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Review Article:

Biofilm formation Inhibition Strategy: Recent Developments

TIROYAONE SHIMANE TSHIKANTWA
MUHAMMAD WAJID ULLAH
GUANG YANG[1]
Department of Biomedical Engineering
Huazhong University of Science and Technology
Wuhan, P.R China
National Engineering Research Centre for Nano-Medicine
Huazhong University of Science and Technology
Wuhan, China

Abstract:

Bacterial biofilms can be seen as a particular kind of tireless bacterial disease. After beginning intrusion, microorganisms can join to living and non-living surfaces, for example, prosthetics and indwelling therapeutic gadgets, and shape a biofilm made out of extracellular polysaccharides, proteins, and different segments. Biofilm development may trigger medication protection and aggravation, bringing about determined diseases. The clinical viewpoints of biofilm development and driving procedures for biofilm inhibitors will be talked about in this mini review.

Key words: Biofilms, Quorum Sensing, anti-QS peptides, exopolysaccharides

[1] Corresponding author: yang_sunny@yahoo.com

INTRODUCTION:

Biofilms are gatherings of microorganisms in which cells adhere to each other on a surface. A biofilm, here and there alluded to as zone, is a polymeric blend for the most part made out of extracellular DNA, proteins, and polysaccharides [1]. Bacterial polysaccharides are a noteworthy part of the extracellular polymeric substance or network of biofilms and intervene the majority of the cell-to-cell and cell-to-surface communications required for biofilm arrangement and adjustment [2]. Biofilms may frame on living or non-living surfaces, on strong or fluid surfaces and additionally on delicate tissue in living life forms, and are commonly impervious to traditional strategies for sterilization. Dental plaque, disgusting covering in tanks, and algal tangles on waterways are cases of biofilms. Biofilm might be inconvenient or gainful. While biofilms are for the most part pathogenic in the body, causing more maladies, they can be utilized valuably in treating sewage, modern waste, and defiled soil. Biofilm development secures and empowers single-cell living beings to expect a multicellular way of life, in which "amass conduct" encourages survival in unfriendly situations [3]. Progress from planktonic development to biofilm happens because of ecological changes, and includes different administrative systems, which make an interpretation of signs to a coordinated quality articulation changes. Four noteworthy antibiofilm techniques are concentrated to counteract unfavorable biofilm arrangement. These include: 1) Prevention by anti-infection prophylaxis and focusing of surface atoms. 2) Weakening by corruption of extracellular grid, hindrance of efflux pumps, or focusing of extracellular and intracellular flagging atoms. 3) Disruption by mechanical ways, organic interruption with compounds, or focusing of extracellular and intracellular flagging particles. 4) Killing by focusing of the bacterial film or focusing on subpopulations with various classes of anti-toxins

[4]. This survey manages biofilm development, featuring a few therapeutically imperative pathogens, and examines late advances on novel systems for biofilm dispersal and restraint.

A BRIEF HISTORY AND DEVELOPMENT:

Antonie van Leeuwenhoek (1684) was the first to show the animalcule (microscopic organisms) found in the plaque of teeth, and depicted in an answer to the Royal Society of London. In 1940, H. Heukelekian and A. Heller expounded on the advancement of bacterial ooze and frontier development appended to surfaces. Zobell (1943) revealed about seawater and depicted huge numbers of the key attributes of appended microbial groups. Since such groups were portrayed and named biofilms in 1978, biofilm science and biofilm designing wind up dynamic fields of study [5]. Microorganisms constitute the best type of life on earth and influence human presence and prosperity either specifically by affecting human wellbeing and sicknesses, or by implication, via doing forms in the characteristic or man-made situations [5].Bacteria frame a structurally complex group (Figure 1) to control cell destiny [6]. Biofilms harbor various cell composes, and it has been suggested that inside biofilms singular cells take after various formative pathways, bringing about heterogeneous populaces [6]. Essentially, mutants don't create extracellular network, i.e., mutants are commonly inadequate in adherence and biofilm development (Figure 2). Understanding the procedures that control biofilm arrangement is essential for improvement of techniques expected to control perpetual contaminations [7]. Cell separation is pervasive and encourages division and advancement. Microbes are fit for multicellular practices that advantage the bacterial group all in all. A striking case of cell separation in microscopic organisms is the development of a biofilm [6,8]. Miniaturized scale settlements of bacterial cells encased in polysaccharide network are isolated from each other

