Microbial Biofilms in Pharmaceuticals: Challenges, Mechanisms, and Innovative Solutions

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ABSTRACT

Microbial biofilms are complex and structured communities of microorganisms encased within an extracellular matrix and have secured attention due to their widespread presence and significant impact on various industries, including pharmaceuticals. The present study aimed to investigate the challenges, mechanisms, and innovative solutions in the application of microbial biofilms in pharmaceuticals. Biofilm formation involves a sequential process of attachment, colonization, maturation, and detachment, driven by intricate microbial interactions and the secretion of extracellular polymeric substances (EPS), discussed in detail. In pharmaceuticals, biofilms pose a notable challenge in multiple aspects. One of the most critical concerns is the elevated resistance of biofilm-associated microorganisms to antimicrobial agents. The EPS matrix acts as a barrier, impeding drug penetration and protecting cells from the effects of antibiotics. This resistance contributes to persistent infections associated with medical devices, chronic wounds, and various biofilm-mediated diseases. In pharmaceutical manufacturing, biofilms can contaminate production premises, equipment, and pharmaceutical products, leading to compromised drug quality and safety. Further, the presence of biofilms creates complexities in drug testing and development. Conventional methods, primarily focused on planktonic cells may not accurately predict the efficacy of new drugs against biofilm-related infections, requiring the development of innovative testing approaches. To address these challenges, professionals are actively exploring strategies to prevent, manage, and treat biofilm-associated issues. These approaches encompass disrupting biofilm formation, enhancing drug penetration through the EPS matrix, and developing novel antimicrobial agents specifically targeting biofilms. Additionally, advancements in imaging techniques and biomaterial design offer promising avenues for monitoring and preventing biofilm formation in the pharmaceutical industry.

Key Words: *Cystic fibrosis, Microbial biofilm, Microcolony formation, Minimum inhibitory concentration, Quorum sensing*

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INTRODUCTION

A biofilm is a cooperative assembly of microorganisms, like bacteria, with the ability to exist and propagate as a unified entity recognized as a colony. These cells adhere both to each other and frequently to a surface. These attached cells become enclosed within a gel-like extracellular matrix, primarily consisting of extracellular polymeric substances (EPS). The EPS is a complex mixture of polysaccharides, proteins, lipids, and nucleic acids that provide the biofilm with structure, protection, and communication channels [1]. It also serves various functions within the biofilm, including offering structural support, protection against antimicrobial agents and the host's immune responses, facilitating the adhesion and aggregation of biofilm cells, enhancing resistance to desiccation, and enabling the uptake of diverse substances. Additionally, EPS can act as a carbon source during nutrient-scarce conditions. They are referred to as 'cities for microbes' because of their three-dimensional structure

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and representation of a collective existence for microorganisms. Biofilms can develop on both living (biotic) and non-living (abiotic) surfaces [2]. This matrix, often referred to as the biofilm matrix, adheres the microorganisms to surfaces and protects them from environmental stresses, such as antibiotics and host immune responses. The epidermis, salivary mucosa, and digestive system contain the bulk of the human body's microbes. They have vital roles in several physiological activities in different segments, ranging from innate immunity to metabolism. But occasionally, these beneficial bacteria can grow out of control, which can result in diseases that encourage the development of biofilms. Bacteria have undergone two separate stages of development during their embryonic life: planktonic, which is characterized by free-floating microorganisms, sessile, and adherent to surfaces [3]. Because of a swift change in the expression of many genes related to the development and synthesis of exopolysaccharide (EPS), often known as 'slime' or bacterial EPS, bacteria exhibit unique properties as they arrive from the planktonic to the sessile stages. This transition initiates the prompt generation of a protective barrier upon bacterial colonization of both living (biotic) and non-living (abiotic) surfaces [4]. This protective shield serves to safeguard the bacteria from the host's natural defense mechanisms and external threats, including antibiotics. The onset of the disease process is triggered by biofilm formation, which involves multiple mechanisms, a few of these are the separation of bacteria or communities of related cells, the discharge of endotoxins, strengthened evasion of the host's immune system, and the creation of a barrier that prevents the development of organisms immune to the host's defenses. The stationary complex structure known as a biofilm is made up of host cells, cellular waste products, and one or more varieties of microbes. The bacteria-produced extracellular polymeric material encloses the cells and keeps them securely attached to the substrate. An optimal setting for biofilm formation is a surface offering moisture and nutrients. Biofilms can be positive, negative, or neutral [5]. Those inherent to a natural environment are neutral, while those developing on wounds post-infection are detrimental. Biofilms might contribute positively to addressing soil contamination caused by an oil spill.

