Review Article

Biofilms: A Silent Threat to Human Life

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ABSTRACT

Microbial biofilms are communities of microorganisms encased within a gelatinous matrix of extracellular polysaccharides, which is purported to be a threatened source of chronic infections in man. It is only at the beginning of the 20th century that man came to study extensively on these casings which are found adhered to both biotic and abiotic surfaces as a dreadful barrier which we need to fight against. Besides, the molecular level resistance, these microbial communities do exert community-level resistance which we cannot struggle with mere antibiotics alone. Some new methodologies have been tried to combat this biofilm. Hence this review mainly focuses on the mechanism behind biofilm formation, its composition, threats and antibiofilm strategies that inhibit the biofilm formation by altering the physicochemical properties of the surfaces on which it initiates, inhibiting the intercellular signalling of microorganisms and to eradicate the preformed biofilms by inducing it's dispersal, persisters eradication, and employing various drug delivery systems.

Abbreviations: AMP-Anti Microbial Peptide, QS- Quorum Sensing, EP- Efflux Pump, ETC- Acyl Homoserine Lactone, ROS- Reactive Oxygen Species.

Keywords: Biofilm, Quorum Sensing, Antibiofilm.

INTRODUCTION

Biofilm is defined as the sessile community of microorganisms like bacteria, algae, protozoa, and fungi that are attached to the biotic surface and embedded within a matrix of extracellular polymeric substances produced by the inhabitant microorganisms. Van Leeuwenhoek assigned for the discovery of microbial biofilms. The beneficial effect of biofilm includes it increases the biomass concentration and facilitate biomass liquid separation and improve cell productivity. Biofilms may form a wide variety of surfaces including living tissues, indwelling medical devices, industrial or potable water system pipings or natural aquatic systems.

Quorum sensing is a signaling mechanism through which many bacteria are known to regulate their cooperative activities and physiological process. It is the process in which bacteria communicate together. Many species of bacteria used it to coordinate their gene expression according to the local density of population. The anti-biofilm strategies mainly include reversible and irreversible inhibition, QS inhibition, non- pathogenic bacteria colonization, and vaccination.

Biofilm is defined as the sessile community of microorganisms like bacteria, algae, protozoans, and fungi that attach to the biotic or abiotic surface and embedded within a matrix of extracellular polymeric substance produced by the inhabitant microorganisms.[1]

Structure of biofilm

The extracellular polymeric substance (EPS) or matrix of biofilm is composed of extracellular polysaccharides, DNA, proteins, and channels that allow water, air, and nutrients to get to all parts.

Exopolysaccharides: These are linear or branched long strands that are attached to the cell surface and stretched out to form large networks that act as a scaffold for other carbohydrate, proteins, lipids etc.

eDNA: Extracellular DNA is critical for biofilm attachment and is either actively secreted or remain as left over from hybrid cells.[2,3]

Formation of Biofilm

Biofilm formation begins with the transition of bacteria from a planktonic stage. Biofilm formation comprises mainly three steps:

1. Attachment: Introduction of bacteria to a surface is driven by Brownian motion, gravitational forces. Bacteria have to overcome the attractive van der Waals forces between the bacterial cell and surface with the formation of flagella to provide mechanical attachment to the surface. The attachment steps could be Reversible attachment/ Irreversible attachment
2. Maturation: In Maturation 1 phase, a matrix of extracellular polysaccharide substances (EPS) is produced. Microcolonies increase and become multi-layered, and their thickness is up to 10

micrometers. This phase lasts for 3 days. In Maturation 2 phase Bacterial micro-colonies grow to their maximum size and their thickness is about 100 micrometers. This phase lasts for six days.

3. Dispersion: In this phase, microcolony structure changes since the bacterial cells situated in their central part regain their mobility and detach from the previously formed structure. During this phase, water channels form between microcolonies & it last for 9-12 days.[2]

Biofilm Resistance

Compared to free swimming or planktonic bacteria those that are in a biofilm are more resistant to antibiotics or host immune responses. A resistant rate upto10, 000 times has been estimated for the cell in biofilm than the planktonic bacteria. Various mechanisms involved in this resistance are Prevention of Access to Target By Reducing Permeability, Increased efflux pump, Reduced growth rate, Persister cells, Degradation and modification of antibiotics, Enzymatic hydrolysis, Group Transfer, Horizontal gene transfer etc. [4,5].