by water channels [9]. Fluid stream happens in water channels, permitting dissemination of supplements, oxygen, and even antimicrobial specialists. Microorganisms exist in two foremost structures as free cells (planktonic state) or in biofilms. Biofilm enables cells to shape long haul connections, communicate with each other and build up metabolic collaboration. The relationship of microbes with a surface and the advancement of a biofilm can be seen as a survival component, with microscopic organisms profiting by securing supplements and assurance from biocides [5].

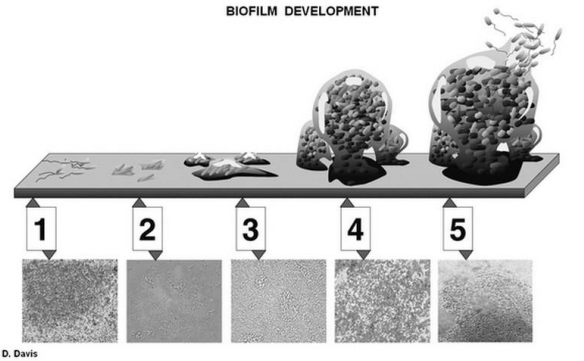

Figure 1: Five stages of bacterial biofilm development

QUORUM SENSING AS TARGET TO CONTROL BIOFILM INFECTION

QS inhibitors and anti-QS peptides

It was well demonstrated 10 years ago that target of QS with synthetic furanones significantly attenuated the lung infections of *P. aeruginosa in vivo*.(10) The recent analyses of synthetic molecules by O′Loughlin *et al.*(11) disclosed the inhibition of the two *P. aeruginosa* QS receptors, LasR and RhlR by synthetics. Their most effective compound, meta-bromo-thiolactone, significantly inhibits the production of virulence factor pyocyanin and biofilm formation. *Caenorhabditis elegans* and human lung epithelial cells were protected from the killing of *P. aeruginosa* by treatment with meta-bromo-thiolactone. They

further found the relevant target was RhlR, not LasR *in vivo*. It has been confirmed in guinea pigs study that a novel QS inhibitor coded as 'yd 47', showed an effect against otitis media and biofilm formation induced by *S. pneumoniae* on Cochlear implants.(12) The combination of QS inhibitor FS3 and daptomycin was investigated for the prevention of prosthesis biofilm in a rat model of staphylococcal vascular graft infection. Both values of MIC and MBC for daptomycin were lower in the presence of FS3 at an *in vitro*study. The combination of FS3 and daptomycin exhibited significant synergy efficacy when compared to any single treatment.(13)

RNAIII-inhibiting peptide was reported to suppress staphylococcal TRAP/agr systems and to reduce biofilm formation *in vivo*. The results indicate the importance of quorum sensing in biofilm infection in the host. The treatment with RNAIII-inhibiting peptide in rats has been found to strongly prevent methicillin-resistant *S. aureus* graft infections, and suggesting that RNAIII-inhibiting peptide can be expected as an anti-QS or/and anti-biofilm agent(14). LoVetri and Madhyastha(15) reported the effects of anti-QS peptides and analogs on the growth of biofilm formation in oral bacteria. It is interesting that a natural QS peptide, competence-stimulating peptide, produced by *Streptococcus mutans*, could kill their own cells at higher concentrations than normal. In addition to cells-killing, KBI-3221, an analog of competence-stimulating peptide developed by various *Streptococcus* species, was shown to decrease biofilm formation.

Attenuation of bacterial QS by furanones, ginseng, garlic and azithromycin significantly improved the immune clearance and the effects of antibiotics *in vitro* and in the animal models of *P.aeruginosa* biofilmpneumonia.(16,17,18,19,20,21) Brackman *et al.* (22) demonstrated that QS inhibitor increased the susceptibilities of both Gram-positive and -negative bacterial biofilms to antibiotics *in vitro* and *in vivo*. Azithromycin has been actually applied routinely to the CF patients as an anti-

QS treatment in several CF centers around the world including the Danish CF Center in Copenhagen.

