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Strategies for combating antibiotic resistance in bacterial biofilms

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Biofilms, which are complexes of microorganisms that adhere to surfaces and secrete protective extracellular matrices, wield substantial influence across diverse domains such as medicine, industry, and environmental science. Despite ongoing challenges posed by biofilms in clinical medicine, research in this field remains dynamic and indeterminate. This article provides a contemporary assessment of biofilms and their treatment, with a focus on recent advances, to chronicle the evolving landscape of biofilm research.

KEYWORDS

biofilm, infection, bacteriophage, antibiotic resistance, treatment

1 Introduction

A biofilm is an immobile, three-dimensional matrix of microscopic organisms that have aggregated onto a surface to form a colony (Sharma et al., 2019). The organisms secrete adhesive proteins and extracellular matrix which help cement the cells to a surface and protect the colony from decussation, environmental hazards, host defenses, and antimicrobial compounds (Jacqueline and Caillon, 2014). One of the key issues with using antibiotics to treat biofilms is achieving the required minimum inhibitory concentration (MIC) of drug at the infection site. The MIC for a biofilm can be between 100-800x greater than the MIC for planktonic cells (Jacqueline and Caillon, 2014). In addition, singular bacteria within biofilms that have been exposed to high concentrations of antibiotics can persist and reestablish a more resistant biofilm, a phenomenon known as recalcitrance (Ciofu et al., 2022). Consequentially, biofilms are frequently refractory to antibiotic treatment and, thus, may require surgical intervention. However, surgery may still prove ineffective, resulting in significant morbidity and mortality, with biofilms implicated in over 500,000 deaths per year in the United States alone (Charani and Holmes, 2019).

Biofilms are known to occur in every human organ system, ranging from the respiratory and digestive tracts to the heart, eyes, and ears (Perry and Tan, 2023). Indeed, biofilms have been implicated in 65% of all bacterial infections (Jamal et al., 2018) and nearly 80% of chronic wounds (Malone et al., 2017). Interestingly, the incidence of biofilm-associated infections is on the rise (Cámara et al., 2022). Many such biofilms exhibit resistance to typical antibiotics and, thus, delay healing time and may require invasive interventions to resolve infection (Metcalf and Bowler, 2013). Furthermore, biofilms present important challenges for the design and use of invasive medical products and prosthetics. For example, biofilms are frequently implicated in catheter-associated infections (Gominet et al., 2017), where they complicate decontamination and treatment of the infection (Ielapi et al., 2020). Biofilms present similar complications in other life-saving interventions, such as endotracheal intubation (Diaconu et al., 2018). Importantly, biofilms commonly affect implanted devices—such as prosthetic joints and pacemakers—and are frequently refractory to pharmacological treatment, ultimately requiring removal of the device (Santos et al., 2011; Tande and Patel, 2014). As a result, recent analyses have estimated the global impact of biofilms to be upwards of $280 billion (Cámara et al., 2022).

Given such significant human and financial costs, there is an increasingly urgent need to develop novel strategies for the clinical management of biofilms. In this review, we focus on the formation and structure of biofilms, the mechanisms of antibiotic resistance within these systems, and highlight emerging non-antibiotic mechanisms of biofilm control.

2 Formation of bacterial biofilms

Biofilm formation is initiated by a complex series of environmental and genetic triggers, primarily involved in stress responses. External factors such as pH, temperature, nutrient availability, and environmental hazards all play a role in causing a planktonic microorganism to shift into an adherent state (Rather et al., 2021). The first step of biofilm formation is reversible adherence, where microorganisms use attachment devices, such as flagella, pili, and fimbriae, to glue themselves to an available substrate. During this stage, the microorganisms are free to abandon their attachment site and return to planktonic life or commit to irreversible attachment (Toyofuku et al., 2016). During irreversible attachment, the microorganisms upregulate various adhesion molecules and glycoproteins. From here, cells undergo division and microcolony formation. Bacteria in the colony communicate through quorum sensing, a process dependent on the synthesis, detection, and regulation of autoinducing molecules (Figure 1). This communication directs the rate of cell division and production of extracellular polymeric substance (Asma et al., 2022)—which accounts for over 90% of the dry mass of mature biofilms (Toyofuku et al., 2016).