Both surface-associated and implant-associated microbial accumulation can cause diseases such as prosthetic valve endocarditis. Surface-associated microbial accumulation exhibits itself in the formation of biofilms on orthopedic, implant dentistry, dialysis peritoneal catheters, and catheters for the urinary tract. Alternatively, it can be non-surface-associated, as evidenced by biofilms in chronic infections such as cystic fibrosis (CF), and microbial accumulation found in marine environments, freshwater, and water treatment systems [6, 7]. Numerous environmental factors, including pH, temperature, salinity, ionic strength, the medium's flow rate (hydrodynamics), and the availability of nutrients, influence the formation of biofilms [8]. As a basic component of microbiology, they have significant effects on several disciplines, including the fields of ecology, the fields of biotechnology, and healthcare [9]. The microbial cells within a biofilm exhibit physiological differences compared to the individual planktonic cells of the same species. Planktonic cells are single cells that may drift or move within a liquid medium, while biofilms are structured communities.

The present study aimed to investigate the challenges, mechanisms, and innovative solutions in the application of microbial biofilms in pharmaceuticals.

RESULTS AND DISCUSSION

Microbial biofilms: formation and dynamics

The construction of the three-dimensional structure of a biofilm is a multistep procedure that includes adsorption, adhesion, microcolony formation, maturation, and dispersion. The point where a biofilm surface intersects with a liquid medium (like blood or water) provides an ideal environment for microbe attachment and growth. Cells in a biofilm colony are close to one another, which promotes the establishment of gradients in nutrition, genome exchange, and quorum sensing (QS). The formation and growth of biofilm follow five distinct stages shown in **(Figure 1)**: (i) the initial adherence (both reversible and irreversible) of individual bacteria, (ii) the clustering of bacteria, (iii) the formation of microcolonies, (iv) maturation, and (v) dispersion or detachment [10, 11]. The first phase is marked by the production of bacterial adhesions, crucial for facilitating adhesion to surfaces. Planktonic bacteria that float freely are responsible for the first colonization of any surface, which covalently clings to the surface, proliferates, transitions to a sessile state, and develops various additional traits from their surroundings.

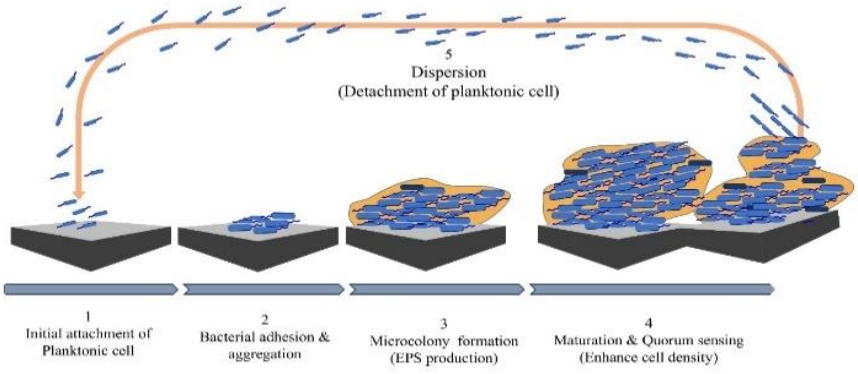

Figure 1. The formation and growth of biofilm go through five distinct stages

Initial attachment

Their attachment to the surface is mediated by either external factors or bacterium extensions such as flagella and pili. Many variables, including the content of materials, bacterial cell surface characteristics, temperature, and pressure, affect bacterium adhesin to its surface [12]. Van der Waals, hydrophobic, steric, electrostatic, and protein adhesion are some of the factors that can regulate the extent of adhesion. The combined impact of these forces enables the bacteria to resist the repulsion forces and continue adhering to the surface, overcoming repulsion forces, and forming a monolayer that is inevitably attached [13].

