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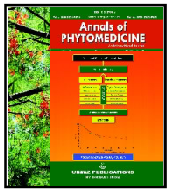

Original article

Enzymes as competent tool for efficient management of pathogen's biofilms

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Abstract

Microbial biofilm is a microbial assemblage which is formed by bacterial adhesion, growth and expansion, enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface. Biofilms are group or micro-organisms in which microbes produced extracellular polymeric substances (EPS), such as polysaccharides, proteins and extracellular microbial DNA. The biofilm can consist of one or more microbial (bacterial or fungal) species and formation of biofilm is a survival strategy for bacteria and fungi to adapt to their living environment, especially in the hostile environment. Bacterial biofilms are normally beyond the access of antibiotics and human immune system and antibiotic treatment is currently most effective measure for the control of microbial infections. However, antibiotic treatments are almost impossible to remove biofilm infections as the pathogenic bacteria in biofilms are resistant to current therapeutic regimes due to their resistant phenotype. The efficient eradication of biofilm is major concern in healthcare sector, especially in living system where use of harsh chemicals and high temperature are unthinkable. However, milder reagents such as enzymes can be of great help as their actions are highly specific to target molecule and have capability to disrupt the structural integrity of the biofilm matrix. The degradation of extra polymeric substance exposes the pathogenic bacterial cells to antibiotics, which along with host immune response acts more efficiently to clear the infectious agents. The major enzymes used to degrade biofilm are alginate lyase, DNase I, α-amylase, protease and dispersin B. The knowledge of chemical nature of the EPS in biofilm helps in deciding that requirement for the use of single enzyme or combination of various enzymes for efficient dispersion of microbial biofilms.

Keywords: Microbial biofilms, EPS, pathogens, enzymes

1. Introduction

Microbial biofilm is a structural community of bacterial cells, surrounded in a self-produced polymeric matrix attached to an inert or living surface. The self-produced extra-polymeric matrix facilitates the survival of bacterial cells in an adverse environment. The matrices contain polysaccharides, proteins, and extra cellular microbial DNA. The biofilm can consist of one or more microbial (bacterial or fungal) species (Aleksandra *et al.*, 2012). Biofilms comprise multiple microorganisms that are found to be associated with the biotic and abiotic surfaces. Biofilms can be either single or multilayered and can have either homogenous or heterogeneous populations of bacteria which remain in the matrix made up of extracellular polymeric substances, secreted by constituent population of the biofilm (Gupta *et al.*, 2016). Biofilms can easily develop on the inert surfaces of medical devices, contact lenses, and catheters or living tissues, as on epithelium of the lungs (particularly in cystic fibrosis patients), on the endocardium and wounds (Aleksandra *et al.*, 2012; Awoke *et al.*, 2019). Biofilm was reported to form in diseases like endocarditis, periodontitis, rhinosinusitis and osteomyelitis, but most frequently it is seen in medical implants and urinary catheters (Table 1). These infections can generally been treated by removal of the implant which subsequently increase the trauma to the patient and the cost of treatment. The major reason for failure of antimicrobial therapy is the formation of microbial biofilms. The biofilm generally cannot be treated by antibiotic therapy alone because the microorganisms in it remain unaffected by given treatment. The biofilm infection indications are recurrent even after several antibiotic therapy cycles and the only successful means of eradicating the cause of the infection is the removal of the implanted device or the surgical removal of the biofilm that has formed on live tissue (Aleksandra *et al.*, 2012).

Further, due to ubiquitous nature of biofilms, it is difficult to eradicate them. Many infectious diseases harbor biofilms of bacterial pathogens as the reservoir of continuous infections which can prove fatal at times (Gupta *et al.*, 2016). Growing microorganisms cause chronic infections with characteristics, like persistent inflammation and tissue damage. A large number of chronic bacterial infections include bacterial biofilms, making these infections very hard to be eradicated by conventional antibiotic therapy (Aleksandra *et al.*, 2012). The biofilms differ from their free-living counterparts in their growth rate, composition, structure and increased resistance to therapeutics and antibodies by virtue of upregulation and/or down regulation of approximately 40% of their genes. This makes them highly resistant to the therapeutic doses of antimicrobial agents (Prakash *et al.*, 2003).

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The fraction of bacteria evolve as persister cells (metabolically inert, replicate slowly, modulate toxin-antitoxin system, upregulate DNA repair and antioxidative machinery, have enhanced phosphate metabolism and exhibit unresponsiveness towards minimal inhibitory concentrations of antibiotics) are genetically similar but are physiologically different compared to parent cells (Lewis, 2010). Majority of biofilm cells and planktonic cells normally kills by drug treatment. However, drug tolerant persisters repopulate the biofilm, disseminate into single microbial cell and start a new cycle of biofilm development (Lewis, 2010; Keren *et al.,* 2011; Zhang, 2014) that increases the duration of treatment of diseases caused by biofilm forming pathogenic microorganisms. It has been observed that bacteria residing within biofilms is antibiotic tolerant and susceptible to antibiotics or other chemical upon dispersal from biofilm which suggest that resilience towards antibiotics is due to phenotypic adaptability and not essentially due to genetic adaptability (Anwar *et al.,* 1989). Factors such as mechanical stress, enzymatic digestion, pH, oxygen availability, temperature and limiting nutrition trigger dispersal of cells from the biofilm. Biofilms induced due to low oxygen condition whereas normoxia decreases biofilm formation (Totani *et al.,* 2017). Enhanced bacterial respiration reduces the persisters in bacterial population (Vilcheze *et al.,* 2017; Kumar *et al.,* 2017).

