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The role of bacterial biofilms in pathogenesis: Insights from a systematic review

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Abstract

Bacterial biofilms are complex communities of microorganisms, enclosed in a self-produced polymeric matrix that adheres to surfaces. Biofilms contain group(s) of microorganisms that are found to be associated with the biotic and abiotic surfaces. Their role in pathogenesis is increasingly recognized as a significant factor contributing to chronic infections and antimicrobial resistance. Aim of this systematic review aims to synthesize current knowledge on the impact of bacterial biofilms in various infectious diseases, focusing on their mechanisms of pathogenesis, clinical implications.

Method: systematic review analyzed studies published in the last two decades, highlighting the mechanisms by which biofilms evade host immune responses and resist antibiotic treatment. Our findings indicate that biofilm formation is associated with a variety of clinical conditions, including cystic fibrosis, chronic wounds, and medical device-related infections. Furthermore, we discuss the implications of biofilm-associated infections for public health and the need for innovative therapeutic strategies to disrupt biofilm integrity. This review underscores the critical need for continued research into biofilm biology to inform clinical practices and improve patient outcomes in infections associated with bacterial biofilms. This review looks at the primary aim of this systematic review is to comprehensively evaluate and synthesize the current literature on the role of bacterial biofilms in the pathogenesis of infectious diseases. Specifically, this study seeks to

Keywords: Bacterial Biofilm; Pathogenesis; Biofilm Formation; Infection; Antibiotic Resistance and Biofilm

1. Introduction

Bacteria form biofilms as part of their survival mechanisms, and biofilms are thus ubiquitous in nature. In 1683, Antoni van Leeuwenhoek observed and described biofilms by using his primitive microscope on matter from his own teeth [1]. Anton Van Leuwenhoek described biofilm for the first time in the late seventeenth century. Bacterial cluster that can aggregate to a surface in a hydrated anion polymeric matrix synthesized by them [2]. Many bacterial species, such as Staphylococcus spp. and Pseudomonas aeruginosa have adopted biofilm as their natural mode of existence, as in the case of advanced-stage Cystic fibrosis patients; P. aeruginosa is the primary bacterium in the lungs [3], and evidence is accumulating that biofilms contribute to the pathogenesis, especially in chronic infections [4].

Bacterial biofilms are clusters of bacteria that are attached to a surface and/or to each other and embedded in a self-produced matrix. The biofilm matrix consists of substances like proteins (fibrin), polysaccharide (alginate)[5]. Bacteria in biofilms can employ several survival strategies to evade the host defense systems. By staying dormant and hidden from the immune system, they may cause local tissue damage and later cause an acute infection. Within the biofilm, the bacteria adapt to environmental anoxia and nutrient limitation by exhibiting an altered metabolism, gene expression, and protein production, which can lead to a lower metabolic rate and a reduced rate of cell division [6], that adaptations

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make the bacteria more resistant to antimicrobial therapy by inactivating the antimicrobial targets or reducing the requirements for the cellular function that the antimicrobials interfere with [7] .

A biofilm infection, activation of both innate and acquired host immune responses which are able to eliminate the biofilm pathogen, but instead accelerate collateral tissue damage [8]. Biofilm-related diseases are persistent infections that develop slowly, are rarely resolved by the immune system, and respond incons. Biofilms have been seen to be present on liquid surfaces as a floating mat and in submerged state also (Vasudevan 2014). Biofilms contain either homogenous or heterogeneous communities of bacteria, embedded on a matrix of extracellular polymeric substances (EPS). EPS mainly consist of polysaccharides biomolecules like proteins, lipids and nucleic acids are also present in EPS (9). Analysis of the EPS coat present in the biofilm has led to the discovery that biofilms are technically hydrogels which exhibit viscoelastic behaviour (10) There is a continuing debate about determining factors that contribute to the formation of biofilms, both genetic and environmental factors contribute towards the microbial biofilm formation. Bacteria can adapt to different environmental conditions by modulating their biofilm structure (11).

Microbial communities of the biofilms usually take part in the production and degradation of organic matter, the remediation of many environmental recalcitrant pollutants, the cycling of nitrogen, sulphur and many metals. Existing literature revealed that microbial biofilms are involved in the purification of sewage. It has been reported that in the treatment of groundwater contaminated with petroleum (11), and in the process of nitrification, microbial biofilms play a major role. Microbial biofilm in rhizospheric soil has also been found to increase the soil fertility and plant growth (12). In extreme acidic environment, such as in acid mine drainage (at a pH of 1), microbial biofilm plays an important role in the cycling of sulphur(13).

1.1. Variability Across Pathogens

There is limited understanding of how different bacterial species and strains form biofilms and their varying pathogenic mechanisms. More comparative studies are needed to assess how biofilm characteristics differ across pathogens and conditions, influencing clinical outcomes (14).