2.1 Environmental control

Specifically, a biofilm's extracellular polymeric substance (EPS) matrix, which is composed of proteins, polysaccharides, extracellular DNA, and lipids, allows it to withstand challenges like fluid shear and mechanical pressure. While increased EPS production can only be speculated for environmental challenges like fluid shear, it was found that in staphylococcal biofilms, increased mechanical pressure stimulated the EPS of the biofilm to produce more polysaccharides (Hou et al., 2018). Further, hypoxic conditions may foster formation of bacterial biofilms—particularly for those involving *Staphylococcus aureus*. In the case of *S. aureus* CIP 53.154, hypoxia results in a 21-fold increase in biofilm production, associated with concomitant downregulation of *lexA*—a stress-response-related gene—indicating the hypoxic conditions were favorable for growth (Lamret et al., 2021). Similarly, Aristotelous (2022) found biofilms were unable to thrive in well-oxygenated environments, likely due to enhanced phagocytosis by neutrophils; however, under hypoxic conditions, biofilm-secreted virulence factors decreased the effectiveness of neutrophil phagocytosis and promoted bacterial persistence (Aristotelous, 2022). These studies illustrate how harsh environments—e.g.,

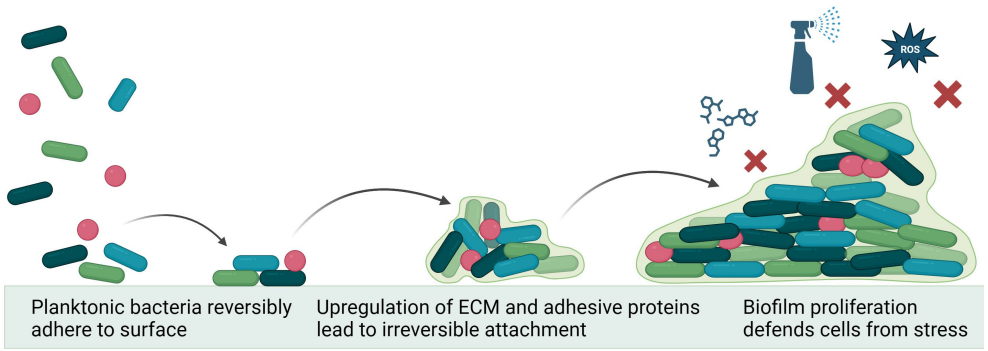

FIGURE 1
Mechanism of biofilm formation. Environmental conditions lead planktonic bacteria to utilize adhesion machinery to attach to a surface. Quorum sensing between colony members drives upregulation of extracellular matrix formation and changes in metabolic function, irreversibly cementing biofilm to surface and protecting the colony from environmental hazards (e.g., antiseptics, reactive oxygen species (ROS) and antibiotics. Figure made using Biorender.com.

those with fluid shear, mechanical pressure, and hypoxia—are quite habitable environments for many forms of biofilms.

3 Current management and treatment of biofilm infections

The cohesion of microorganisms leading to biofilm formation autonomously generates an extracellular matrix, establishing environments that promote bacterial tolerance and resistance to antibiotics through diverse mechanisms contingent upon factors, such as biofilm composition and prevailing growth conditions. Although many studies have studied drug penetration through the biofilm barrier, the underlying mechanisms remain inconclusive. Thus, understanding the mechanisms underlying biofilms' contribution to antibiotic tolerance and resistance is crucial for devising innovative strategies to combat these infections.

3.1 Structure: density and penetration

The efficacy of biofilm treatment is linked to the ability of the antimicrobial agent to penetrate the heterogenous biofilm structure. It has been shown that the capacity of the drug to penetrate the biofilm is highly dependent upon biofilm structure, bacteria genus and strain, and selected antibiotic (Singh et al., 2016). Extracellular DNA, a constituent of the structural framework of the biofilm, has been demonstrated to induce antibiotic resistance (Panlilio and Rice, 2021). Furthermore, the resistance of biofilms to antibiotics is significantly influenced by the bacterial exopolysaccharide (EPS) matrix, a key component in biofilm formation and maintenance (Liu et al., 2017). The production of EPS serves as an adaptive mechanism, with bacteria synthesizing them under stressful conditions, including exposure to antibiotics (Vazquez-Rodriguez et al., 2018). The reduced penetration through the EPS matrix constitutes a mechanism through which biofilms resist antibiotics (Yasir et al., 2018). Factors affecting antibiotic penetration include increased biofilm thickness, drug diffusion efficacy, and the concentration and duration of the administered antibiotic (Hall and Mah, 2017). Additionally, the slow or incomplete diffusion of antibiotics can trap them within the biofilm, resulting in their inactivation by extracellular matrix enzymes (Pinto et al., 2020).