Table 1: The common objects and sites for biofilms generation and their related infections

Objects for infection	Major biofilm generating bacterial species	Location of infection
Living objects		
Native valve endocarditis	*Viridans* group *Streptococci*	Inner surface of heart
Cystic fibrosis pneumonia	*P. aeruginosa* and *Burkholderia cepacia*	Lungs
Meliodiosis	*Pseudomonas pseudomallei*	Lungs, heart
Dental caries	Acidogenic Gram-positive cocci (*e.g., Streptococcus*)	Tooth
Periodontitis	Gram-negative anaerobic oral bacteria	Gum
Otitis media	Nontypable strains of *Haemophilus influenza*	Middle ear
Bacterial prostatitis	*E. coli* and other Gram-negative bacteria	Prostate gland
Biliary tract infection	Enteric bacteria (*e.g., Escherichia coli*)	Biliary tract
Pentile prostheses	*S. aureus* and *S. epidermidis*	Penis
Peritoneal dialysis peritonitis	A variety of bacteria and fungi	Site where the catheter is inserted to carry the cleansing fluid
Exit sites	*S. epidermidis* and *S. aureus*	Anal
Non- living objects		
Orthopedic devices	*Hemolytic streptococci, Enterococci, P. mirabilis, Bacteroides* sp., *P.aeruginosa, E. coli*	Inside the device
Contact lens	*P. aeruginosa* and Gram-positive cocci	Surface of lens
Schleral buckles	Gram-positive cocci	Deep behind the eyelids under the muscles
IUDs	*S. epidermidis, S. aureus, Corynebacterium* sp., *Micrococcus* sp., *Enterococcus* sp., *Candida albicans,* Group B *Streptococci.*	Intra uterine devices
Urinary catheter cystitis	*S. epidermidis, K. pneumoniae, E. faecalis, Proteus mirabilis*	Surface of catheter
Endotracheal tubes	A variety of bacteria and fungi	Inside the tube
Central venous catheters	*S. epidermidis, S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa, C. albicans*	Surface of catheter
Mechanical heart valves	*Viridans streptococci, Enterococci*	Surface of valves
Vascular grafts	Gram-positive cocci	Surface of grafted material
Biliary stent blockage	A variety of enteric bacteria and fungi	Inside *biliary stents*
Arteriovenous shunts	*S. epidermidis* and *S. aureus*	Surface of shunts
Sutures	*Staphylococcus epidermidis* and *S. aureus*	Surgical site

The host immune system react to various bacterial infections by activating several signalling cascades, complement activation, cytokines and expressing genes associated with stress management (Hartmann and Schikora, 2012; Hartmann *et al.,* 2014). However, host immune responses are not much more effective against bacterial biofilms in comparison with their single microbial cell counterpart (Schultz *et al.,* 2010). Many bacterial pathogens that are initially considered as strictly extracellular can continue to exist inside the host body by the evolution of biofilm through the process of adaptation that result in the evasion of the bacteria from innate immunity of the host. The evasion of biofilms from host innate response proves harmful to the host, as the inflammatory influx released by the body in response to the bacterial infection may harm the host tissues (Archer *et al.,* 2011; Gupta *et al.,* 2016). Sub-population of persister cells is tolerant to high levels of antimicrobial agents. Therefore, antibiotics such as β-lactams which are only active against dividing cells are not very efficient at eradicating biofilm infections (Hoiby *et al.,* 2010). The EPS matrix also acts as a diffusion barrier to delay the infiltration of some antimicrobial agents (Xu *et al.,* 2000). The reactive chlorine species in most of these agents get deactivated at the surface layers of the biofilm because they are not able to disseminate easily into the interior of the biofilm (de Beer *et al.,* 1994). A study showed that oxacillin, cefotaxime, and vancomycin had reduced the penetration throughout *S. aureus* and *S. epidermidis* biofilms (Singh *et al.,* 2010). However, with the emergence of multidrug resistant *S. aureus*, the desire for more effective treatments of biofilm-associated infections becomes imperative (Kalia and Purohit, 2011; Pooi and Yien, 2014).

2. Mechanism of antibiotic resistance of biofilm-associated bacteria

The biofilm matrix is composed of DNA, proteins, extracellular polysaccharides and this make pathogens residing inside resistant to antibiotics. The disruption of the biofilm structure could be achieved *via* the degradation of individual biofilm compounds by various enzymes (Aleksandra *et al.,* 2012).