Biofilms and Antibiotic Resistance: Bacteria in biofilms can be up to 1000 times more resistant to antibiotics than their planktonic counterparts (15). Increased antibiotic resistance of biofilm bacteria makes it difficult to eradicate biofilm infections such as chronic lung infections in cystic fibrosis patients, periodontal disease, and bacteremias resulting from in-dwelling medical devices. The well characterized mechanisms of antibiotic resistance such as target mutations (16)

1.2. Biofilm development

is not a random process, when bacteria encounter a surface, when bacteria are transported to the surface by sedimentation, liquid flow, or active swimming, they first make weak and transient attachments. The next phase, irreversible attachment, depends on the properties of both the surface to be colonized and the bacterial cell surface. If the physicochemical conditions e.g., hydrophobicity or hydrophilicity (17).

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. The microorganisms account for less than 10% of the dry mass, whereas the matrix can account for over 90%. There are a variety of mechanisms by which different microbial species are able to come into closer contact with a surface, attach firmly to it, promote cell–cell interactions and grow as a complex structure (18).

Presently five simple generalized stages are shown for formation of biofilm. Step-1 planktonic cell attaches with the substrate by adhesion mechanism, Step-ll cell starts adsorption and multiplication, Step-lll early development of biofilm architecture, production of cell-cell signaling molecule and f inally produce firmly mature biofilm architecture with extracellular polymeric substances (EPS) and Step-V dispersion of single cell from the biofilm. Literature review showed that both genetic and environmental factors contribute towards the microbial biof ilm formation (19). EPS have been called 'the dark matter of biofilms' because of the large range of matrix biopolymers and the difficult to analyzed. EPS mainly consist of polysaccharides and other biomolecules like proteins, lipids and nucleic acids etc (20). Polymers like glycopeptides, lipids and lipopolysaccharides form a scaffold and hold the biofilm together (21). The complexity of biofilm structure and metabolism has led to the analogy of biofilms to tissues of higher organisms (22).

1.3. Molecular basis of biofilm formation

The development of a biofilm and the release of cells (either individually or in clusters) can be regulated by population densitydependent gene expression controlled by cell-to-cell signaling molecules such as acylated homoserine lactones (AHLs) for Gram negative bacteria, and specific peptides for Gram-positive bacteria (23).

1.4. Gene Activation During Biofilm Development:

Research on *Pseudomonas aeruginosa* has shown that upon surface attachment, there is a rapid and specific transcriptional response. Within 30 to 60 minutes post-attachment, significant changes in gene expression occur, indicating that the transition from planktonic to biofilm states is initiated swiftly after surface contact. Mutations in genes regulated upon surface attachment have been observed to decrease biofilm development, underscoring their importance in this process (24).

In *Escherichia coli*, the SdiA protein, a LuxR-type transcriptional regulator, has been implicated in biofilm formation. Recent findings suggest that SdiA positively regulates the expression of the small RNA CsrB, which in turn modulates biofilm formation. This regulatory pathway highlights the intricate control mechanisms bacteria employ to adapt to environmental changes (26).

1.5. Extracytoplasmic Function (ECF) Sigma Factors and Biofilm Formation:

In *Pseudomonas aeruginosa*, the ECF sigma factor AlgU (also known as σ^E or σ^22) has been identified as a key regulator in biofilm development. AlgU controls the expression of genes involved in the production of exopolysaccharides, such as the psl operon, which are crucial for biofilm matrix formation and stabilty. Mutations in algU result in defective biofilm formation, highlighting its pivotal role in surface attachment and biofilm maturation (28).

Understanding these intricate regulatory networks and resistance mechanisms is crucial for developing effective strategies to prevent and treat biofilm-associated infections. In response to antibiotics, bacteria can accumulate high levels of beta-lactamases. Scanning confocal laser photomicrographs of Pseudomonas aeruginosa PAO1-J32 biofilms have shown beta-lactamase gene (ampC) promoter activity via expression of the green fluorescent protein (GFP) reporter gene, 6 days after exposure to high dose ceftazidime (100 mg/ ml for 4 h) (29).

2. Method

A systematic review method is ideal for providing a comprehensive and unbiased synthesis of existing literature on the role of bacterial biofilms in pathogenesis. Below is an outline of the typical steps you can use to develop a methodology for this review:

2.1. Research Question Development

- Databases Collection: Select relevant academic databases such as PubMed, Scopus, Web of Science, and others related to microbiology, infection, and medical sciences.
- inclusion/exclusion criteria to focus the search on peer-reviewed articles, reviews, clinical trials, or case studies published within a specific timeframe (the last 10 years).
- Inclusion Criteria: Studies focusing on the role of bacterial biofilms in pathogenesis, articles with experimental data, clinical research, or reviews on bacterial biofilm-associated infections, and human and animal studies.
- Exclusion Criteria: Non-peer-reviewed studies, editorials, and commentaries, studies focusing only on non-pathogenic biofilms.