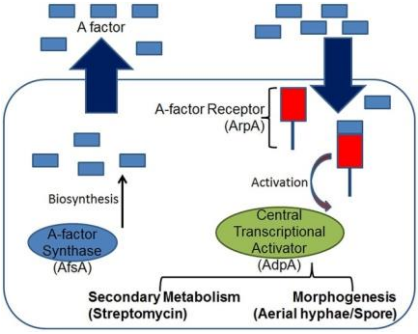

Figure 2: Similar to Gram-negative bacteria, at threshold concentrations the diffusible A-factor (a γ-butyrolactone) binds the intracellular receptor ArpA and activates expression of the transcriptional activator AdpA which in-turn regulates multiple phenotypes either indirectly via a multi-step cascade, such as the development of aerial hyphae and sporulation, or directly, such as the production of secondary metabolites like streptomycin.

TREATMENT OF MICROBIAL BIOFILM INFECTIONS:

As revealed in a few papers, that biofilm contaminations are hard to deal with and are regularly anti-infection treatment alone insufficient. For the most part, the methodologies can be separated into including a remote body or not. If not including a remote body, long haul treatment with high measurements and regularly utilizing mix of anti-microbials with various killing instruments can at times dispense with the disease. Be that as it may, if a remote body is included, evacuation of the material is much of the time important for an effective result. In different cases, just diminishment of the biofilm is conceivable trailed by interminable biofilm suppressive treatment or sitting tight for the biofilm to backslide with a worsening. Here we might want to impart our clinical encounters to our associates in blend with the most recent pertinent writing.

Removal of foreign bodies and abscess

It has been demonstrated that high inoculums (108 CFU·mL−1; CFU, colony forming units) of *Staphylococcus aureus* in animal soft tissues could not create any abscesses in the absence of foreign body, whereas 102 CFU·mL−1 of *S. aureus* were sufficient to induce an infection with foreign body in 95% of the cases despite significant presence of polymorphonuclear leukocytes,34 and this might be associated with the fact that the presence of foreign body significantly downregulated the phagocytosis and intracellular bactericidal effects of polymorphonuclear leukocytes.35 Obviously, foreign body provides an ideal surface for bacteria to attach to, whereas polymorphonuclear leukocyte functions are injured due to the presence of foreign body. Thus, the presence of foreign body increased significantly the possibility of biofilm infection. According to the biofilm characters of antibiotic resistance, it is currently difficult to eradicate biofilm infections by conventional antibiotic treatments. Therefore, the removal of a foreign body becomes an important prerequisite for the eradication of such biofilm infections. It is thus highly recommended to remove the infected indwelling devices implanted into patients for medical reasons or replace the infected device with a new one, if we hope to cure the biofilm infections. In case not possible to remove the infected foreign body, an attempt to reduce the biofilm burden with antibiotics followed by continued suppressive antibiotic treatment to prevent regrowth of the biofilm could be suggested.

Change of the infected central venous catheter (CVC) or dialysis catheter

When bacteria form biofilm on CVC or dialysis catheter, an intermittent bacteraemia with an identical bacterial stain could be expected. In addition, the positive rate of blood cultures sampled from the infected catheter is usually higher than that from the peripheral veins and the time to positivity is at least

two hours shorter if the blood is taken through a CVC containing a biofilm compared to a simultaneously blood culture taken through a peripheral vein.36,37 To cure such catheter biofilm infections, change of the infected catheter is crucial, followed by a short time treatment of sensitive antibiotic intravenously to remove the bacteria released into blood stream from the infected catheter. In case change of catheter is not possible temporarily, antibiotic and other lock therapy may help to minimize the release of planktonic bacterial cells from the catheter biofilm, which means instillation of high concentrations of antibiotic with or without anti-coagulant or 70% ethanol or hydrochloric acid (2 mol·L−1 HCl) into the lumen of CVC.38,39,40,41 In our clinical practice, vancomycin (1 mg·mL−1) is used to the catheter infection with Gram-positive bacteria and gentamicin (2 mg·L−1) is used to the Gram-negative bacteria. In alternative, 70% ethanol or 2 mol·L−1 HCl lock therapy can also be considered.