Various hypotheses have been proposed which try to explain the possible mechanism of antibiotic resistance of biofilm-associated bacteria. The first hypothesis suggests that the antibiotic may not be able to penetrate completely into the deep of biofilm (Stewart and Costerton, 2001). Sometimes, if the antibiotic gets degraded while penetrating the biofilm, their action decreases rapidly. Antibiotics may get adsorbed on the extracellular polymeric surfaces of the biofilm which can diminish the penetration of the antibiotic (aminoglycosides) (Kumon *et al.,* 1994; Shigeta *et al.,* 1997). Sometimes, the negatively charged molecules of the biofilm matrix can bind to positively charged antibiotics in nature. This interaction and binding, thereby hampers the passage of the antibiotic to the biofilm depth (Gordon *et al.,* 1988; Nichols *et al.,* 1988).

Another theory suggests that the biofilm changes their microenvironment rapidly that resulted in the malfunction of the antibiotics. In deep layers of the biofilm, there is no consumable oxygen left and the niche becomes anaerobic (de Beer *et al.,* 1994). It has been reported that a class of antibiotics, namely; aminoglycosides are not effective in anaerobic environmental condition (Tack and Sabath, 1985). It has also been found that the increase in amount of acidic waste accumulation inside a biofilm changes the pH of the environment and subsequently may reduce the action of some antibiotics (Stewart and Costerton, 2001). The accumulation of toxic waste or limitation of necessary substrate can lead the bacterial population to remain in a dormant, non-growing form which can protect the bacteria from certain antibiotics like cell wall inhibiting agents and penicillin (Tuomanen *et al.,* 1986). The biofilm population decreases the abundance of porins in the bacterial membrane under osmotic stress that consequence in the reduction in the transport of some antibiotics inside the cell (Stewart and Costerton, 2001).

It has also been proposed that a small population of the bacteria residing in a biofilm may adapt a protective phenotype that result in the development of drug resistance in biofilm population (Gupta *et al.,* 2016). Antibiotics and chemical treatment may sometimes disturb the gut microflora and cause susceptibility to infection caused by *Clostridium* sp. (Buffie *et al.,* 2012). The symbiota of gut (probiotics) has an important role in maintaining microbial composition, metabolism and immunity of gut by immune modulating systemic immunity and pH (Singh *et al.,* 2013). Gut microflora compete with pathogens for binding sites and neutralize toxins released by pathogens. Microbiota as probiotics have potentials for use against biofilms associated with dental plaque, chronic wounds and urogenital infections (Singh and Hasnain, 2014; Vuotto *et al.,* 2014).

3. Major types of biofilms

3.1 Biofilms formed by *Pseudomonas aeruginosa*

In cystic fibrosis (CF) patients, the principal pathogen in the lungs is *P. aeruginosa*. Bacterial chronic colonization leads to progressive lung damage and eventually respiratory failure and death in most CF patients. In *P.aeruginosa*; a complex quorum sensing hierarchy plays a central or very important role in the regulation of virulence and contributes to the late stages of biofilm maturation. Antibiotic therapy in patients colonized with *P. aeruginosa* often gives a measure of relief from symptoms but fails to cure the beset ongoing infection. This is because the antibiotic therapy cannot eliminate the antibiotic resistant sessile biofilm communities (Aparna and Yadav, 2008).

3.2 Biofilms formed by *Staphylococcus*

The major cause of medical device related infections is the intercellular adhesions of *Staphylococcus epidermidis* with in polysaccharide intercellular adhesin (PIA) biofilms (Gotz, 2002). This polysaccharide is composed of beta-1, 6- linked N-acetyl glucosamines with partly diacetylated residues and the cells embedded in it are protected against the host's immune defense and antibiotic treatment. The genetic and molecular basis of biofilm formation in *staphylococci* is multifactorial. Various proteins such as the staphylococcal surface protein, the accumulation-associated protein, the biofilm associated protein and the clumping factor A are involved in biofilm formation of *S. epidermidis* (Aparna and Yadav, 2008).

3.3 Dental biofilms

The most well studied natural biofilm in human is dental biofilms, commonly called plaque. Development of dental biofilms follows a sequence of events and involves hundreds of species of bacteria. The tooth enamel becomes coated with a variety of proteins and glycoproteins of host origin and this coating is called as acquired

pellicle. The primary colonizers, first *streptococci* and later actinomycetes, colonize the surface of the teeth by adhesion molecules and pilli and undergo cell-to-cell interaction *via* quorum sensing. A number of *streptococci*, including *Streptococcus mutans* and related organisms, begin to synthesize insoluble glucan *via* glucan binding protein. Bridge bacteria (members of the genus *Fusobacterium*) form aggregates with primary colonisers. The late colonisers form aggregate with bridge bacteria. The biofilm primarily consists of non-pathogen at this point of time. However, in the presence of dietary sucrose and other carbohydrate, acids are produced *via* fermentation, which leads to demineralisation of the tooth enamel, over the time, caries. The microbial flora continues to change, if the plaque is allowed to remain undisturbed on the teeth for several days. The last colonisers of the biofilm are considered pathogenic because of their role in periodontal disease. The most important pathogens include *Porphyromonas gingivalis, Bacteriodes forsythus, Actinobacillus actinomycetiemcomitans* and *Treponema denticola* (Rosan and Lamont, 2000).