3.2 The metabolic environment within biofilms

The heterogeneity bacterial population observed in biofilms gives rise to metabolically distinct microcolonies. Various mechanisms have been postulated to explain the observed heterogeneity in biofilms. According to the zone model, each bacterium responds to its microenvironment, leading to diverse physiological states within the same biofilm (Kirketerp-Møller et al., 2020). Differences in physiological activity have been shown to be due to differences in pH, hydrogen peroxide, and noncellular materials (Jang et al., 2016; Wu et al., 2018; Ghosh et al., 2019). It has been shown that the deepest layers of the biofilm are exposed to more nutrient-depleted conditions when compared to upper layers of the biofilm due to diffusion barrier and consumption of nutrients carried out by cells in the periphery of the biofilm (Liu et al., 2015). These nutrient-deficient zones have also been identified as a primary source of resistance in bacteria (Liu et al., 2022). Furthermore, nutrient-deficient zones promote the emergence of persister cells, dormant cells that exhibit slow growth and resistance to antibiotics (Olsen, 2015; Miyaue et al., 2018). Therefore, the existence of diverse zones results in a myriad of genotypes and phenotypes coexisting within a local environment. This phenomenon accounts for the emergence of unique metabolic pathways that contribute to drug resistance.

3.3 Efflux pumps

Efflux pumps have conventionally been associated with multidrug resistance due to their capability to extrude diverse antibiotics from bacteria (Nishino et al., 2021). Moreover, these pumps are known to play a crucial role in biofilm formation—particularly in the context of biofilm-associated drug assistance. The physiological heterogeneity within biofilms explains the observed patterns of efflux pump gene expression. For instance, Babin et al. noted the upregulation of specific antibiotic resistance pumps in the upper region of biofilms, while downregulation or no change was observed in the middle of the biofilm (Babin et al., 2017). In the case of *Pseudomonas aeruginosa*, it has been demonstrated that hypoxia enhances antibiotic resistance by altering the composition of multidrug efflux pumps (Schaible et al., 2012). Furthermore, efflux pump inhibitors have been shown to block the antibiotic tolerance of biofilms and completely abolish biofilm formation (Kvist et al., 2008; Zimmermann et al., 2019).

3.4 Quorum sensing

Biofilm formation is partly regulated by quorum sensing (QS), a mechanism through which bacteria employ signaling molecules to enhance communication and survival (Preda and Săndulescu, 2019). QS has been demonstrated to directly impact the regulation of biofilm resistance to antibiotics; specifically, QS regulates expression of various efflux pumps, subsequently influencing the QS system itself (Wang et al., 2019). Further, QS plays a critical role in formation of both gram-positive and -negative biofilms, albeit through slightly different mechanisms. While gram-negative bacteria employ acyl-homoserine lactones within their QS system, gram-positive bacteria employ larger oligopeptides. Both molecules, however, contribute to biofilm formation, thereby hindering antibiotic penetration (An et al., 2019; Gimza et al., 2019).

4 Clinical management of biofilms

The mechanical barrier assembled by biofilms shield constituent microorganisms from antimicrobial agents, thereby presenting significant issues for the clinical management of biofilms. Presently, biofilm management relies on antimicrobial agents and surgical debridement; however, inconsistent treatment outcomes persist. Thus, research in this field is essential to advance the strategies for eradicating biofilms.

4.1 Antibacterial therapies

The heterogeneity of biofilm formation presents a significant challenge in biofilm management. While cells within biofilms exhibit a much higher minimum inhibitory concentration of antibiotics, topical administration allows for delivery of elevated antibiotic concentrations to target biolfilms (Overhage et al., 2008; Yang et al., 2017). Antimicrobial agents have shown high efficacy against biofilm-associated bacteria. However, due to antibiotic resistance, combination therapy emerged as a therapeutic strategy for treating biofilm infections. Combining antibiotics with other agents, such as N-acetylcysteine and recombinant deoxyribonuclease I, has been shown to significantly reduce biofilms (Belfield et al., 2017). Furthermore, certain agents, including catechin, protocatechuic, and vanillic acids, exhibit synergistic effects when combined with antibiotics, inhibiting bacterial adhesion and, thus, biofilm formation (Bernal-Mercado et al., 2020). However, the eradication of biofilms using traditional antibiotic therapy remains challenging, as the large doses required to reach a concentration sufficient to eliminate biofilms frequently cause detrimental side effects to the patient (Ciofu et al., 2017).