2.1.1. Study Selection Process

- Screening: Two independent reviewers should screen the titles and abstracts to identify potentially relevant articles. Disagreements will be resolved by a third reviewer.

- Full-Text Review: Full-text articles of the selected studies will be reviewed to ensure they meet the inclusion criteria.
- Data Extraction Develop a standardized data extraction form to collect the following information

Author(s), publication year, study type, types of pathogens and biofilm characteristics, mechanisms of biofilm formation and resistance, clinical implications (infection type, severity, treatment challenges), Major findings and conclusions.

2.1.2. Quality Assessment

Assess the quality and risk of bias in the selected studies using standardized tools like the Newcastle-Ottawa Scale (for observational studies) or the Cochrane Risk of Bias Tool (for randomized trials).Studies will be categorized as high, medium, or low quality based on their design, methodology, and reporting standards.

2.1.3. Data Synthesis and Analysis

- Qualitative Synthesis: Summarize and discuss the key findings of the selected studies regarding biofilm formation, pathogenicity, and clinical relevance. Identify patterns and trends.
- Quantitative Synthesis (if applicable): If sufficient quantitative data are available, a meta-analysis could be performed to estimate the overall effect of biofilms on specific pathogenic outcomes (e.g., infection rates or resistance levels).

3. Discussion

DNA-binding regulatory protein (brlR) involved in the biofilm-specific antibiotic tolerance of P. aeruginosa (10). BrlR acts as a repressor of phoPQ expression with increased colistin susceptibility (i.e. higher membrane susceptibility) and tobramycin resistance (12). Sigma factor (sigma 22) encoded by algT/algU which is inhibited by the anti-sigma factor MucA and activated in response to cell wall stress, which contributes to antibiotic tolerance. In biof ilms, a small subpopulation of bacteria can reversibly enter a slow-growing or starved state. These cells are known as persisters or dormant cells and highly resistant to killing by antibiotics (22). There are now growing evidences that one of the main factors leading to persisters formation is nutritional stress, with a major effector molecule, ppGpp, the mediator of stringent response (18). Biofilms are not only recalcitrant to antibiotics, but also evade host immune-responses (15). Phagocytic cells seem not only to be unable to physically engulf the biofilm structures but also to be impaired in their activities (14). Even though there are several outstanding reviews on antimicrobial resistance in biofilms (19.20,and 23), most of these focus on a specific organism, a specific mechanism.

Common process for biofilms control, Searching remedy for biofilm infection is one of the most diff icult and challenging tasks in antibacterial drug development, because different bacteria were used chemically diverse molecules to establish biofilms. Thus, there is a great problem with marketability, even if such an approach could succeed, because only specific bacteria could be targeted. Strategies to plan against bacterial biofilm must be achieved by prevention of biofilm formation rather than dispersal of the formed biofilm. The biofilms can be eliminated by adopting different strategies like Mechanical, physical and chemical methods.

Mechanical control , anther chanical strategies that are being adopted for removal of biofilm-associated bacteria are: avoidance of attachment of the bacteria to the surface, Surface charge and Hydrophobicity, use of compounds that can disrupt the biofilm formation, induction of dispersion or degradation of the formed biofilm (**20).** Surface roughness can also affect biofilm adhesion. Rough surfaces are more conducive to biofilm formation and maturation, while smooth surfaces are less susceptible to biofilm adhesion. The roughness of a surface can affect the hydrophobicity or hydrophilicity of the contacting substance, which in turn affects its ability to adhere (4). Modification of the surface charge of polymers has also proven to be an effective means of biofilm prevention. Positively-charged polycationic chains enable the molecule to stretch out and generate bactericidal activity (9) Hydrophobicity also plays significant role in determining the ability of bacteria to form biofilms. Some species are not able to attach to a surface and are sometimes able to establish themselves directly to earlier colonists (26).

Physical control Theoretically, biofilm formation on medical devices can be prohibited by altering the device's surface to prevent bacterial attachment, or by including antibacterial therapeutics in the device to prevent early stages of biofilm formation. The physical methods used for the regulator of biofilms include super-high magnetic fields (Pothakamury et al., 1993), ultrasound treatment (3), high pulsed electrical on their own (12), low electrical fields both on their own (29). Low currents of 200 and 400 mA, using silver, carbon and platinum electrodes killed planktonic cells of Gram-positive and Gram-negative bacteria and Candida albicans (10). In combination with antibiotics and low electrical currents was successfully employed for biofilm control (17 and 11). Nano-plasma trimethyl silane (TMS) coating can be used on stainless steel or hydrophilic surfaces to prevent S. epidermis biofilms (Ma et al., 2012). Silane xerogel coatings can provide super hydrophobic coating and act as anti-adhesion agent against biofilm-forming bacteria (5). A technique named antimicrobial lock technique (ALT) can be used to inhibit biofilm formation in catheters (26 and 28).