Biofilm inhibition methods

I. Reversible inhibition

A) Surface modification

Inhibitions of reversible adhesion of bacteria by altering the physicochemical properties of biomaterial or medical devices. Approaches followed are:

a) Bacteriostatic/Bactericidal coating: Coating of indwelling medical devices by bacteriostatic or bactericidal agents to either inhibit or arrest the growth of bacteria.

1. Heavy metal silver: The positively charged silver ions deposited on the surfaces can attract electrostatically the metal and negatively charged bacterial membrane facilitating its uptake and lethality of silver to bacteria is attributed due to the inactivation of enzymes by the reaction of thiol group in cysteine residue thereby Inhibits DNA replication, Inhibits expression of ribosomal & other cellular proteins and or Interferes the ETC & cell division. [3]

2. Polymethacrylate derivative with cationic side chain: About 99.9% bacteria that were initially found attached to the costing was found to be dead during the 1 hr exposure & over the course of 2-8 days the coating slowly hydrolyzed releasing 98% of microbial cells exhibiting their non-fouling nature that further prevents attachment because the surface hydration layer serves as a physical & energetic barrier to protein adsorption &bacterial adhesion.

3. Furanones: Halogenated furanones derived from red algae *Deliseapulchra*was coated onto surfaces by physical adsorption & inhibit the biofilm formation & fouling of the surface.

4. QAS: Covalently bonded 3-(trimethoxysilyl)-propyl dimethyl octadecyl ammonium chloride (QAS) to silicone rubber will generate quaternary ammonium groups on the surface that exerts antimicrobial activity. [3,6,7]

b) Anti-adhesion coating

The surface properties of biomaterials can be modified by creating desired anti-adhesion characteristics without altering the bulk properties of the material. Major anti-adhesion coatings are Trimethylsilane plasma coatings (TMS), Silica colloid doped fluorinated silanexerogel., Surface roughness, Organoselenium coating and coating with biosurfactants.[7,8]

II) Irreversible Inhibition

A) Adhesion interaction blocking

The first step in the biofilms formation is the adhesion of a cell to a surface. Blocking this initial step would be an effective method to prevent biofilm infection.

Mannoside, Pilicides, Curlicides

Mannosides: The Fim H adhesion bound to mannose have been used to design drug molecules called mannosides which competitively inhibits the Fim H binding to the receptor. Mannoside administration as prophylaxis for UTIs interfered UPEC adherence & invasion reducing IBC formation & attenuating UPEC during the acute infection stages.

Pilicides & Curlicides: Microorganisms rely upon proteinaceous appendages like pili, curli to adhere to surfaces. Pilicides mainly act by inhibiting the Chaperone-Usher Pathway which is responsible for the formation of surface structures. Curli are functional amyloid fibers and is the main protein component found in the matrix of biofilm that contributes to immune system activation, host colonization &cell invasion. Compounds that can interfere with the polymerization of the curli subunit for the amyloid fiber formation are called curlicides. [3]

Polysaccharides: Exopolysaccharides mediate cell to surface & cell to cell interactions responsible for biofilm formation & maintenance. But some bacterial Exopolysaccharides do inhibit or destabilize the biofilm formation. [3,9]

B) Chelators

Chelating agents show biofilm dispersing property because the integrity of the biofilm matrix is mainly held by ions like calcium, Magnesium, Iron. Phosphate is involved in solidifying the biofilm architecture. EDTA-EDTA weakens the structure of biofilm allowing the immune system or antibiotics to gain access to the microbes hiding deep inside the biofilm. Other chelators include disodium & tetrasodium derivatives of EDTA, N acetyl-L-cysteine. [3]

III. Quorum Sensing Inhibitors

The interruption of QS signaling pathways can prevent initial biofilm formation and modify its progression by the inhibition of secretion of adhesins which affect bacterial motility, adhesion to surfaces, cell auto & co-aggregation, the formation of micro-colonies and Inhibition of EPS production.[5,6,10]