3.4 Biofilms formed by *Candida*

The common candidiasis manifestations are associated with the formation of *Candida* biofilms on surfaces and it is also associated with infections at both mucosal and systemic sites. *Candida* biofilms share several properties with bacterial biofilms and its formation has three distinct developmental phases: early, intermediate and mature. The detailed structure of mature *C. albicans* biofilms consists of yeast, hyphae and pseudohyphae. This mixture of yeast, hyphae and matrix material is not seen when the organisms is grown in liquid culture or on an agar surface, which suggests that morphogenesis is triggered when an organism contacts a surface (Ramage *et al.,* 2001; Douglas, 2002; Douglas, 2003). The *C. dubliniensis* has the ability to adhere to and form biofilms with structural heterogeneity and typical microcolony and water channel architecture similar to bacterial biofilms and *C. albicans* biofilms (Ramage *et al.,* 2001; O'Toole *et al.,* 2000).

4. Process of biofilm formation

Biofilm formation is a dynamic process and different mechanisms are involved in their attachment and growth (Sadekuzzaman *et al.,* 2015). The biofilm-forming pathogens possess mechanisms for initial attachment to a surface, subsequently form microcolony which leads to development of mature biofilm. In most biofilms formation, unicellular organisms come together to form a community that is attached to a solid surface and covered in an exo-polysaccharide matrix. In a biofilm, the microorganisms account for less than 10% of the dry mass, whereas the matrix can account for over 90%. Biofilm growth is guided by a series of physical, chemical and biological processes (Gupta *et al.,* 2016) and formation can be divided into three main stages: early, intermediate and mature (Aleksandra *et al.,* 2012). Biofilm formation and maturation are sequential, dynamic and complex processes, which depend on the substratum, the medium, intrinsic properties of the cells, signaling molecules, cellular metabolism and genetic control. The process of biofilm formation begins with a conditioning layer of organic or inorganic matter on a surface. This conditioning layer alters the surface characteristics of substratum which eventually favors microorganisms to colonize on surface (Sadekuzzaman *et al.,* 2015).

4.1 Steps involved in biofilm formation

Initially, bacterial cells attach reversibly *via* weak interactions (such as van der Waal forces) with an abiotic or biotic surface (Bos *et al.,* 1999; Donlan, 2002). The bacteria cells attach reversibly to a solid living or non-living substratum (O'Neill *et al.,* 2008) by van der Waal forces, steric interactions, and electrostatic (double layer) interaction, collectively known as the DLVO (Derjaguin, Verwey, Landau, and Overbeek) forces (Garrett *et al.,* 2008). The surface of the substratum is conditioned by the host matrix proteins (fibrinogen, fibronectin, and collagen), forming a conditioning film that facilitates adhesion by the bacteria (Francois *et al.,* 2000; Pooi and Yien, 2014). In this stage, microbial cells adhere to the surface either by physical forces or by bacterial appendages such as Pilli or flagella (Figure 1). 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.

The irreversible attachment to the surface *via* hydrophilic/ hydrophobic interactions by means of several attachment structures (flagella fimbriae, lipopolysaccharides, or adhesive proteins) (Bos *et al.,* 1999; Donlan, 2002). A number of the reversibly adsorbed cells remain immobilize and as a result of the hydrophobic and hydrophilic interaction between the bacteria and the surface, they become irreversibly adsorbed (Liu *et al.,* 2004; Pooi and Yien, 2014). The irreversibly attachment occur when the attractive forces are greater than repulsive forces (Garrett *et al.,* 2008). It has been reported that the physical appendages of bacteria like flagella, fimbriae and pili overcome the physical repulsive forces of the electrical double layer of the cell and the surface and consolidate the interactions between bacteria and the surface (Kumar and Anand, 1998). Cell surface hydrophobicity also plays a crucial role in biofilm formation when the bacteria adhere to a hydrophobic nonpolar surface because the hydrophobic interaction between the surface and the bacteria reduces the repulsive force between them (Tribedi and Sil, 2014). Therefore, in the first and second stages of biofilm development, microbial cells initially loosely associate with the concerned surface, succeeded by specific and strong adhesion (Hall-Stoodley *et al.,* 2004; Gupta *et al.,* 2016).

The proliferation and production of a self-produced extracellular polysaccharide (EPS) matrix mainly composed of polysaccharides, proteins, and extracellular DNA and ultimately the development of the biofilm architecture (Branda *et al.,* 2005; Flemming *et al.,* 2007). The microbial cells communicate among each other by the production of auto inducer signals (Davies *et al.,* 1998; Vasudevan, 2014) that result in the expression of biofilm-specific genes. In this stage, microorganisms secrete a matrix of EPS to stabilize the biofilm network. It was found that *P. aeruginosa* makes and releases three polysaccharides, namely; alginate, Pel and Psl which provide the stability to the biofilm. Alginate interacts with nutrients and water and supplies nutrients to the biofilm (Rasamiravaka *et al.,* 2015). Pel (glucose rich polysaccharide) and Psl (pentasaccharide) act as a scaffold for the structure of the biofilm (Colvin *et al.,* 2011; Franklin *et al.,* 2011). It has been reported that eDNA is also responsible for cellular communication and stabilization of *P. aeruginosa* biofilm (Gloag *et al.,* 2013). Young *Pseudomonas* biofilms are more susceptible to DNase treatment compared to mature biofilm which suggest the stabilizing role for eDNA during the initial biofilm stages

when EPS components are less (Whitchurch *et al.*, 2002). The biofilm at this stage becomes multi-layered and their thickness increased up to 10 µm (Gupta *et al.*, 2016).

