Recent Advancements in Biofilm Inhibition

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Biofilms are communities of bacteria that adhere to a surface and are encased within a self-produced matrix of substances called extracellular polymeric substances. Biofilm bacterial infections are difficult to treat due to their complex matrix. The biofilm matrix does not allow antibiotics to penetrate, which in turn promotes antimicrobial resistance. Antimicrobial resistance occurs when different types of bacteria, such as those forming biofilms, resist the effects of antimicrobial drugs. Up to 80% of human bacterial infections are biolm-associated, which is why biolms pose a major threat in healthcare and why antimicrobial-resistant drugs are a hot topic in current medical advancements. This review paper aims to answer the questions, How do biofilms play a role in bacterial resistance to antibiotics? Why are they able to evade treatment, and what are the most promising therapies to overcome this type of resistance? This paper goes into three areas of advancements within biofilms: immunotherapies, peptides, and bacteriophages. This paper will go into specific examples of viable treatment options for biofilm-associated infections, the pros and cons of each therapy, and why scientists believe in each therapy

Introduction

A biofilm is a complex, three-dimensional structure composed of bacterial communities that adhere to many surfaces and produce a protective extracellular matrix [1]. Biofilms are commonly found on natural and artificial surfaces, some including rocks in streams, plant roots, water pipes, and medical devices, which form slimy, slippery coats. Bacteria survive in biofilms, promoting resistance to antibiotics [1]. The extracellular polymeric matrix of a biofilm holds together microbial cells and acts as a barrier that reduces the effectiveness of antibiotics and other antimicrobial agents [1]. Inside a biofilm, unique microenvironments coexist with significantly lower oxygen level, which in turn changes pH and limits nutrient availability. As a result, these harsh microenvironments reduce the efficacy of antibiotics [1].

Due to these unfavorable environments, treatments for biofilm-associated infections are becoming increasingly complicated to treat. As a result, this encourages the formation of resistant strains. Biofilms contribute to recurring infections and heightened antibiotic resistance, displaying their significant threat in healthcare [2]. Limited antibiotic penetration at the biofilm exterior, unique microenvironments within biofilms that hinder antibiotic effectiveness, and specialized persister cells that withstand antibiotic exposure are only some of the mechanisms by which resistance arises [2].

Biofilms often create pain and inflammation in patients and further complicate treatment efforts. Biofilms require new treatment methods to control effectively. Drug resistance causes antibiotics and other antimicrobial medicines to become ineffective. As a result, infections become increasingly complicated or untreatable, which increases the spread of disease, severe illness, disability, and fatality in patients [2]. This is a problem because antimicrobial resistance is one of the top global public health and development threats, also being directly responsible for around 1.27 million global deaths in 2019 and contributing to 4.95 million deaths [2].

Due to biofilm's major role in antimicrobial resistance and persistent infections, they are critical targets for future research and innovation. Different strategies to address biofilm-associated infections include the development of antibiofilm agents, such as enzymes that destroy the extracellular matrix created by biofilms, and employing nanoparticles to enhance antibiotic delivery [1]. These approaches, however, are still emerging, and more research is needed to overcome the challenges presented by biofilm-associated infections.

In this review article, we will answer two crucial questions about biofilm technologies and their medical advancements: How do biofilms make bacterial infections resistant to antibiotics, and what are the best ways to break them down? In this article, we will address alternate methods to interrupt biofilms and make antibiotics effective again in the human body by deeply understanding the structural and molecular mechanisms that break down biofilms. The alternate therapies being explored are immunotherapies, peptides, and bacteriophages.

Immunotherapy

A method to address the challenges of antimicrobial resistance in biofilm-associated infections is Immunotherapy. Immunotherapy manipulates the bodys immune system to aim and attack

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biofilm communities, in turn, reducing the need for antibiotics and debilitating resistance.

An example of immunotherapy in biofilm treatment involves using monoclonal antibodies, otherwise known as mAbs. mAbs are a laboratory-made protein that imitates antibodies in the body's immune system[3]. These antibodies can be engineered to recognize and bind to specific biofilm components, such as surface antigens of bacteria within the biofilm matrix. By binding to these antigens, mAbs enhance the ability of immune cells, such as macrophages and neutrophils, to recognize and attack biofilm-embedded bacteria, leading to overall more effective clearance[3].