Bacterial adhesion and aggregation

Specific adhesins and the outermost layer are involved in a genetically integrated interaction that occurs during the second phase of attachment, also known as the latching or anchoring phase [14]. In this stage, organisms produce EPS that interact with surface substances or receptor-specific molecules on pili, fimbriae, and fibrillae to help them become more strongly bonded to surfaces. This results in irreversible adhesion, and the organisms stably aggregate on the surface, resembling the attachment of a cocoon to a leaf. Different species may use different adhesins to attach to surfaces, depending on their needs. In this adhesion process, planktonic microbes can adhere to each other and various surface-bound organisms, leading to aggregation on the substratum. It is remarkable to consider that some microorganisms can encourage the adhesion of other microbe species when they are present on the exterior surface. Different adhesins are produced by each bacterium, certain of which are genetically regulated. This enables organisms to transition between a planktonic organism and a sessile form in response to environmental conditions [15]. For instance, *S. epidermidis* generates polysaccharide intercellular adhesin (PIA), which is essential for the production of biofilms and the adhesion of cells [16].

Microcolony formation

After initial bacterial adherence, the subsequent phase involves the multiplication and division of cells, resulting in the development of microcolonies. Certain chemical signals in the EPS and microenvironments cause this process to proceed [17]. Bacterial colonies in a biofilm usually consist of a variety of micro-communities working together to exchange substrates and facilitate the movement of essential products from metabolism, while regulating the removal of metabolic byproducts. The collaboration among these micro-communities is crucial; for instance, a minimum of three bacterial species is necessary for anaerobic digestion, breaking down complex organic matter into CH_4 and CO_2. Once this breakdown is complete, methanogens acquire energy by breaking down acetate, carbon dioxide, and hydrogen into methane, while fermenting bacteria form acid and alcohol, which are consumed by acetogenic bacteria. A biofilm is an ideal environment for the development of syntrophic associations, which generates relationships between metabolically different bacteria that depend on one another to use certain substrates for their power requirements [18].

Maturation (EPS) production and matrix formation

The EPS matrix is comprised of organic molecules like polysaccharides (e.g., alginate), lipids, and proteins, including extracellular DNA and exopolysaccharides. Numerous environmental parameters, including temperature, pH, and nutrition availability, control the intricate process of producing extracellular solids [4]. One common mechanism involves the polymerization of sugar monomers, such as glucose, fructose, and galactose. These monomers are transported across the cell membrane and then linked together by enzymes known as glycosyltransferases. Numerous studies suggest that the formation of biofilms is attributed to polysaccharide intercellular antigen (PIA), although there is additional evidence indicating that *S. aureus* can develop biofilms independently of PIA. The matrix of *K. pneumoniae* biofilms is constituted by proteins, DNA, exopolysaccharides, and lipopeptides. In addition to these elements, the bacteria may be greatly protected by the

polysaccharide capsule, contributing to the inhibition of complement deposition and the evasion of processes such as phagocytosis and opsonization [19]. The biofilm's cells can be shielded from antimicrobial agents and other dangerous compounds by the EPS matrix.