EPS are responsible for binding of cells and other particulate materials together (cohesion) and to the surface (adhesion) (Boyle, 1989; Sutherland, 2001; Allison, 2003). The general composition of bacterial EPS comprises polysaccharides, proteins, nucleic acids, lipids, phospholipids, and humic substances (Jahn and Nielsen, 1998; Sutherland, 2001). According to Tsuneda *et al.* (2003), proteins and polysaccharides account for 75-89% of the biofilm EPS composition, indicating that they are the major components which form a gel phase where microorganisms live inside (Sutherland, 2001). The EPS matrices act as a barrier and have protective effect on biofilm microorganisms against adverse conditions. The EPS matrix either delays or prevents the antimicrobials from reaching target pathogens within the biofilm by causing diffusion limitation and/or chemical interaction with the extracellular proteins and polysaccharides (Heinzel, 1998; Mah and O'Toole, 2001). Lipids and nucleic acids might significantly influence the rheological properties and, thus the stability of biofilms (Neu, 1996). The extracellular DNA is required for the initial establishment of biofilms by *P. aeruginosa* and possibly for biofilms formed by other bacteria that specifically release DNA (Whitchurch *et al.*, 2002).

The next phase in biofilm formation is the maturation phase; bacteria grow, multiply and form microcolonies or mature biofilm (Stoodley *et al.*, 2008). The mature biofilm contains water channels that effectively distribute nutrients and signaling molecules within the biofilm (Hall-Stoodley *et al.*, 2004; Dufour *et al.*, 2012). Once microcolonies are formed in optimal growth conditions, the biofilm undergoes the maturation stage where a more complex architecture of biofilm is established with water channels equipped to aid the flow of nutrients into the deep interior of the biofilm. The cells from different regions of a biofilm can show different gene expression patterns due to the different physicochemical conditions in terms of oxygen availability, diffusible substrates and metabolic side products, pH and cell density (Pooi and Yien, 2014). The size of the microcolony at this stage increases and its thickness reaches to about 100 µm. Microcolonies in biofilm quiet often consist of diverse microbial communities. Therefore, multispecies micro-consortia function in relatively complex manner (Gupta *et al.*, 2016). Their close proximity enhances substrate exchange, distribution of metabolic products and removal of toxic or waste end products (Davey and O'toole, 2000).

The dispersion of microbial cell marks the shedding of the biofilm and return of sessile cells to the motile form (Hall-Stoodley *et al.*, 2004). The detachment of biofilm cells takes place individually or in clumps due to intrinsic or extrinsic factors. The biofilm spreads and colonizes to the new surfaces to form biofilm. The microbial community inside the biofilm produces different saccharolytic enzymes which break the biofilm stabilizing polysaccharides and, thereby releases surface bacteria residing on the top of biofilm structure for colonization to a new surface (Gupta *et al.*, 2016). The *P. fluorescens* and *P. aeruginosa* release various enzymes such as alginate lyase, *E. coli* releases N-acetyl-heparosan lyase and *Streptococcus equisimilis* produce hyaluronidase for the breakdown of the biofilm matrix (Sutherland, 1999). Moreover, at this stage, microorganism upregulate the expression of the flagella proteins which make the organisms motile and bacteria can move to a new site. Disruptive forces are also important in biofilm cycle as detachment of cells from the biofilm helps in spreading the infection from the biofilms to other sites (Otto, 2013).

Finally, the cells get dispersed from biofilms and subsequently colonize at other niches (Srey *et al.*, 2012; Sadekuzzaman *et al.*, 2015). The dispersed bacterial cells from the biofilm, either by physical detachment or signalling events followed by the hydrolysis of EPS, return to the mobile state to enable the occupancy of new niches. The subsequent biofilm formation occur in similar manner but at new site (Boles and Horswill, 2011; Pooi and Yien, 2014).