Change of the infected urinary catheter (UC)

Catheter-associated urinary tract infections are the most common nosocomial infection, which associated with the formation of microbial biofilm in UC. In addition to intermittent urinary tract infections with the identical pathogen, it can also result in urosepsis. Change of the infected UC is not difficult; however, the time to change is important. It is recommended to change the infected UC after 48 h of adequate and sensitive antibiotic treatment to minimize the bacterial concentration in bladder and urinary tract; otherwise, the new UC would be colonized quickly by the bacteria to form new biofilm.

Change of the infected joint prostheses

Prosthesis-related infection is a serious complication in patients with joint replacement and it has been demonstrated as a

biofilm correlated infection with poor prognosis.13,42 In case the prosthesis infection is diagnosed, change of the infected prosthesis in most of the cases becomes the only choice. If the prosthetic implants are loosening due to biofilm infection, staged exchange of prosthesis in combination with sensitive and aggressive antibiotic treatment is recommended.13,42

Changes of other infected indwelling devices

Endocarditic patients with prosthetic heart valves or cardiac pacemakers are at risk of intermittent sepsis, cardiac insufficiency and infective embolic complications. 43,44 Therefore, change of the infected prosthetic heart valves or cardiac pacemakers in combination with aggressive and sensitive antibiotic therapy becomes necessary.44 For the patients with biofilm infections in biliary stents, endotracheal tubes, dead bones (chronic osteomyelitis), biliary and urinary stones (biliary and urinary tract infections), effective antibiotic treatments and removal of the infected foreign bodies are crucial to cure the infections.

Empty of abscesses

Abscesses are not biofilm, but they have some kinds of connections with biofilm.45 When an abscess is formed, it becomes difficult for antibiotic to penetrate through the wall of abscess into the focus. Therefore empty of abscess is necessary.

Early and aggressive antibiotic treatments against biofilm infections

In vitro experiment showed that young biofilm could be easily cleared by antibiotic treatment compared to the matured biofilm.5 Therefore early and aggressive antibiotic treatments are recommended for biofilm infections.4 However, early diagnosis of biofilm infection is currently difficult and most of the clinical biofilm infections are actually matured biofilms which are usually difficult to eradicate with antibiotic treatment.4,5,6,46 It is therefore important and crucial to

legitimately apply currently available antibiotics in the treatment of biofilm infections. On the basis of removing foreign bodies and combined with the results from our previous studies,3,4,5,6,8,13,46 the following factors should be taken into account when an antibiotic treatment against biofilm infection is to be decided:

Selection of antibiotics

Treatment of biofilm infection requires sensitive and well-penetrating antibiotics to ensure a sufficient concentration of effective antibiotic at the site of biofilm infection. In general, macrolides, lincosamides, tetracyclines, rifamycins, quinolones, fusidic acid, nitroimidazole, sulfonamides and oxazolidinones penetrate better in tissues and cells than beta-lactam (including penicillins, cephalosporins and carbapenems), aminoglycosides, glycopeptide and polymyxin. It is well known that infection could lead to inflammation, which results in faster metabolism and significant consumption of oxygen locally or systemically. If oxygen supply could not meet the demand, glycolysis will be activated leading to acidosis, and the effects of antibiotics could be affected by pH values. It has been reported previously that low pH value (pH 5.2) could decrease the effects of ß-lactam antibiotics and increase effects of rifamycin SV.47 Therefore antibiotic treatment and correction of acid-base balance disorders could be important for the treatment of biofilm infections.

Table 1: Effects of different antibiotics family against *Staphylococcus* biofilms.

