it is similar to biofilm structure. For this reason, plaque is be considered as biofilm according to many studies conducted in recent years. When viewed as whole, biofilm contains approximately 700 types of bacteria. New species are still being isolated [38-39].

When food biofilm is be investigated, a single powerful biological agent can weaken biofilms, but is insufficient to destroy mature biofilms formed by bacterial species in foods. The right combination of two or more control approaches, called “barrier technology”, is be thought to overcome this problem [40].

In *P. aeruginosa* infection, mechanical (sonication, etc.) destruction/removal of medical biofilm, immune modulation (low dose azithromycin, doxycycline), local antimicrobials (silver or tobramycin), drugs with good biofilm penetration (e.g. rifampicin (combined), daptomycin, echinocandin use) are eradication methods [28].

Toushik et al. 2020 was reported that "Fighting with old foes: The pledge of microbe-derived biological agents to defeat mono- and mixed-bacterial biofilms concerning food industries" entitled manuscript. Microbe-derived biological agents constitute a “green” biofilm-suppressor approach. Biofilm agents as an alternative to a physical or chemical treatment approach. Biological compounds are effective in preventing both mono-bacterial and mixed-bacterial biofilms. A mixture of biological agents can exhibit broad-spectrum anti-biofilm efficacy.

Foods safety are obligatory for survive. It has been estimated that 250 known and many unknown diseases are transmitted to humans majority by foods unsafety contaminated with pathogenic microorganism, causing diseases of more than 600 million people globally, including 420,000 deaths annually. [41]. The National Institutes of Health in the USA reported that 65% of microbial and 80% of chronic infections, including major foodborne diseases, are primarily caused by microorganisms associated with biofilms [25].

Destroying natural biofilm is much more complex than examining single-species biofilms created experimentally. However, biofilms must investigated in order to successfully apply the knowledge obtained from the experimental environment to the industrial environment. Better model systems and more reliable techniques for evaluating control strategies must be developed. Technological developments in preventing biofilm or eliminating the biofilm that has formed show the importance and currency of the biofilm issue.

6. Conclusion

In 17th century, Antoine Von Leeuwenhoek, for the first time observed a type of creature on his own teeth, discovery considered to biofilm [42]. Zobell in 1943 stated that “the surrounding sea water have less number of bacteria than on the surface” [43]. Even at the end of 1960 and the start of 1970, physical and chemical properties of biofilms were not investigated [44]. [45] observed “Bottle Effect”

of marine microorganisms – the growth and activity enhances when they are attached to a surface [45-25].

Counting live bacteria is important in monitoring biofilm [46]. Adversely, the large doses of antibiotics used to treat biofilms clinically have also contributed to the development of antibiotic-resistant bacteria strains. Also it has been seen that some bacteria within biofilms, called “persister cells” are dormant variants that exhibit antibiotic tolerance and can become active when the therapy is withdrawn [47].

Papadopoulos et al. 2024 was reported that further use X-ray computed microtomography to image spatial distribution of biofilms and computational fluid dynamics to link biofilm. By combining the advantages of additive manufacturing for the creation of reproducible 3D porous microarchitectures with flow control and instrumentation accuracy of microfluidics, system provides a platform to study dynamics of biofilm in 3D porous media and to rapidly test in process engineering [48].

Watnick and Kolter, 2000 ‘Biofilm, city of microbes’ title manuscript this concept, which was termed for the first time, is very impressive, emphatic and shed light on this compilation [49].

Bacteria living in biofilms produce a protective matrix, which makes them difficult to kill. Due to the widespread distribution of biofilms in diseases and their resilience to numerous antimicrobial treatments, biofilm research is receiving more attention. Owing to increasing antimicrobial resistance, the focus of current research is shifting from targeting bacterial growth/division that causes cell death or dormancy, towards novel approaches. Recently, biofilm-related infections have been increasing and have become almost unpreventable. So, new treatment, biofilm drugs and prophylaxis strategies are been needed.

Biofilms are associated with two third of all infections especially chronic and device-related infections. The effects of antibiotic applications on resistance genes should be examined in infection modeling with in vivo model organisms.

QS, major features of biofilm, such as surface adherence, EPS structure, architectural of biofilm, cell features within biofilm and highly regulated biofilm maturation - dispersal are being examined as targets for biofilm-specific treatments.

This very dangerous biofilm formation, which has existed for a long time and whose research continues to this day, has been studied by many scientists. It seems that this vital virulence force, the biofilm, will continue to be a system where bacteria socialize, communicate, easily transfer genes, and in short, will continue to increase their risk of disease.

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