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AN OVERVIEW TO EXPLORING BIOFILM AND ANTIBIOFILM FORMATION APPROACHES

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Abstract

Bacteria that form sticky material around the surface and aggregate into matrix with each other are known as biofilms. They comprises different components like clumps of microbes, their flocculants and porous material in which they adhere and form a unique structure. Only some natural multicellular communities have ability to produce the biofilms. Biofilm is a sticky material, and it is organized by micro- and macro-colonies that cannot be easily removed without rinsing. At first stage of attachment, bacteria produce extracellular polymeric substances (EPS). In some cases, biofilm formation is not considered as a good property of bacteria. Therefore, some antibiofilm formation approaches (Enzyme action, Photodynamic therapy, Nanoparticles and Aptamers) are utilized to stop this process. Production of antibiofilm formation agents with antibacterial activity is the most significant approach that can be utilized for various purposes.

Keywords: Biofilm, Extracellular Polymeric Substances, Microbial Colony, Nanoparticles, Aptamers

Introduction

The aggregation of microbes in extracellular material produced by themselves is called as biofilm that is present naturally in our ecosystem. After discovery of biofilms formation ability of bacteria, it has become an emerging issue (Vestby *et al*., 2020) and is recognized as a persuasive matter (Totsika *et al*., 2013). In last decades, during Louis Pasteur discoveries

biofilms were investigated as a new horizon which describes that bacteria have different growth phases which are totally different from planktonic growth.

When bacteria form biofilms, their cellular morphology becomes changed due to attachment of cells with their surfaces (Tuson & Weibel, 2013; Yang *et al*., 2016). In early stage, similar bacterial species aggregate with each other and then collaborate with other bacterial species to form the micro- and macro- colonies (Muhammad *et al*., 2020). Afterwards, extracellular polysaccharides are produced that provide microenvironment for bacterial colonies (Dominique *et al*., 2015). Each bacterium responds according to its requirement against specific environment (Muhammad *et al*., 2020) Then it gradually develop the biofilm structure to fix its place in this structure and to establish strong connection with other partners so that colony becomes stabilized (Roy *et al*., 2018).

Biofilm Formation- Characteristic of Bacteria

Bacteria produce biofilms, which are multicellular populations of microorganisms held together by a matrix. Diverse bacteria have different processes for forming biofilms, which are exclusively determined by environmental circumstances and strain uniqueness. *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* are four well-studied model systems used in this review to get an insight of how different species participate in biofilm development. These bacteria are used as an example to demonstrate biofilm development and the mechanisms involved in the activation of extracellular signaling patterns. Biofilm formation has a significant impact on humans in a variety of ways, including natural, industrial, and medical settings. Biofilm deposits on medical or surgical devices, such as implants or catheters, for example, are frequently observed and cause difficulty in treating chronic infections (Stoodley *et al*., 2004; Donlan, 2008; Hatt & Rather, 2008).

Infections linked to biofilm development have also been reported in human body such as the urinary tract, teeth, and skin (Hatt & Rather, 2008). Despite this, biofilms on human surfaces are not always toxic. Dental plaque biofilms, for example, are made up of dozens of species, and the makeup of these biofilms might tell you whether or not you have a disease. The colonization of dental plaque progresses, but the presence of beneficial species prevents detrimental germs from colonizing (Kreth *et al*., 2008). Biofilms, on the other hand, can be found almost everywhere. Biofilms, for example, can build on ship hulls and inside pipelines, causing

significant damage (Carvalho, 2007). Many natural settings have shown mutualistic relationships with biofilms. Ants, for example, keep pathogen-free fungal gardens alive by growing *Actinobacteria* (Currie, 2001; Danhorn & Fuqua, 2007). A biofilm can provide a wide range of benefits and drawbacks, so understanding how bacteria form in these communities is vital [(Mah & Toole, 2001; Matz & Kjelleberg, 2005; Anderson & Toole, 2008)] Several researchers have found that biofilms were resistant to various antimicrobials protecting them from host defences. One of the likely causes appears to be an increase in the percentage of persisted cells within the biofilm (Lewis, 2005). Persister cells are antibiotic-resistant cells that are genetically identical and do not divide. Persister cells are expected to be immune to antibiotics because they have toxin or antitoxin systems that block antibiotic targets through toxin modules (Lewis, 2005). Extracellular matrix protects constituent cells from external damages, in addition to being a persister. Anderson and Toole (2008) and, Stoodley (2009) found that extracellular matrices provide a diffusion barrier for tiny compounds. As a result of the slower diffusion of nutrients, co-factors and vitamins in biofilms, some of the cells in the bacterial population exhibit dormant metabolic responses.