4.2 Surgical debridement

The current best treatment to eradicate biofilms involves surgical debridement (Rodríguez-Merchán et al., 2021). This type of debridement uses sharp instruments to remove non-viable and possibly viable tissue surrounding a wound and requires properly trained medical providers and pain control options (Tran et al., 2023). Surgical debridement allows the wound to be more receptive to antibiotic therapies which increases the likelihood of eradicating the biofilm from the wound (Ousey and Ovens, 2023). While this form of debridement is the standard care for many open wound infections, it is unlikely that complete removal of the biofilm will occur, and new strategies including using surgical debridement with meshed skin graft simultaneously may have better outcomes related to healing and infection rates (Namgoong et al., 2020).

4.3 Alternative treatments

Due to the challenges seen with treatments with antibiotics, both as standalone and in combination, research has explored alternative approaches for biofilm eradication. More recently, quaternary ammonium compounds have exhibited high potency and a broad spectrum of activity for biofilm elimination; however, certain analogs have raised concerns regarding toxicity (Saverina et al., 2023). Elevated concentrations of antimicrobial lipids have also been shown potential in eradicating biofilms. In a related study, lipid-coated hybrid nanoparticles were utilized to enhance biofilm penetration for antibiotic treatment (Lee et al., 2022). Additionally, secondary metabolites, such as phenazines and quinolines, have demonstrated complete eradication of certain biofilms with the added benefits of low toxicity; however, it is worth noting that these metabolites have been found to trigger formation of biofilm, dependent on species and strain (Huigens, 2018). For antibiotic resistant biofilms that are challenging to treat, anticancer drugs, such as mitomycin C and cisplatin, have been successfully used as therapies, though clinical toxicity remains a concern (Wakharde et al., 2018). A deeper understanding of these alternative treatments holds potential to pave way for the development of new antibiotics and agents for effective biofilm treatment.

5 Novel strategies for eradication

Given the challenges biofilms pose to conventional treatment strategies, there is increasing interest in exploring novel therapeutic therapies. Such strategies aim to exploit various aspects of biofilm—such as the extracellular matrix—without relying on the metabolism of the cells themselves. These techniques are being investigated both for the prevention of biofilm formation on biotic and abiotic surfaces, as well as for the treatment of active infections.

5.1 Light-based strategies

The use of Ultraviolet Light as an anti-bacterial and anti-biofilm therapy is promising as UV light non-specifically targets DNA and RNA to assist in elimination of bacteria regardless of antibiotic resistance (Conner-Kerr et al., 1998). It plays a role in synthesis of cyclobutene pyrimidine dimers that disrupt cell growth and proliferation. The power of antibacterial photodynamic therapy (APDT) can be enhanced further through the use of photosensitizer molecules (PS), such as phenothiaziniums, tetrapyrroles, hypericin, and curcumin. Irradiation causes the electrons within a PS to enter higher energy orbitals. Upon return to ground state, these electrons can react with organic compounds inside cells, leading to free radical generation. These free radicals cause oxidative damage to the cell, promoting apoptosis (Ghorbani et al., 2018). It is unlikely that development of resistance to APDT would occur due to the non-specific nature of the target. Clinical application of this anti-bacterial method is limited to surface infections or medical device sterilization due to the difficulty of delivery and limited penetration of light through host-tissue (Argyraki et al., 2018). In addition, UV light is potentially carcinogenic to host-tissue, but has been shown to cause minimal damage when used at appropriate fluences (Barnes et al., 2018). More targeted treatment strategies utilizing light-based technology such as photodynamic therapy can further reduce host-tissue damage (Yin et al., 2013).