TRL1068 is an example of a human mAbs that can be used against a biofilm anchoring protein, specifically those in the DNABII family[4]. Burke and colleagues implanted stainless steel into a mouse and examined biofilm formation on its surface[4]. To visualize the biofilm, the TRL1068 was stained with a fluorescent molecule, which, when present, indicated TRL1068's co-localization with biofilm eDNA. This signal was observed in comparison to the control group, which had no significant fluorescent signals. In addition, scanning electron microscopy was used to observe biofilms on explant pins after 8 days. In the control group that had sterile pins implanted, there were no biofilms on the surface. On the infected, positive control, however, there was a thick and complex biofilm structure with both bacteria and host cells. For another control, a non-specific antibody was used and had similar results to the infected controls, with dense biofilm structures present. When compared to TRL1068, there were no observable biofilms similar to the sterile control group, thus concluding that TRL1068 disrupted the biofilm structure effectively. This demonstrates the ability of TRL1068 to reduce the total bacterial load, making biofilms more susceptible to antibiotic treatments and promoting more diverse options for patients. Overall, the fluorescently tagged antibody known as the control antibody group (CAb) group was similar to infected controls and had dense biofilm structures present. Compared to TRL1068 on day 8, there were no observable biofilm or bacteria. This concludes that TRL1068 reduced the bacterial load, making biofilm structure effective, and also reduced the bacterial load, making biofilms more susceptible to antibiotic treatments if needed, allowing more options to treat patients. This further proves how TRL1068 is an example of a human mAbs that can be used against a biofilm anchoring protein.

The use of vaccines is another immunotherapeutic strategy that can be used against biofilm formation. Vaccines allow the bodys immune system to recognize biofilm-forming bacteria before an infection occurs, particularly in individuals at high risk of biofilm-related infections, such as those with implanted medical devices[5]. By invigorating an immune response early, these vaccines help prevent bacteria from establishing biofilms, cumulatively reducing the risk of chronic, resistant infections. One example of a vaccine is the use of a chimeric peptide vaccine that boosts the immune system to prevent biofilm formation caused by Haemophilus influenzae[4]. DNABII proteins are a family of bacteria essential for maintaining biofilms. The study explored using the chimeric peptide vaccine to disrupt DNABII. The vaccine worked by targeting a specific part of the DNABII protein, known as the B-tip region[4]. Overall, research showed that targeting DNABII proteins with antibodies or vaccines can effectively disrupt biofilms and treat biofilm-associated infections. It is promising for future clinical trials and offers a potential vaccine to prevent infections caused by persistent biofilms, particularly Haemophilus influenzae.

A molecular mechanism in immunotherapy treatment is epitope targeting. Epitope targeting occurs when antibodies bind to specific amino acid regions (epitopes) on the alpha toxin (AT) protein produced by bacterial targets, such as S. aureus (Staphylococcus aureus), to prevent biofilm-associated damage[6]. This works by two portions of antibodies binding to monomeric AT. The two ATs bind onto opposite sides while strategically targeting host cells and AT monomer assembly into functional pores. Epitope therapies have been seen to neutralize AT, prevent cell lysis, and disrupt biofilm development by limiting toxin-driven tissue damage. This means that anti-biofilm interactions could be a promising solution to the problem of biofilms.

Another molecular mechanism is antibody-biofilm interactions. Antibody-biofilm interactions are when antibodies physically and functionally interfere with the formation, maintenance, and stability of bacterial biofilms. Antibodies interact with biofilms in three steps, preventing attachments by using monoclonal antibodies, disrupting the matrix formation, and collapsing the existing structure, which overall leads to biofilm disintegration. They prevent attachments by using mAbs such as anti-ClfA and anti-FnBP to block S. aureus from binding to host surfaces[6]. Next, they reduce biofilm anchoring, allowing them to disrupt the matrix formation by anti-PNAG antibodies binding the matrix polysaccharide and disrupting cell-to-cell cohesion within the biofilm. Lastly, they collapse the existing structure by anti-DNABII antibodies targeting HU proteins, which stabilize eDNA scaffolding, which ultimately leads to biofilm disintegration[6]. Resulting in the reason why antibody-biofilm interactions are a promising method to combat AMR.