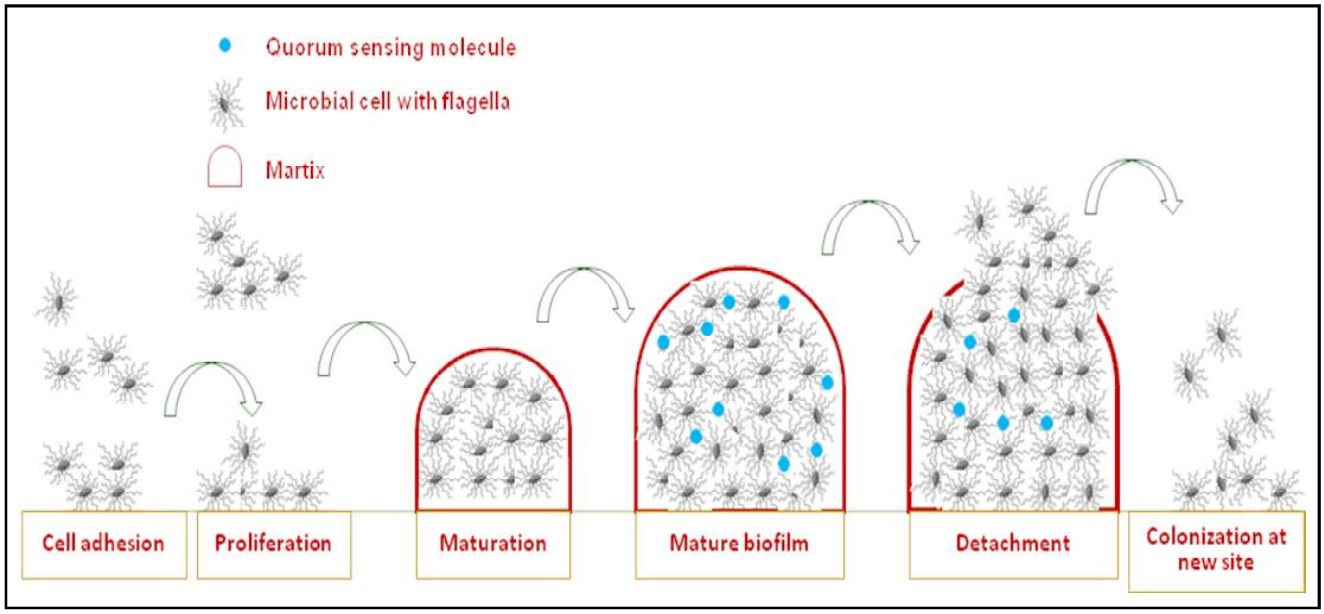

Figure 1: The schematics of biofilm formation.

5. Dispersion of biofilm by enzymes

Different antibiotics and various chemical reagents have been used to control the growth of pathogens and removal or dispersal of biofilms (Akgunlu *et al.*, 2016; Garg and Azmi, 2017; Gezici *et al*., 2017; Kanwar *et al.*, 2018; Das and Gezici, 2018). In *P. aeruginosa*, clarithromycin blocks biofilm matrix formation (Yasuda *et al.*, 1993). The overall thickness of the biofilm reduces by ciprofloxacin and exposes the immature biofilm to phagocytosis by polymorpho nuclear neutrophils and the matrix polymer of biofilm in *S. aureus* was dissolved by streptokinase (Nemoto *et al.*, 2000). The acyl-homoserine lactone interferes with cellular signalling mechanisms which have been used for quorum sensing adversely affects normal biofilm formation (Parsek and Greenberg, 2000). However, due to the antibiotic resistance of biofilm-associated bacteria, alternate and efficient tools are needed to overcome this limitations and the use of different enzymes is one of them.

The composition of the EPS matrix formed by bacteria such as *P. aeruginosa*, *Bacillus* sp, *staphylococcus* sp, *streptococcus* spp. has been studied extensively. The constituent of extracellular matrix depends on the environment and the bacetria present within the biofilms. The main component of biofilms is DNA, polysaccharides, proteins, and EPSes. The degradation of matrix components can weaken or disperse biofilms and studies show that the complete and effective disruption of the biofilms architecture could be done by various enzymes (Fleming *et al.*, 2017). The common enzymes used for disruption of the biofilms are deoxyribonucleases, proteases, glycoside hydrolase, lysostaphin, alginate lyase and lactonase.

5.1 Use of deoxyribonucleases

The use of deoxyribonuclease was found to be effective against the biofilms formed by both Gram +ve (*S. aureus* and *S. pyogenes*) and Gram -ve (*Acinetobacter baumanii, H. influenza, K. pneumonia, E. coli, and P. aeruginosa*) bacteria (Table 2). Researchers showed that the DNase is highly effective at the concentration of 5 µg/ml and able to significantly degrade 24 h active biofilms biomass by approximately 40% (Tetz *et al.*, 2009). They also notice synergistic effects of DNase1with antibiotics (azithromycin, rifamycin, levofloxacin, ampicillin). Table 2 summarizes many of the DNase that has been shown to have biofilm-disrupting activity.

5.2 Use of proteases

Proteases cleave the matrix or surface proteins and inhibit dispersal of established biofilms or interfere with biofilm formation (Pooi and Yien, 2014). Extracellular proteins are a major EPS component that can represent a substantial portion of the biofilm's dry mass (Lasa *et al.*, 2006; Jiao *et al.*, 2010; Muthukrishnan *et al.*, 2011; Speziale *et al.*, 2014). The *S. aureus* alone secrete ten proteases and four of those have been shown to involve in biofilm disruption (Shaw *et al.*, 2004; Abraham *et al.*, 2012; Mootz *et al.*, 2013; Loughran *et al.*, 2014). Exo-proteins are essential for the ability of microbes to sustain and modify the EPS (Zhang and Bishop, 2003; Kaplan *et al.*, 2010) and certain proteins, such as DNA-binding proteins, functional amyloids/amyloid-like proteins and other biofilm-associated proteins, are vital contributors to surface and EPS adhesion and the overall physical stability of the biofilm matrix (Lasa *et al.*, 2006). Thus, enzymatic degradation of EPS exoproteins has the potential to cause a massive dispersal event (Table 2).