Quorum sensing

Bacteria communicate information about their population density with one another through a mechanism called quorum sensing, which enables them to modify gene expression in response [20]. Through this technique, bacteria can express energy-intensive functions collectively, but only when their effects on the surroundings or a host are at their maximum. In the context of biofilm formation, quorum sensing plays a critical role in the transition from the initial attachment of cells to the surface to the formation of a mature biofilm. As bacteria accumulate on a surface, they begin to produce and release signal molecules. These signal molecules accumulate in the environment, and when they reach a certain threshold concentration, they trigger a coordinated response in the bacterial population. The specific response triggered by quorum sensing depends on the type of bacteria and the specific signal molecules involved. Quorum sensing controls the development of biofilms and pathogenicity in *P. aeruginosa*. Quorum-sensing components in *P. aeruginosa*, like N-acyl homoserine lactones 76, are implicated in biofilm formation and pathogenic factor expression [21]. Autoinducing peptides (AIP) are a different kind of signaling molecule that is synthesized inside the cell and then transferred into the extracellular environment [22]. AIPs are essential for regulating the replication of genes in response to changes in the density of the bacterial population. This phenomenon is essential for controlling the development of biofilms, antibiotic resistance, and pathogenicity, among other bacterial activities.

Dispersion

Biofilm dispersion is the process by which microorganisms detach from a biofilm and enter the planktonic state. A typical biofilm consists of two distinct layers. The foundational layer serves as the primary residence for bacteria, while the surface layer acts as a dispersal zone, allowing them to spread into their surroundings and maintain a sustained presence [12]. This stage can lead to chronic infections and severe conditions like embolic problems, necessitating prompt medical attention. Commonly referred to as metastatic seeding, in which the individual cells or small clusters of cells are released to form a biofilm. This mechanism occurs as resources deplete with the aging of the biofilm, leading to the accumulation of toxic metabolic byproducts. To survive, microbial cells spread out into other sections of the medical device or the infected host, looking for nutrition and relieving stressful conditions and waste products. The dispersion process begins when individual cells or groups of cells separate from the biofilm. Some experts suggest that in these instances of aerobic bacteria, this process was anticipated and initiated by oxygen consumption or nutritional deficiencies. The secretion of enzymes by cells of bacteria that facilitate saccharide lysis is one of the processes that support the digestion of EPS in conjunction with gene regulatory pathways [23]. The polysaccharide matrix that holds the biofilm together is broken down, releasing the bacterial outermost coat. Following their release, such bacteria can either form new biofilms in multiple organs in the body or proliferate on surfaces by stimulating the synthesis of proteins that improve their ability to propagate.

Biofilm-associated challenges in pharmaceuticals

Biofilms are often more resistant to antibiotics than their planktonic (free-floating) counterparts. The protective matrix and altered metabolic state of biofilm cells make them less susceptible to the effects of antimicrobial agents. Biofilm-associated infections can become chronic and recurrent. The ability of biofilms to resist immune responses and antimicrobial treatments contributes to prolonged infections, leading to increased healthcare costs and patient morbidity. Medical devices, such as catheters, implants, and prosthetics, provide surfaces for biofilm formation. Biofilm-associated infections on these devices can lead to complications, including device malfunction, tissue damage, and the need for device removal or replacement. They often require extended hospital stays, additional medical interventions, and increased use of antibiotics, and detecting biofilm-associated infections can be challenging. Conventional diagnostic methods may not be as effective in identifying biofilm-related issues, leading to delayed or inadequate treatment. Developing effective strategies to prevent and treat biofilm-related infections requires ongoing research and development efforts and finding ways to inhibit biofilm formation on medical devices without compromising their functionality is a complex task [24].

Device-related biofilm infection

Urinary tract infection (UTI)

A device-related biofilm UTI refers to an infection that occurs in the urinary tract as a result of the formation of biofilms on medical devices such as catheters and intra-uterine devices (IUDs). Indwelling urinary catheters are commonly used in healthcare settings to manage various conditions, such as urinary retention, urinary incontinence, or during surgical procedures. The prolonged presence of a catheter provides an ideal environment for bacteria to

adhere to its surface and form biofilms [24]. They may also appear as a result of bacteria migrating from the urethra to the ureters. Some individuals possess a genetic predisposition for developing UTIs. Gram-negative bacterial strains are more commonly accountable for this ailment. Uropathogenic *E. coli* (UPEC) strains have been identified as the primary responsible, especially since they exist in the intestinal region however they may transit to the urinary system and cause invasive infections [25].