This figure demonstrates the protein structure of ClfA and its functional mechanism. The last molecular mechanism being discussed is immune cell recruitment, which is another molecular mechanism of immunotherapies. Immune cell recruitment is when engaging host immune effectors via Fc (tail region of an antibody) complement proteins, and immune cells like macrophages and neutrophils[6]. For instance, when antibodies bind bacterial targets, their Fc regions point outward, and Fcs are recognized by phagocytes, which engulf the bacteria and destroy them. Fc regions also activate the complement pathway, where proteins such as C1q bind. Complement proteins also

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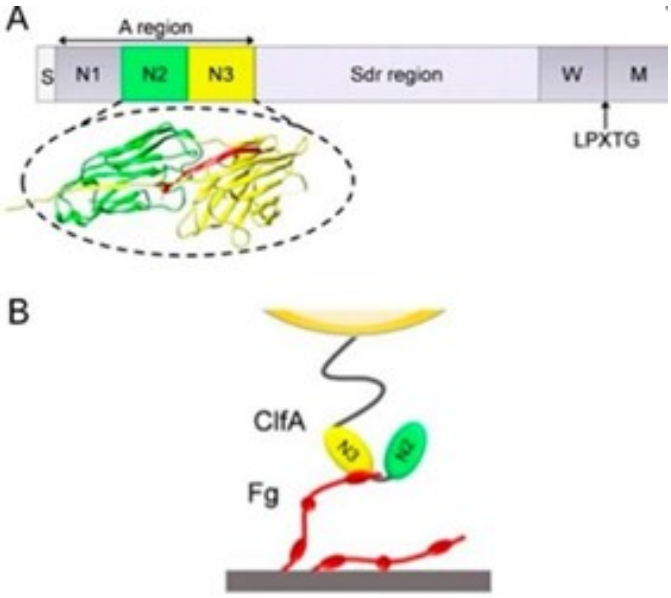

coat the bacteria or form membrane attack complexes to lyse them[6]. Leading to why immune cell treatment is a postive way to combat AMR.

Some notable advantages of using immunotherapies to combat antimicrobial resistance in biofilms are their long-term survival rate, high accuracy, and wide adaptability. Additionally, several studies highlighted the strengths of this approach, even obtaining positive results in treatment trials. However, there have been a few individual cases where immunotherapies can be seen to cause fatal adverse reactions, leading to some controversy when treating patients with immunotherapy[7]. Overall, immunotherapy treatments offer susceptible ways to combat antimicrobial resistance.

Peptides

Another emerging therapy for biofilm-associated infections is antimicrobial peptides (AMPs). AMPs are molecules that are naturally occurring and disrupt bacterial cell membranes, making them effective against antimicrobial resistance associated with biofilms. Peptides are small in size; this property allows them to penetrate through biofilm structures and target bacteria directly. This displays AMP's potential as a promising complement to traditional antibiotics, which often struggle to fully abolish bacteria associated with biofilms.

AMPs benefit from their unique mechanism of action. Unlike traditional antibiotics, which typically disrupt bacterial metabolism or protein synthesis to eliminate bacteria, AMPs directly disrupt the bacterial cell membrane[8]. This action is effective against biofilm-forming bacteria, as AMPs penetrate the biofilm's extracellular matrix, reach bacterial cells more effectively, and aim to target biofilm-bacterial infections.

LL-37 is a peptide that exhibits many antibiofilm properties and effectively breaks down biofilms[9]. LL-37 penetrates biofilm matrices, triggering bacterial cell death by increasing membrane permeability. LL-37 effectively inhibits a bacterium called Pseudomonas aeruginosa biofilm formation without affecting planktonic bacterial growth[9]. LL-37 stops biofilms from forming and breaks up biofilms that are already there.

Synthetic and engineered peptides are currently being developed to improve the stability, specificity, and effectiveness of AMPs. During intubation, bacteria like Staphylococcus can adhere to the surface of endotracheal tubes (ET), forming biofilms and posing a threat to patients[10]. The antimicrobial peptide, Lasioglossin-III, is delivered via drug-eluting ET tubes. The Lasioglossin-III coating on ET tubes makes them very effective at killing airway microbes. These airway microbes are common bacteria that exist to form biofilms. Finding biofilms on ET tubes demonstrates the importance of keeping bacteria from sticking to ET tubes, which is key in biofilm formation. Lasioglossin-III restricts bacteria from attaching to the surface of ET tubes, and this stops biofilm from forming and restricts the growth of bacteria that would normally cause biofilm to build up. This indicates that the Lasioglossin-III coating on ET tubes abolishes biofilm formation, which is a big problem in intubation-related infections. This prevents bacteria from sticking to the ET tubes and prevents them from performing their function. These engineered peptides can be made to target specific types of bacteria or biofilm structures. This capability makes it easier for them to break down biofilms without damaging human cells. The versatility of peptides makes them an adaptable and valuable tool in combating biofilm-associated infections, especially as antibiotic resistance continues to rise, as well as the molecular mechanisms of peptides.