5.2 Antimicrobial peptides

Antimicrobial peptides (AMPs) have gained increasing attention due to their ability to decrease cell adhesion and reduce the thickness of a broad spectrum of biofilms (Shahrour et al., 2019). AMPs can be classified based on their secondary structure as either α-helical, β-sheet, loop, and extended peptides. To date, α-helical AMPs—such as Magainin-2 and LL-3—are the most well studied. The cationic amphipathic structure of these AMPs allows them to interact with negatively charged bacterial membranes, causing membrane lysis or invasion to carry out non-membranolytic mechanisms (Di Somma et al., 2020). AMPs exhibit additional antimicrobial activity as a result of non-membranolytic mechanisms, which are particularly useful in disruption genes or proteins that are essential for biofilm formation, function, and virulence (Luo and Song, 2021; Castillo-Juárez et al., 2022). There has been recent interest in isolating particular AMPs from plant essential oils. Eugenol derivatives from clove, bay, and pimento berry oils have been found to inhibit *Escherichia coli* O157: H7 biofilm formation by downregulating attachment proteins (Kim et al., 2016). Unfortunately, like antibiotics, AMPs are susceptible to intrinsic and acquired AMP resistance via various mechanisms, such as a more positively charged lipid membranes or efflux pumps—which may perpetuate selection for multi-drug resistant pathogens (Andersson et al., 2016).

5.3 Bacteriophage therapy

Additionally, bacteriophage therapy shows great promise as a specific, targeted option for treatment of biofilms, given their inherent antibacterial activity and minimal adverse effects. Bacteriophages are viruses that follow a lytic life cycle and infect specific strains of bacterial species, making them useful for targeting specific bacterial infections. Their lytic life cycle allows bacteriophages to replicate and spread through many bacteria, efficiently clearing infections. More importantly, the selective targeting of bacteria by bacteriophages spares human cells, thus, resulting in relatively few documented adverse events (Cesta et al., 2020). Due to coevolution with biofilm producing bacteria, bacteriophages have developed the ability to infect and lyse bacteria within biofilms through enzyme mediated degradation of biofilm ECM and can even infect cells during dormancy, causing lysis upon metabolic reactivation (Doub, 2020). Though bacteriophage resistance poses a challenge for therapy, bacteriophage "cocktails"—specific for multiple strains of a bacteria species—can be administered to reduce rates of resistance as well as help ensure the infecting pathogen is covered (Clarke et al., 2020). Furthermore, combination of phages and antibiotics has yielded promising results, even against multidrug-resistant biofilms (Akturk et al., 2019). In particular, pre-treatment of biofilms with phages has been shown to enhance the effects of antibiotics. (Townsend et al., 2020). Moreover, genetically engineered phages have also demonstrated the capacity of biofilm degradation and inhibitory effects (Li et al., 2020).

5.4 Immunotherapy

Several immunotherapeutic options have been explored with vaccination strategies and monoclonal antibodies being potential options. In the case of *S. aureus*, significant efforts have been made to develop a vaccine, but factors such as a lack of understanding of conserved antigens between strains and the need to account for both planktonic and biofilm components to fully eliminate infection have made a vaccine elusive (Bhattacharya et al., 2015). Monoclonal antibodies have had similar complications as preclinical and clinical trials fail to mitigate infection via passive immunity, however, application of monoclonal antibodies conjugated to antibiotics could provide another avenue for exploration as a way to concentrate antibiotics to the site of infection and increase their effectiveness (Speziale and Pietrocola, 2021).

6 Conclusion

As the average age of the US population increases, and the capacity of biomedical technology expands, so does the rate of hospitalization and surgical intervention (Pallin et al., 2014). Between 2005 and 2030, the number of total knee and total hip arthroplasties are predicted to increase by 174% (Antonelli and Chen, 2019). The number of artificial heart valve implantations is increasing by 5-7% every year (Saksena et al., 2019). These numbers only scratch the surface. Without urgent intervention, we can anticipate the rate of biofilm infections and antibiotic resistance to likewise multiply. Modern medicine is facing a microbial arms race, one which will require novel approaches, beyond conventional antibiotic therapy, to win. Inventions such as UV radiation, antimicrobial peptide design, phage therapy, and immunotherapy offer some possibilities to combat and control pathogenic biofilms and deserve further clinical investigation. Moreso, both public and private sector health entities would be wise to invest in both technology and training for clinicians involving biofilms. We are currently 20 years into the advent of antimicrobial stewardship programs and have deepened our understanding of microbial resistance and control (Charani and Holmes, 2019). By expanding these programs to explore biofilm regulation and resistance, medicine can enter the next generation of antimicrobial dominion to the benefit of patients worldwide.

Author contributions

KG: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. JK: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. DR: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. MK: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. AM: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. PL: Writing – review & editing. AK: Writing –

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Conflict of interest

YL is an executive editor for Journal of Cellular Biochemistry.

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