5.3 Use of glycoside hydrolase

The major EPS constituents of most biofilms are secreted extracellular polysaccharides, or exopolysaccharides (Wingender *et al.*, 2001; Flemming *et al.*, 2010; Bales *et al.*, 2013). They perform many important functions for the establishment and persistence of biofilms including, structural stability, physical and chemical defense against antimicrobials and the host immune system, adhesion and aggregation of microbial cells, desiccation tolerance, sorption of organic and inorganic compounds, and can provide a carbon source in times of nutrient starvation (Flemming *et al.*, 2010; Limoli *et al.*, 2015; Watters *et al.*, 2016). Attemts have been made to target exopolysaccharides due to their importance for the establishment and maintenance of biofilm architecture with glycoside hydrolases as a means for dispersing biofilms (Table 2). The α-amylase is one of the examples of glycoside hydrolases and its biological function was investigated for inhibition and removal of *S. aureus* biofilms (Craigen *et al.*, 2011). The results indicate that amylase could be used in the near future to control of *S. aureus* biofilm infection (Sadekuzzaman *et al.*, 2015). Cellulase from *Penicillium funiculusum* was effective in degrading mature biofilms of *P. aeruginosa*; and it was also useful in degrading the exopolysaccharides of *P. fluorescens* (Loiselle *et al.*, 2003; Vickery *et al.*, 2004). Dispersin B, which has been produced by a periodontal pathogen *Actinobacillus actinomycetecomitans* is used as biofilm-releasing enzyme. It eliminates the biofilm in half of the catheter tested in a sheep model for port-related bloodstream infection (Kaplan *et al.*, 2004).

5.4 Use of lysostaphin

Lysostaphin is a naturally occurring staphylococcal endopeptidase with ability to effectively penetrate or invade into biofilms (Belyansky *et al.*, 2011; Belyansky *et al.*, 2011). This enzyme is a glycyl-glycine endopeptidase which specifically cleaves the pentaglycine cross-bridge in the staphylococcal peptidoglycan and disrupts the extracellular matrix of *S. aureus* biofilms. The activity of lysostaphin toward biofilms was investigated on clinical and reference strains of *S. aureus* and *S. epidermidis* (Walencka *et al.*, 2005). It was observed that lysostaphin is capable of effectively eradicating the biofilms of all *S. aureus* and *S. epidermidis* strains (Sadekuzzaman *et al.*, 2015). The lysostaphin markedly reduced biomass thickness when applied to biofilms of *S. aureus* clinical isolates grown *in vitro* (Wu *et al.*, 2003; Kokai-Kun *et al.*, 2009). It has been demonstrated that lysostaphin is effective in treatment of established biofilm infections on implanted jugular veins catheters in mice, particularly in combination with nafcillin (Pooi and Yien, 2014). The antimicrobial properties of lysostaphin along with its biofilm inhibitory concentration for *S. aureus* and *S. epidermidis* clinical strains were also determined (Walencka *et al.*, 2005; Aleksandra *et al.*, 2012).

5.5 Use of lyase and lactonases

The co-administration of a lyase with an antibiotic was found to inhibit and eradicate microbial biofilms (Alkawash *et al.*, 2006). The researchers assessed a combined effect of alginate lyase and gentamycin on a biofilm of mucoid *P. aeruginosa* strains. Their results revealed that the combined treatment caused liquefaction of the biofilm matrix and complete eradication of the biofilm structure and living bacteria (Sadekuzzaman *et al.*, 2015). Lactonase was also examined as a potential antibiofilm enzyme and it was found that treatment with of lactonase reduced biofilm formation by *P. aeruginosa* strains (Kiran *et al.*, 2011). Further, treatment with lactonase also disrupted the biofilm structure and increased the sensitivity to antibiotics ciprofloxacin and gentamycin (Kiran *et al.*, 2011; Sadekuzzaman *et al.*, 2015). The role of lactonase as a potential antibiofilm agent was also established by Aleksandra *et al.* (2012).