A molecular mechanism in peptide treatment is the membrane disruption mechanism. The membrane disruption mechanism is when the peptide DMS-PS2 from its peptide family, dermaseptin, acts by damaging processes through coil-to-helix transition upon binding to lipid bilayers[11]. Specifically, dermaseptins attach to bacterial membranes, aggregate, and disrupt them by covering their surface like a carpet. This leads to cell lysis and bacterial death without needing to enter the cell or act on intracellular targets. Leading to why molecular mechanisms enhance peptide treatment and make them a viable option in treating AMR .

Another molecular mechanism in peptide treatment is specificity determinants. This is when peptide DMS-PS2 selectively targets bacterial membranes due to charge differences. Its selectivity avoids harming human cells, especially red blood cells. Selective binding to anionic bacterial membranes as opposed to neutral mammalian ones. Coil-to-helix transition only activates in target (bacterial) membrane environments. Weak toxicity to mammalian red blood cells confirms functionality and specificity[11].

The last molecular mechanism for peptide treatment is the penetration mechanism. Penetration mechanisms are when dermaseptins, beyond surface disruption, self-aggregate into supramolecular structures that enhance delivery or retention at the infection site. Dermaseptins facilitate translocation into biofilm matrices and bacterial cytoplasm through temporary pore formation and direct transmembrane translocation. DMS-PS2 shows aggression within 3H, suggesting early formation of complexes that enhance interaction with membrane lipids and biofilm EPS (extracellular polymeric substances)[11]. Overall, penetration mechanisms help strengthen peptides as a treatment against AMR.

Some problems with AMPs are that they are not very stable in physiological environments, they are expensive to make, and they can harm healthy cells when they are not supposed to[12]. Moreover, delivering these peptides effectively to the site of infection remains a significant challenge. Overall, AMPs show promise as a way to address the problems caused by biofilm-associated antimicrobial resistance. Synthetic and engineered peptides offer a complement to antibiotics by directly attacking and breaking down the extracellular matrix of biolms, thus making them safer and viable treatments for biolm infections.

Bacteriophages

The last emerging therapy for biofilm-associated infections is phage therapy, which has gained traction as a great method for combating biofilm-associated infections. Viruses that infect and kill bacteria are known as bacteriophages, or phages.

Unlike traditional antibiotics, bacteriophages specifically target bacterial cells, including those embedded within biofilms, making them a tool in addressing the challenges of antimicrobial resistance[13]. This cycle of analyzing infection and destroying is highly specific to the pathogenic bacteria, causing less harm to beneficial bacteria or host cells[3].

For example, bacteriophages targeting a group of bacteria called Pseudomonas aeruginosa have shown significant success in breaking down biofilms and reducing bacterial populations[14]. The study conducted by researchers focusing on biofilm-associated infections focuses on the understanding of the composition and structure of P. aeruginosa biofilms, which includes extracellular polymeric substances (EPS), exopolysaccharides (Psl, Pel, and alginate), and extracellular DNA (eDNA). P. aeruginosa biofilms are difficult to treat because they protect bacteria from antibiotics and immune responses. When bacteriophage strategies were applied to combat the resistance, efficiency was demonstrated. Particularly, bacteriophages could specifically target biofilm matrix components such as polysaccharides and eDNA and could enhance the susceptibility of bacteria to antibiotics and immune responses[15].

There are 3 molecular mechanisms in bacteriophage treatment. The viral life cycle in biofilms is a molecular mechanism. The viral life cycle in biofilms occurs when bacteriophages enter the biofilms by infecting individual bacterial cells. They replicate and increase local phage numbers. This allows for the local amplification of infectious particles throughout the biofilm. Lytic Bacteriphage, then replicate inside host bacteria, causing cell lysis, releasing new phages which continue infecting nearby bacteria within the biofilm. As a result, they infect the persister cells, which are cells resistant to antimicrobial resistance[16]. This molecular mechanism proves how the viral life cycle strengthens bacteriophage treatment as a promising option.