Antibiotic		Species	Assay	Effect on biofilm
Beta-lactams	Penicillins and most cephalosporins	*Staphylococcus aureus*	*in vitro*	Induction of biofilm formation at Sub-MICs
	Ceftaroline			Bactericidal anti-biofilm activity after prolonged exposure
Rifampicin		*S. aureus*	*in vitro*	Anti-biofilm activity, synergistic with fusidic acid and tigecycline
		S. epidermidis		High anti-biofilm activity alone or in combination with vancomycin or daptomicin
Vancomycin		*S. aureus*	*in vitro*	Promotion of biofilm formation through an autolysis-dependent mechanism
		S. epidermidis		Induction of eDNA release at sub-MICs leading to increased biofilm formation
Daptomycin		*S. aureus*	*in vitro*	Induction of viable but non-cultivable cells in biofilm at low concentrations
				Anti-biofilm effect in monotherapy
			in vivo	Prevention of the emergence of rifampin resistance mutants
Fosfomycin		*S. aureus*	*in vitro*	Anti-biofilm activity synergistic with linezolid or minocycline or vancomycin

Administration of antibiotics

We have previously demonstrated that combination therapy of antibiotics against biofilm infection was significantly better than antibiotic monotherapy.48 Antibiotic combination therapy is therefore recommended for the treatment of biofilm infections. According to the character of antibiotic tolerance and resistance in biofilm and the high MIC and MBC of biofilm cells demonstrated in experimental studies,5,6 high dosages of antibiotics under the safe range of renal and hepatic functions are suggested. In addition, a proper duration of antibiotic treatment is also important. For the patients with biofilm infections suitable for topical treatment of high concentrations of antibiotics, systemic combined with topical antibiotic treatment can give better effects against biofilm infections, such as antibiotic inhalation or direct administration for airway biofilms 8,6 and bladder irrigation with high concentration of antibiotics against biofilm urinary tract infections.

The pharmacokinetics (PK) and pharmacodynamics (PD) of antibiotics in biofilm infections

Bacteria growing in a biofilm could become 10–1 000 times more resistant and tolerant to antibiotics compared with their planktonic counterpart.50,51 Antimicrobials available for the

treatment of highly resistant bacterial infections are limited;52,53 therefore, dosage optimization of currently available antibiotics becomes extremely important to improve anti-infection outcomes and to prevent further development of antimicrobial resistance and tolerance. The PK and PD of antimicrobial agents can be used reliably to predict the effect of antimicrobial regimens to achieve maximum bactericidal effect against infections. Several recent studies have shown the different PK and PD profiles of antibiotics between planktonic and biofilm infection.5,6 PK and PD information of antimicrobial agents on biofilm-associated bacteria can be applied to optimize the dose regimens on biofilm infections.6 Minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration are two PD parameters for antimicrobials in biofilm infections.5 The application of biofilm growing bacteria in the susceptibility tests of clinical laboratory, with MBIC and minimum biofilm eradication concentration, is useful to obtain a better outcome of antimicrobial chemotherapy, compared with the traditional susceptibility test based on planktonic bacteria.5 In our previous PK/PD study, colistin showed a concentration-dependent killing, and imipenem showed a time-dependent killing on biofilm bacteria *in vivo*.6 The elimination of *P. aeruginosa*biofilm bacteria in the lungs of our experimentally infected animals was best correlated to AUC/MBIC of colistin (AUC, the area under the concentration-time curve), and T>MBIC of imipenem (T>MBIC, the duration of time a drug concentration remains above the MBIC).(6)

CONCLUSION:

Biofilm development empowers bacterial pathogens to colonize a wide assortment of host specialties and hold on in unforgiving situations, making their destruction especially troublesome. Biofilm qualities ‘decide’ if, to what degree, and which

antimicrobial medications might be viable. The age and structure of the biofilm are the main considerations impacting the weakness of the inhabitant microorganisms. As the biofilm develops, expanded EPS collection, joined with the supplement and oxygen slopes that influence cell digestion and development rates, result in lessened section and movement of antimicrobial operators making biofilm-framing pathogens dynamically more impervious to anti-toxin regimens. Along these lines, novel procedures, intended to obstruct a particular biofilm advance without executing the microscopic organisms, for example, the utilization of antiadhesion operators, or utilizing normal, bacterially delivered signs to advance bacterial dispersal, are energizing roads for investigation and at last the improvement of quick acting, intense, and bioavailable treatment systems.

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