Bacterial Growth Rate

If cells are connected to a small gap within a biofilm, the bacterial growth rate will be influenced (Stewart & Franklin, 2008). As a result, biofilm formation was predicted by a natural stationary phase of bacterial growth. During stationary phase, bacterial shape changes dramatically due to increased production of secondary metabolites like colors, antibiotics, and other small compounds (Martin & Liras, 1989). Secondary metabolites serve as signaling molecules, allowing other organisms in the same ecosystem to either initiate or prevent the biofilm building process (Lopez & Kolter, 2009). Biofilms assemble a sumptuous meeting of protein, DNA, and polysaccharide in their self-produced EPM (extracellular polysaccharide matrix) and are early found on a variety of surfaces such as living tissues, drinkable water devices, medical units, and so on (Banar *et al*., 2016).

Bacterial biofilms have been extensively researched in terms of cell phagocytosis, antibiotic susceptibility, and specific disinfectant chemicals. Biofilms affect the indoors and outside procedures by changing their architecture. As the cells are in proximity, they alternate their greater chromosomal plasmid, quorum sensing molecules and show splendid persona in respective biofilm community. Regardless of all the special research on architectural facts of

biofilms alongside its mechanisms, composition, advantages and disadvantages, evaluation mainly focuses on the stairs which are involved in biofilm development and antibiofilm methods.

Composition of Microbial Biofilms

Biofilm is a prepared mass of microorganisms living in an extracellular matrix and connected with the residing floor irreversibly (Allesen *et al*., 2006; Anderson & Parsek, 2007). EPS is produced especially in some unspecified time in the future of the attachment phase of a biofilm to the surface. The EPS offers balance and power to the interrelated microorganisms (Allison & Sutherland, 1987; AlFattani & Douglas, 2004; Baidamashina *et al*., 2017). Normally, the thickness of an EPS matrix is in between 0.2 to 1.0μm, despite the fact that measurement of the biofilm does not exceed 10 - 30 nm (Balabanova *et al*., 2017). Almost 5% to 35% of the volume of a biofilm is fed on by means of microorganisms and the rest of volume is of extracellular matrix. The primary composition of this EPS is three-dimensional proteins (Choi *et al*., 2015). Specific possibilities of biomolecules make up the composition of EPS together with proteins which have majority. Exclusive substances along with polysaccharides are approximately 1 to 2%, DNA molecules are less than 1% , RNA is also less than 1% , ions either free or sure and the rest is water 97%. Due to this large percentage of water content, it's feasible to drift nutrients providing detail of biofilm area (Costa *et al*., 2017; Garcia & Pagan, 2015).

Steps in Formation of a Biofilm

According to genetic research, biofilm formation takes place in a lot of steps and ways. The maximum distinct signaling system in biofilms is the signaling of quorum sensing that occurrs amongst the cells of microorganisms. In evaluation of the planktonic kinds of the equal microorganisms, transcriptional genes are required. (Costa *et al*., 2017; Ergin *et al*., 2017). The elastic and viscous facts of extracellular matrix contribute towards mechanical balance to a biofilm (Fleming & Rumbaugh, 2017). Biofilm is complex in nature, but it occurs in few common steps (Franklin *et al*., 2015) i.e.,

1. Early adhesion to the surface
2. Formation of micro-colony
3. formation and maturation of biofilm architecture
4. Detachment of the biofilm

Initial Contact or Attachment to the Surface

The preliminary step in biofilm formation calls for attachment of microbial cells to any surface. Attachment of microbial cells of biofilm is performed with the help of flagella and pili which can be finger-like appendages. They'll additionally get attached via different bodily forces like electrostatic interactions or van der Waal's forces and so on (Garcia & Pagan, 2015; Graham & Cady, 2014). Stable-liquid interface is the primary intent for their attachment (Graham and Cady, 2014). Exclusive motive at the gain of the attachment of microbes is floor hydrophobicity due to the fact that it substantially reduces pressure of repulsion between microorganism and floor (Haris & Khan, 2017; Kumaran *et al*., 2018). Microorganisms have a greater capacity to cling to Teflon or plastics because they are hydrophobic and non-polar surfaces, as opposed to metals or glass, which are polar and hydrophilic (Li *et al*., 2017; Mao *et al*., 2017; Misba *et al*., 2018).