Enzymatic matrix degradation is another molecular mechanism. Enzymatic matrix degradation is when bacteriophages either produce or encode enzymes such as depolymerases, DNases, and endoglycosidases. These break down biofilm matrix components like polysaccharides and extracellular DNA. These enzymes act in a very localized fashion, released only upon infection or lysis, directly degrading nearby matrix components. Different enzymes have different activities. For instance, tail-spike enzymes have hydrolytic activity and aid in the digestion of specific polysaccharide receptors, facilitating phage attachments and penetration. Enzymatic activity also renders the matrix more porous, aiding in phage progeny escape, better diffusion, and potential bacterial migration[16]. To conclude, synergistic mechanism with antibiotics is a molecular mechanism. Synergistic mechanisms with antibiotics are where bacteriophages work, and where antibiotics fail. For instance, even in 100x MIC of amikicin, early biofilm bacteria were resistant to antibiotics, yet still allowed bacteriophage amplification. This means bacteriophages can kill bacteria in antibiotic-tolerant environments When used with antibiotics. Synergistic biofilm formation was driven by plasmid transfer during co-cultivation of genetically diverse E. coli strains. This implies that biofilm expansion through genetic exchange may make bacteria harder to treat with antibiotics alone. Overall, demonstrating biofilm formation is enhanced synergistically by genetic exchange-particular via conjugative plasmids. Combining bacteriophages with antibiotics might be especially valuable in settings where biofilms are enhanced via plasmid-driven synergy[17].

Bacteriophage therapy offers several advantages and disadvantages as a potential treatment option[18]. Some of the advantages include, phages are highly specific, targeting only the bacterial strain responsible for the infection while sparing beneficial bacteria. They can penetrate biofilms through enzymes that degrade the biofilm matrix, enhancing bacterial clearance. Additionally, phages are self-amplifying, replicating at the infection site, and naturally increasing their therapeutic concentration. The risk of resistance is relatively low, as bacteria resistant to one phage can often be targeted by alternative or engineered phages. Moreover, phage therapy is eco-friendly, reducing reliance on synthetic antibiotics and promoting sustainability. However, challenges exist in phage therapy. Phages must be carefully aligned to correct a bacterial strain, meaning good analysis is required. The host

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immune system can also interfere with phages to act effectively. In addition, the production and purification of phages on a large scale is still difficult and costly. Other constraints of phages include the unavailability of phage therapy in many countries due to regulatory issues.

Finally, while promising, the clinical evidence for phage therapy is still limited, thus requiring more research to establish its safety and efficacy. Bacteriophages can be useful in managing biofilm-associated infections. Phage engineering improvements, coupled with changing regulatory environments, could facilitate the use of phages as antibiotics in managing AMR.

Conclusion

In conclusion, biofilms make bacterial infections resistant to antibiotics, and the best ways to break down biofilms involve immunotherapy, peptides, and bacteriophages. Each therapy offers individual strategies to combat biofilm-associated infections. Immunotherapy enhances the body's immune response, reducing reliance on antibiotics, but encounters immune regulation challenges. Peptides provide a low-dosage and low-resistance approach, yet their stability and delivery require refinement. Bacteriophage therapy targets specificity and biofilm-targeting capabilities but is constrained by pathogen specificity and regulatory limitations.

Future research should specifically focus on the stability, delivery, and scalability of these therapies. Additionally, combining these approaches, such as pairing peptides or phages with traditional antibiotics, may offer great solutions to biofilm treatment. If more investment is put into research and clinical trials, it becomes very essential to translate these innovative therapies into practical, effective solutions for combating biofilm-associated infections prone to happen.

Methods

A number of steps were taken to guarantee a thorough and excellent analysis of the research on biofilms and antibiotic resistance. Using the databases Google Scholar and PubMed, a thorough search of peer-reviewed journal publications was first carried out. Numerous important terms were employed, such as peptides, immunotherapy, biofilm, bacteriophage, and antimicrobial resistance. Second, to find more research on biofilm-targeted tactics and antimicrobial processes, the reference lists of pertinent articles were examined. Third, choosing papers from high-impact publications in the domains of immunology, microbiology, and pharmaceutical sciences was emphasized. Studies that looked at new treatments and resistance patterns were included as a result of the search's emphasis on literature released between 2015 and 2024.

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