Table 2: List enzymes that exhibit biofilm-disrupting ability

S.No.	Enzymes types	Target pathogens	References
		DNase used for dispersal of biofilms	
1.	DNase I	*P. aeruginosa, V. cholerae, E. coli, S. pyogenes, S. aureus, S. heamolyticus, K. pneumoniae, Acinetobacter baumannii, Aggregatibacter actinomycetemcomitans, Shewanella oneidensis, Bordetella pertussis, Bordetella bronchiseptica, Campylobacter jejuni, H. influenza, B. bacteriovorus, Enterococcus faecalis, Listeria monocytogenes, Candida albicans* and *Aspergillus fumigatus*	Fredheim *et al.*, 2009; Medina *et al.*, 2009; Whitchurch *et al.*, 2002; Seper *et al.*, 2011; Waryah *et al.*, 2017
2.	DNase 1L2	*P. aeruginosa* and *S. aureus*.	Eckhart *et al.*, 2007
3.	Dornase alpha	*S. aureus* and *S. pneumonia*	Kaplan *et al.*, 2012; Hall-Stoodley *et al.*, 2008
4.	ë Exonuclease	*V. cholera*	Seper *et al.*,2011.
5.	NucB	*B. licheniformis, S. aureus, S. epidermidis, S. salivarius, S. constellatus, S. lugdunesis, S. anginosus, S. intermedius, E. coli, Micrococcus luteus* and *B. subtilis*	Shields *et al.*, 2013; Nijland *et al.*, 2010; Shakir *et al.*, 2012
6.	Streptodornase	*P. aeruginosa*	Nemoto *et al.*, 2003
		Proteases used for dispersal of biofilms	
1.	Aureolysin	*S. aureus*	Loughran *et al.*, 2014
2.	Proteinase K	*S. aureus, Listeria monocytogenes,S. lugdunensis, S. heamolyticus, Gardnerella vaginalis, E. coli, Heamophilus influenza* and *Bdellovibrio bacteriovorus*	Shukla and Rao, 2013; Nguyen *et al.*, 2014; Cui *et al.*, 2016; Chaignon *et al.*, 2007; Patterson *et al.*, 2007; Fredheim *et al.*, 2009; Izano, 2009; Medina *et al.*, 2009;
3.	Spl Proteases	*S. aureus*	Boles and Horswill, 2008; Lauderdale *et al.*, 2009
4.	Staphopain A and B	*S. aureus*	Mootz *et al.*, 2013; Loughran *et al.*, 2014
5.	Streptococcal Cysteine Protease	*S. aureus*	Nelson *et al.*, 2011; Connolly *et al.*, 2011
6.	Trypsin	*P. aeruginosa, S. epidermidis, S. mitis, Actinomyces radicidentis* and *Gardnerella vaginalis*	Chaignon *et al.*, 2007; Patterson *et al.*, 2007; Banar *et al.*, 2016; Niazi *et al.*, 2014
		Glycoside hydrolases used for dispersal of biofilms	
1.	Alginate lyase	*P. aeruginosa*	Lamppa *et al.*, 2013; Hisano *et al.*, 1993; Alkawash *et al.*, 2006; Bayer *et al.*, 1991
2.	α-amylase	*V. cholerae, S. aureus* and *P. aeruginosa*	Kalpana *et al.*, 2012; Craigen *et al.*, 2011; Watters *et al.*, 2016b; Fleming *et al.*, 2017
3	α-mannosidase	*P. aeruginosa*	Banar *et al.*, 2016
4	α-mannosidase	*P. aeruginosa*	Banar *et al.*, 2016
5.	Cellulase	*S. aureus* and *P. aeruginosa*	Fleming *et al.*, 2017
6.	Dispersin B	*S. aureus, A. actinomycetemcomitans, S. epidermidis, A. baumannii, K. pneumoniae, E. coli, Burkholderia*s pp., *A. pleuropneumoniae, Yersinia pestis* and *P. fluorescens*	Waryah *et al.*, 2017; Izano *et al.*, 2007; Kaplan *et al.*, 2004 Izano *et al.*, 2007; Itoh *et al.*, 2005
7.	Hyaluronidase	*S. aureus* and *S. intermedius*	Ibberson *et al.*, 2016; Pecharki *et al.*, 2008

6. Conclusion

The biofilms are the most dominant and safe lifestyle of microorganisms in all environments, either natural or manmade and that's why remain a serious concern in the healthcare, food and marine industries. The formations of biofilms help in microorganism to counter the host immune defenses and conventional antimicrobial therapies more efficiently. The development of effective strategies to combat biofilms (either it's formation or dispersion) is a challenging task. Further, the rise of antibiotic resistance among microbial community has led to a decrease in the efficacy of treatments for the elimination of biofilm related infections. The researchers and clinicians have now begun concentrating their efforts on coupling biofilm destruction with antimicrobial therapy due to the fact that majority chronic human microbial infections are biofilm-associated. The new and advance approaches such as enzyme based therapy gaining more attentions as enzymes weaken the structure of the biofilm by targeting the component of biofilm. These strategies seem to be better for biofilm dispersal as it can more effectively release biofilm-associated microbes from the protection of the EPS. The logical step towards total eradication of biofilm-afforded protection of infectious microorganisms is the uses of enzymes as they can target the EPS on a molecular scale, or cause the microbes themselves to degrade their own biofilms.

7. Future prospective

Biofilm is a reservoir for pathogenic organism and it's major role is in providing antimicrobial resistance especially in chronic diseases. Microbial biofilm research is proceeding on many fronts with particular emphasis on elucidation of the genes specifically expressed by biofilm-associated organism. More study from biofilm perspective is required in the fields of food and water, clinical, environmental and industrial microbiology for better understanding of the various interacting phenomena. The target area of research should be on the development of new methods and strategies for efficient dispersion of microbial biofilms.

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Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. All authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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