IUDs are made of polyethylene, an impermeable polymer that has been incorporated with barium sulfate. Pregnancy-related pelvic inflammation has been linked to the use of IUDs. Anaerobic bacteria such as *beta-hemolytic streptococci, E. coli, S. aureus*, and some have been discovered in IUDs removed from patients suffering from pelvic inflammatory diseases. Other pathogens that have been identified include *S. aureus*, *Candida albicans*, *Corynebacterium* spp., *Micrococcus* spp., *Lactobacillus plantarum*, and *S. epidermidis* [23]. The tail of the IUD is particularly notable as a possible major source of infection. Research has indicated that the proximal regions of the tail, which are in contact with the vaginal microbiome, are where microcolony development is most common.

Prosthetic heart valve infections

This infection comes in two main types: mechanical valves and bio-prostheses, also known as tissue valves. Upon surgical insertion, tissues around the wound may cause an overproduction of platelets and fibrin at the suture site, which increases the risk of microbial colonization. Prosthetic valve endocarditis is an infection associated with artificial heart valves (PVE). After the operation, PVE is categorized as early (≤ 12 months) or late (> 12 months). The pathogenic mechanism may be reflected in the time of infection. Gram-negative bacilli, *S. aureus*, and coagulase-negative staphylococci (CoNS) are the most prevalent causes of infection during valve implantation [26].

Contact lenses infections

Based on factors such as components for construction, design, and frequency of discarding, contact lenses have been divided into two categories: flexible contact lenses and rigid contact lenses. Both kinds of lenses have surfaces that are easily occupied by microorganisms. The kind of substrate, amount of water, the concentration of electrolyte, composition of polymers, strain type of bacteria, etc. all affect the attachment of the lenses. Many bacterial strains and fungal species such as *S. aureus*, *P. aeruginosa*, *Serratia marcescens*, *Aspergillus*, *Fusarium*, and *Candida albicans* are mainly responsible for the development of biofilm on contact lenses and can have negative consequences such as corneal ulcers, inflammation, and vision impairment [27]. There may be signs of ocular infections, including contact lens-induced peripheral ulcer (CLPU), contact lens-induced acute red eye (CLARE), and microbial keratitis (MK) or infiltrative keratitis (IK). Erythrogenic or conjunctival inflammation is the collective term for the co-occurrence of IK, CLPU, and CLARE. Several different techniques may be used to stop biofilm development associated with contact lens usage [28]. These approaches include using antibacterial agents, regularly cleaning lenses, and upholding proper lens hygiene. Utilizing multifunctional contact lens solutions, coating surfaces with antimicrobial agents and biocidal properties, and altering surface properties can also help reduce the production of biofilms. New materials for contact lenses with improved surface qualities have been made possible by advances in material science, to reduce the hazards associated with the development of biofilm. Extracts from *Buddleja salviifolia* and *Calendula officinalis* have shown promising results in defending against biofilms on flexible contact lenses [29].

Catheter-related bloodstream infection

CRBSIs are a major concern in healthcare settings because they increase rates of morbidity, death, and medical expenses. Stays in the hospital for longer increases the possibility of infection, and they are believed to be the most common nosocomial infections [30]. The primary source of CRBSIs is biofilms that form on the outermost layers of intravascular catheters, such as central venous and artery catheters (ACs and CVCs). The biofilm matrix promotes microorganism growth and survival, strengthening their resistance to antibiotic therapies. They serve as a physical barrier that keeps germs safe from the immune system of humans and prevents the penetration of antimicrobial drugs. Microbiological species often associated with Candida infections (CRBSIs) include *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus species*, *C. Albicans*, and *C. parapsilosis* species. Biofilm formation has very negative consequences for catheters [31]. They may lead to chronic bloodstream infections, septicemia, endocarditis, and other systemic illnesses. Additionally, biofilms can serve as an environment for the transfer of pathogens to other parts of the body, which further complicates the clinical course of infections. Preventing biofilm formation on catheters is crucial for reducing the incidence of CRBSIs. To mitigate the formation of biofilms, various approaches have been explored, including meticulous use the aseptic installation methods, keeping hands clean, taking good care of the catheter site, and checking for antimicrobial protection for catheters [32]. The application of anti-infective substances on catheters has demonstrated a reduction in microbial contamination.