Chemical control , chemical modifications are the main strategy for biofilm prevention. Antibiotics, biocides, and ion coatings are commonly used chemical methods of biofilm prevention. N-alkylpyridinium bromide, an antimicrobial

agent, was attached to a poly (4-vinyl-Nhexylpyridine), the polymer was capable of inactivating Z99% of S. epidermidis, E. coli, and P. aeruginosa bacteria (22). Iron-chelating compounds can be used to disrupt Pseudomonas aeruginosa biofilms if used along with aminoglycosides (8). Sodium citrate inhibited biofilm formation by several Staphylococci species in vitro (Shanks et al., 2006). Some detergents are bactericidal and some disinfectants may even depolymerize EPS like peracetic acid (4), hydrogen peroxide (25), iodine (18), chlorine (20). N-acetyl cysteine, a derivative comes from the amino acid L-cysteine, inhibits biofilm formation of S. epidermis (9).

In our systemic review study, has come to understand many things about the biology of bacterial biofilms. Biofilms represent microbial societies with their own defense and communication systems. (26 and 7). There is a continuing debate about determining factors that contribute to the formation of biofilms. We have to rethinking alternate diagnosis and treatment process against biof ilm. The increase of microbial resistance to antibiotics threatens public health on a global scale as it reduces the effectiveness of treatments and increases morbidity, mortality, and health care costs (6). Moreover, the emergence of resistant bacteria to conventional antimicrobials clearly shows that new biofilm control strategies are required (2). So we have to rethinking alternate diagnosis and treatment process against biofilm. Following are the some green technology to fight against bacterial biofilm

Bacteriophages against biofilm, can show high levels of resistance to agents, such as biocides and antibiotics. Bacteriophages are nature that infects bacteria naturally and may provide a natural, highly specific, non-toxic are controlling several microorganisms involved in biofilm formation (9). They can either coexist with their host by inserting themselves into the bacterial genome (lysogenic bacteriophages) or destroy them (lytic bacteriophages; the type most suited to therapeutic use). Phage T4 and E27 are successfully employed against the infection of E. coli and Pseudomonas aeruginosa biofilms (22). Enterobacter agglomerans biofilms degraded by the bacteriophage through cell lysed (3). The synergistic effect of an alkaline cleaner and a bacteriophage in the inactivation of E. coli O157:H7 biofilms formed on stainless steel (5). It has been reported that phages alone can disrupt biofilm colonies of target organisms, such as Staphylococcus epidermidis growing on silicon catheters (9). Phages were efficient in the removal of biofilms in the early stage of development and 5 days old biofilms of P. fluorescens (10). A bacteriophage (L. monocytogenes phage ATCC 23074-B1) was used successfully in L. monocytogenes biofilm inactivation (16).

Enzyme against biofilm, have effective in cleaning the extracellular polymers form the biofilm matrix (5). Enzymatic cleaning products against biofilms formed by microorganisms commonly found in dairy products (Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus) (13). Proteins reached biofilms formed by S. aureus with the help of Bap proteins were susceptible to Proteinase K mediated detachment and dispersal (12). It has been reported that bacteria use extracellular DNA to form biofilms. To disrupt such kind of biofilms, DNase I can be used to degrade the e DNA released by S. aureus (8). The combination of proteolytic enzymes with surfactants increased the wettability of biofilms formed by a thermophilic Bacillus species and, therefore, enhanced the cleaning efficiency (13). Synergistic action of enzymes in combination with surfactants and phenolic antimicrobials (7).

4. Conclusion

In view of the increased resistance of bacterial biofilms to antimicrobial treatments, new strategies should be implemented for the control of biofilms. Current therapeutic approaches for prevention of biofilms is limited to use of antimicrobial agents and post infection remedy lies in surgical removal of the biofilm followed by continued antibiotic administration. Bio control strategy against biofilm should be considered as a supplement to the present treatment process. Multidisciplinary approaches like Scientist, Biofilm researchers, Doctor, Health profession, Instrument engineers etc. will be necessary in the years to come to translate the data obtained from various biofilm researches to clinic to overcome biofilm associated infection. Such multidisciplinary communication can hope that prevention and inhibition of biof ilms by bacteria can be achieved in near future.

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