IV. Bacteriocins

Bacteriocins are antimicrobial peptides or proteins produced by bacteria and are generally active against closely related species hence they can act as narrow-spectrum antibiotics. Bacteriocins that form stable pores on biofilm cells are highly effective against biofilm infections. Eg:nisinA, LacticinQ, and nakasin ISK1.A purified recombinant ColA-43864 bacteriocin gene was highly effective in killing E.coli, Citrobacter species&K. pneumonia cells in the planktonic state as well as in biofilm.

V. Vaccination

The development and use of antibodies or antisera directed against bacterial adhesins as an antiadhesive strategy. Various approaches used are active or passive immunization using bacterial adhesins, an adhesin subunit, or an immunogenic peptide fragment based either on an individual/group of adhesins or immunization with a DNA vaccine encoded with adhesins or part thereof. Active immunization with T2544 or passively with anti T2544 antiserum protected bacterial challenge byS. Typhi causing typhoid fever.T2544 is the S .typhiadhesin involved in bacterial host interaction, pathogenesis, and a potential target for antiadhesion vaccine.[11]

Biofilm eradication methods

I. Dispersal induction

A) Enzymes

An approach for the eradication of preformed biofilm is to destroy the integrity of the biofilm matrix by enzymatic degradation of the components of the EPS. Enzymes perform the functions like biofilm formation inhibition, dispersing preformed biofilm, sensitizes the cells to the action of antibiotics. Some examples are DNAses like DNA thermonuclease, Glycoside hydrolase, Alginate lyase Protease, Serine protease Esp-S. epidermidis, metalloproteases etc.[3,10,12]

B) D- Amino Acids

Exogenous addition of amino acids produced by dispersing B. Subtilis disrupted preformed biofilms by Promoting disassemblage of biofilm by disrupting adhesive fiber interactions or Preventing protein localization to the cell surface. These D amino acids can also prevent the biofilm formation by P. aeruginosa and S. aureus.[3]

II. Bacteriophages

Phage therapy has emerged as an important biocontrol strategy to eradicate the biofilms. Phage-mediated attacks may be an effective method due to various factors such as Phage mobility as an ability of virions once released from parent cells to diffuse to adjacent, potentially sensitive target cells, Ability of phage to disrupt the structure of biofilms via Bacterial lysis etc.[3,13]

III. Persisters eradication

Persistent cells which are more prevalent in biofilms adopt a slow or non-growing phenotype and they are resistant to various environmental stress like an antibiotic challenge, thus creating a reservoir of surviving cells that are able to grow and cause relapsing infections.

1) Antimicrobial peptides

Antimicrobial peptides have emerged as a novel method for targeting for persister eradication. Many AMPs derived from natural sources have found to possess efficient antimicrobial activity on viable cells of biofilm than the persisters. Hence synthetic AMPs have been entering into the scenario can target persister cells too. Amphipathic nature of the AMP help to integrate into the lipid bilayer of the membrane and cause disruption of cell membrane integrity, Inhibition of macromolecule synthesis(Protein, DNA, RNA) and Interaction with other intracellular targets.[14]

2) Metabolic stimulation

Metabolic stimulation to persister cells which potentiate the action of antibiotics is another approach. Aminoglycosides are ribosome targeting bactericidal antibiotics which have weak activity against dormant persister cells though translation rate in these cells is at a low rate of the action of aminoglycoside require a high energy. Stimulation by means of metabolites like glucose, mannitol, fructose (glycolysis) induced the rapid killing of persisters by gentamycin & this is due to the induced high level of aminoglycoside uptake in the cells.[15]

IV. Drug delivery systems

A) Nanotechnology: Nanotechnology has emerged as a new paradigm for the antibiofilm technologies. The superparamagnetic iron oxide nanoparticles were used against biofilm. It exerts the effect by generating hydroxyl radicals that can depolymerize polysaccharides, cause breaks in DNA, inactivate enzymes that make up the EPS matrix of biofilm architecture. It can effectively disrupt cell membranes causing the death of planktonic cells.