Formation of Micro-colony

After the stable attachment section of microorganisms to a dwelling or a nonliving surface, multiplication and division of cells begin as initiated via unique chemical signaling happening in the extracellular polysaccharide matrix. This will sooner or later cause the micro colonies formation (Graham & Cady, 2014; Norris, 2014). Bacterial colonies form many microcommunities in a biofilm, and these communities interact with one another in a variety of ways. For example, complex organic matter is transformed into methane and carbon dioxide during anaerobic digestion. There are three forms of bacterial participation required (Oliver *et al*., 2018), such as:

(i) Chemical compound production, such as acids and alcohols, is initiated by fermentative bacteria and is dependent on the dissimilation of complex organic molecules.

(ii) Acetogenic bacteria then consumed these as their substrates.

(iii) Methanogens obtain energy by converting acetate, hydrogen, and carbon dioxide into methane. Biofilm satisfies the requirement for a full environment for the establishment of a syntrophic relationship (Oliver *et al*., 2018).

Maturation and Architecture of a Biofilm

At maturation level, microbial cells coordinate with the help of car-inducer alerts (Pan *et al*., 2016; Steenackers *et al*., 2012). To attain required microbial density, cellular to cell coordination is an imperative method which is completed by means of quorum sensing this is facilitated by using automobile inducer signaling molecules (Costa *et al*., 2017). An excellent method for structuring EPS expression of positive gene products is required at this stage of biofilm maturation. Three-dimensional shape of a biofilm is maintained by the EPS, consequently interstitial spaces are developed in the matrix. A circulatory device is required to remove waste from the agencies of micro-colonies and to distribute necessary vitamins among the communities of a biofilm, and these channels are filled with water to accomplish this goal (Totsika *et al*., 2013).

Detachment or Dispersion Stage of a Biofilm

In the detachment section, sessile structure of cells in the biofilm is converted into motile form. For that reason, detachment takes area in a natural phenomenon (Graham & Cady, 2014). On the other hand, some bacterial cells, at once, disperse into the surroundings as they just get concerned in mechanical stressing technique now and again (Wang *et al*., 2017). In the biofilm, at some factor of the detachment section, the microbial communities launch one-of-a-kind saccharolytic enzymes that allow microbes to be released to a new location for colonization. For example, alginate lyase is produced by *P. aeruginosa* and *P. fluorescens*, N-acetyl-heparosan lyase is produced by *E. coli* and hyaluronidase is produced by *Streptococcus zooepidemicus* for the breakdown of the extracellular polysaccharide matrix and later detachment (Zaptoczna *et al*., 2017). In this phase, up-law of the expression of a few proteins is achieved by using microbial cells due to proteins that assist in formation of flagella with bacteria moving to a brand-new site. This detachment phenomenon can be the source of spreading of infections (Ribert & Cossart, 2015).

Factors Assisting Biofilm Formation

Many genetic and environmental elements contribute in formation of biofilm as many microbial communities showcase resistance closer to specific factors. Those elements may be physiological and chemical (Livermore, 2003).

I. Some microorganisms enhance resistance genes in their plasmids which cause them to become less willing towards resistance (Webber & Piddock, 2003).
II. Mutation at genomic level and expanded quorum-sensing guidelines contribute towards the elevated formation of biofilm (Hoiby *et al*., 2010).
III. Structural trade of cellular cells, makes bigger attachment picks making it feasible to connect with special surfaces and form biofilms (Mazhar & Asif, 2020).

Consequently, microbes fortify natural resistance before everything in response to exceptional environmental elements and strains. They additionally switch their resistance genes to next era helping biofilm formation. Qne-of-a-kind dyes, enzymes, tablets, structural trade and human sports can minimize this resistance and get rid of biofilm formation (Mazhar & Asif, 2020).

Anti-Biofilm Approaches

Anti-biofilm strategies target the trade of biofilms through changing their complete formation steps and their durability via unique natural steps brought on structures. Anti-biofilm methods can modify both the adhesion level of biofilms or mature biofilms (Miquel *et al*., 2016). The infection purpose at some point of implantation and specific surgical method is because of the reason that biofilm is largely predicated upon the floor and shape of scientific devices. Bacterial cells are available in contact with the scientific device surfaces and exert strong interactions with them. As a result, the surface abilities or compositions of biomaterials are modified so that it will collect appropriate results. Surface engineering of brilliant medical gadgets reduces the probabilities of biofilm formation to cause decline in biofilm infections as this system increases the magnification and biocompatibility of devices towards human beings. Except this, one-of-a-kind techniques are beginning to be introduced that especially target the adhesion and maturation steps. Anti-biofilm sellers may be used as adjuvants in aggregation with antimicrobial shops (Roy *et al*., 2018).

Anti-Adhesion Approaches

This technique can cause either popular or unique inhibition of adhesion counting on its target. Topographical research conferred the nonspecific inhibition of adhesion (Beloin *et al*., 2014; Neoh *et al*., 2017). Engineering or manipulation of surface topography at micro and nanoscale appears to be a fantastic technique as it's non-poisonous and unbiased of fabric type.

Furthermore, chemical amendment is moreover a part of this technique (Graham & Cady, 2014). But this approach has now not been definitely explored (Hsu *et al*., 2013). Lagree *et al*., in 2018 reported the consequences of surface topography at the *Candida albicans* biofilm formation. Polydimethyl siloxane (PDMS) solids of distinctive sizes were coated on the floor of biofilm throughout its formation. They discovered that greater biofilm formation was determined on surfaces that were covered with debris with a size range of 4 to 8 µm when compared to surfaces that were lined with debris with a size range of 0.5 to 5 µm.

Perera-Costa *et al*. (2014) pronounced that 3 bacterial strains (*S. epidermidis, E. coli* and *Bacillus subtilis*) exhibit reduced adhesion phenomena whilst biofilms grown on spatially organized micro-topographic surface were handled with polydimethyl siloxane by using 30 to 40% more as compared with easy surfaces. The inhibitory effect of ground topography has been attributed to the presence of fewer binding internet web sites whilst in assessment to flat surfaces. Moreover, the strong surfaces on which bacteria have a tendency to generate biofilm trapped air which decreases its effectivity of attachment to the robust ground (Lagree *et al*., 2018).

Photo-Dynamic Therapy (PDT)

It is primarily based on the use of a secure PlayStation (photosensitizer) that activates upon exposure to a distinct wavelength. The activation of this photosensitizer produces unique cytotoxic reactive oxygen species, that in turn damage the sub cellular factors of microorganisms. PDT has a big spectrum interest in opposition to biofilm microorganisms which include resistant pathogens. Photosensitizers target unique web sites of microorganisms alongside the elements of the biofilm matrix. After penetrating the cytoplasmic membranes, they cause damage to the phone floor or intracellular damage (Hu *et al*., 2018). Misba *et al*. (2016) conjugated a photosensitizer toluidine blue O (TBO) with silver nanoparticles (AgNPs). *S. mutans* biofilm repressed upon exposure to laser moderate (630nm) of conjugate. Conjugate movement appears to be far superior to TMO because it increases cell substance leakage and results in more obvious down regulation of biofilm associated genes. Pourhajibagher *et al*. (2016) reported the impact of sublethal doses of PDT using ICG (indocyanine inexperienced), TBO (toluidine blue O) and MB (methylene blue) on *E. faecalis* biofilms. The sub-lethal dose reduces biofilm formation by, 19.5, 42.8 and 22.6 percent, respectively. The acquired consequences factor out that ICG-PDT demonstrated better antibiofilm workout in comparison

with distinct photosensitizers. Chiniforush *et al.* in (2016) examined the outcomes of ICG (Indocyanin inexperienced) on biofilms via *E. faecalis*. ICG-mediated photodynamic therapy significantly reduced bacterial counts and inhibited biofilm formation.

Nanoparticles

Any material whose fundamental unit in the three-dimensional space is in the range of 1 to 100nm or one dimension is in the nanometer scale range is referred to as nanoparticle. Nano debris having unique characteristics which include massive surface region to quantity ratio has advanced the physical and chemical houses. Because of these functions, they may be used as antibacterial agents against different microorganisms over a broad-spectrum variety. In addition to their antibacterial pastime, nanoparticles have currently come to be a promising preventing method towards biofilms (Wang *et al*., 2016).