B) Microemulsions: Microemulsions of ethyl oleate with Tween 80 as an emulsifier &n-pentanol co-emulsified with TritonX 100 found to reduce the preformed biofilms of Salmonella species. The bactericidal activity of this microemulsion is due to n-pentanol which disrupts bacterial cell membrane, leading to leakage of ions & metabolites.[13,16]

CONCLUSION

From the study, we made on microbial biofilms and various antibiofilm strategies we came to the conclusion that biofilms are playing a vast role in creating chronic infections which we cannot conquer by the mere antibiotic use alone. In addition to the centrally playing antimicrobial compounds, adjuvant therapies that aim at inhibiting the nascent biofilm formation and the eradication of preformed biofilms are also inevitable.

As we see from the above discussion, by the judicious use of antibiotics along with other supportive therapies which may include various quorum sensing inhibitors, persister eradicators, biofilm disrupting agents, penetration enhancers can bring an exit to the ongoing era of chronic infections can be prevented and the fear of developing menacing antibiotic resistance can be ruled out.

REFERENCES

1. DenkhausE, Meisen S, TelgederU, WingenderJ. Chemical and physical methods for characterisation of biofilms. Microchim Acta., 158, 2007, 1–27.
2. Nira Rabin, Yue Zheng, Clement Opoku-Temeng, Yixuan Du, Eric Bonsu & Herman O Sintim. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem., 7, 2015, 493-512.
3. Kostakioti M, Hadjifrangiskou M, Hultgren S.J. BacterialBiofilms: Development, Dispersal, and Therapeutic Strategies in the Dawn of the PostbioticEra. Cold Spring Harb Perspect Med, 2013, 3, a010306.
4. Borges A, Abreu A C, Dias C, Saavedra M J, Borge F, Simoes M. New Perspectives on the Use of Phytochemicals as an Emergent Strategy to Control Bacterial Infections Including Biofilms. Molecules. 2016, 21(7).
5. Penesyan A, Gillings M, Paulsen I T. AntibioticDiscovery: Combating bacterial Resistance in cells and in Biofilm Communities. Molecules, 20, 2015, 5286-5298.
6. Sadekuzzaman M, Yang S, Mizan M F R, Ha S D. Current and Recent Advanced Strategies for Combating Biofilms. Comprehensive Reviews in Food Science & Food Safety, 14, 2015, 491-509.
7. Chen M, Yu Q, Sun H. Novel Strategies for the Prevention and Treatment of Related Infections. International Journal of Molecular Sciences, 14, 2013, 18488-18501.
8. Ibrahim Banat, Mayri Diaz De Rienzo, Gerry Quinn. Microbial biofilms: biosurfactants as antibiofilm agents. Applied Microbiology and Biotechnology, 98, 2014, 9915–9929.
9. Rabin N, Zheng Y, Temeng C O, Du Y, Bonsu E Sintim O H. Agents that inhibit bacterial biofilm formation. Future Medicinal Chemistry, 7, 2015, 647-671.
10. Blackledge M S, Worthington J W, Melander C. Biologically Inspired Strategies for combating Bacterial Biofilms. Current Opinion in Pharmacology, 13, 2013, 699-706.
11. Krachler A M, Orth K, Targeting the bacteria-host interface Strategies in antiadhesion therapy, 4, 2013, 284-294.
12. Stewart P S. Prospects for Antibiofilm Pharmaceuticals. Pharmaceuticals, 8, 2015, 504-511.
13. Miquel S, Lagrafeuille R, Forestier C. Anti-biofilm Activity as a Health Issue. Front Microbiol, 7, 2016, 592.
14. Jorge P, Lourenco A, Pereira. O. New trend in peptide-based anti-biofilm strategies: review of recent achievements and bioinformatic approaches. The Journal of Bioadhesion and Biofilm Research, 2012, 1033-1061.
15. Allison K R, Brynildsen M P, Collins J J. Metabolite Enabled eradication of Bacterial Persisters by Aminoglycosides. Letter, 473, 2011, 216-219.

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