The silver nanoparticles developed by Namasivayam *et al.* (2013) could reduce the macromolecular content of biofilm matrix, thereby weakening biofilm formation and allowing medicine penetration. GPA NPs (aggregate of Au (gold) nanoparticles with gentamicin) produced by Mu *et al*. (2015) efficiently dented the established biofilms of Gram-negative microorganism i.e. *S. typhimurium*, *P. aeruginosa*, and *E. coli* and Gram positive *L. monocytogenes* and *S. aureus*. Costa *et al*. (2017) produced poly chitosan nanoparticles which have proven bactericidal interest and anti-adhesive property. Furthermore, they reduce the biofilm formation by means of the Methicillin prone and resistant *S. aureus* traces. The silver nanoparticles produced by Kyaw *et al*. (2017) were able to save the biofilm formation with the aid of *B. subtilis, S. typhimurium, P. aeruginosa* and *E. coli*. They ruined *B. subtilis, Salmonella* and *Pseudomonas* biofilms at concentrations starting from 25-50 ppm. Ramachandran and Sangeetha (2017) assessed the antibiofilm potential of AgNPs against *P. aeruginosa*, *A. baumanni*, *S. pneumonia* and *E. coli*. The estimated variety of about 12.5 - 100µg/ml of AgNPs successfully limited biofilm formation of the examined bacteria. Ravindran *et al*. (2017) additionally tested AgNPs synthesized by using the aqueous root extract of *V. zizanioides* which seem to be perfect anti-qouroum sensing and anti-biofilm agents in opposition to *S. marcenscens*. Oliver *et al*. (2018) prepared AgNPs by using polycat, cat-borax or catechin. AgNPs synthesized by the usage of polycat exhibited superior antibacterial effect highlighting the anti-biofilm property against *Pseudomonas aeruginosa* biofilms. Li *et al*. in 2018 studied block copolymer nanoparticles, which are novel polymeric NPs, that displayed the phenomena of dispersal upon

binding to cells of numerous clinically significant Gram-negative microorganism, which can be resistant to many drugs, such as *Enterococci, E. faecalis* and *S. aureus*. Slomberg *et al*. in 2013 examined the results of nitric oxide (NO) liberating silica nanoparticles on biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They stated that in assessment to round fashioned NPs, rod fashioned NPs have been more powerful in handing over NO to the biofilms and prompted extra antibacterial movement. As a result, nanoparticles having antibacterial traits in addition to other residences (extra surface prices, small sizes and shapes) fall inside the class of anti-biofilm agents which brings about accelerated penetration capacity and makes them strong drug shipping retailers (Hu, 2017).

Aptamers

Aptamers include single stranded (ss) DNA or RNA sequences that can primarily bind to and inhibit their targets. Only in few studies, aptamers have been investigated as anti-biofilm technique. Aptamer is a promising method which is used to prevent biofilm formation by blocking the flagellar motility. Ning *et al*. (2015) advanced a single stranded DNA aptamer that specially centered *S. choleraesuis* flagellin protein. Formation of mature biofilms is prevented by the characterized aptamers as they limited the early attachment of microbes by preventing the cell aggregation. Synergistic effect was shown when flagellin aptamer was integrated with ampicillin antibiotic. Cheng *et al*. (2017), in addition, upgraded the flagella concentrated on aptamer with the aid of linking it with ampicillin. So, in comparison with one-at-a-time carried out additives, the conjugate had a unique antibacterial interest and better anti-biofilm pastime which makes it greatly approachable. It ensures facilitated access of ampicillin into the biofilm which reduces its antibiotic tolerance. Microorganisms lose their motility because of flagellin aptamer as it reduces the matrix adherence ability and the developed aptamer may act as server for an antibiotic provider, allowing ampicillin to breach the biofilm, eliminate its cells, and overcome drug tolerance in the biofilm. Wang *et al*. (2017) reported an aptamer that focused on *P. aeruginosa* biofilms. The aptamer which acted as a targeted transport agent expanded to two complexes, aptamerSWNT and aptamerciprofloxacin-SWNT. The former complex showed better inhibition of biofilm upto 36% when compared to SWNT. The 3-issue complicated established higher antibiofilm interest than that after the complicated additives applied separately or as an issue complicated. Mao *et al* (2018) studied *S. typhimurium* biofilms with the conjugate of graphene oxide aptamer and graphene oxide. The ST-3-cross conjugate

repressed and detached biofilms up to 93.5 and 84.6%, respectively. ST-3 aptamer might have eased the entry and induced a lower mobile membrane capability.

Conclusion:

Biofilms are described as microbial groups that are aggregated to each other or to diverse surfaces and embedded in extracellular matrix which is self-produced. They also constitute microbial aggregates, adherent populations, and floccules within the porous media. Biofilm formation is not an amazing component in lots of methods because it harms medical devices, reasons dental consists of water infection etc. So, some method to stop the formation of biofilm is required but there are a few antibiofilm procedures and by using them it is possible to prevent the boom of biofilms. Several strategies encompass photodynamic therapy, aptamers, nanoparticles, enzyme treatment, anti-adhesion processes etc are in use to suppress the biofilm formation. The enhancement of anti-biofilm agents in opposition to different microbial objectives and their subsequent application as adjuvants with antimicrobial sellers seems to be greener.

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