Device-related biofilm infection

Cystic fibrosis (CF)

A hereditary condition mostly affecting the respiratory system and digestive tract is called cystic fibrosis (CF). Thicker, more viscous mucus may constrict ducts and airways in many organs, and this condition is brought on by alteration in the CFTR gene. Biofilms, communities of microorganisms embedded in a slimy matrix, contribute to the development of pulmonary infections in individuals with CF. Biofilms are often formed by bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* [33]. These biofilms create a protective environment for bacteria, making them more resistant to the immune system and antibiotics. The thick mucus in the airways of patients suffering from CF provides an ideal substrate for biofilm formation, contributing to chronic respiratory infections. Biofilms in CF exacerbate the cycle of inflammation and infection, leading to progressive lung damage. Strategies to manage CF often include addressing biofilm-related challenges through a combination of antibiotics, mucolytic agents, and therapies aimed at improving airway clearance [34].

Periodontitis

Dental biofilm, also known as dental plaque, is an intricate microbial colony that adheres to the surface of teeth. Biofilm is a complex microbial community that forms on tooth surfaces, composed of bacteria, fungi, and extracellular polymeric polymers encasing more microorganisms [35]. Dental biofilm is the primary etiological factor in several oral diseases, including dental caries (tooth decay, gingiva) and periodontal disease. Teeth can become exfoliated as a result of long-term periodontitis. The most common spot of infection with periodontal disease is the subgingival crevice, which is the canal between the gums and the tooth root. The primary bacteria linked to periodontitis include *Fusabacterium nucleatum*, *Preptostreptococcus micros*, *Eubacterium timidum*, *E. brachy*, and *Pseudomonas anerobicus*. A low pH in the oral cavity can upset the balance of the oral microbiome, causing dysbiosis, which in turn can result in infections [36, 37]. This is caused by poor maintenance of dental hygiene and neglecting oral health. These circumstances turn usually non-pathogenic *Candida* species into pathogenic, which can lead to infections caused by fungi.

Infective endocarditis (IE)

A potentially fatal cardiovascular infection that affects the endocardium, prosthetic valves, implants, and the inside of the heart is called infectious endocarditis. *Staphylococcus*, *Enterococcus*, and *Streptococcus* are responsible for around 80% of IE cases [38]. When infective endocarditis occurs, bacteria attach themselves over the outermost layer of a prosthetic valve, the injured heart endocardium, or the valve sub-endothelium via fibronectin and polysaccharides to initiate the creation of a biofilm. Antimicrobial treatment of IE has become difficult because the biofilm protects the underlying bacteria. The only treatment option left for IE is surgical excision of the biofilms. Phage treatment has been used in patients with prosthetic wall endocarditis and Staphylococcal sepsis, according to a case study published by Gilbey *et al*. [39].

Chronic wound ınfections (CWI)

Healthcare professionals are heavily burdened by chronic wounds. Inherited illnesses such as diabetes, obesity, hypertension, cancer, advanced age, or peripheral vascular disease can all contribute to the delayed healing of wounds. The primary reason they stay in their non-healing condition is that they get stopped in any of the four wound-healing stages (tissue remodeling, hemostasis, inflammation, and proliferation) [40]. Biofilms of several pathogenic bacteria, including fungi (*Candida* spp.), β-hemolytic *Streptococci*, Coagulase-negative *Staphylococci*, bacteria from the ESKAPE group, and *Proteus* spp. are linked to chronic wounds. The presence of necrotic material, an absence of oxygen tension, an immunological response, and wet, nutrient-rich surroundings are favorable factors that lead to the formation of biofilms. Bacterial aggregates are linked to granulation tissue and are distributed across cells that comprise the extracellular matrix, including fibroblasts, keratinocytes, elastin, collagen, and fibronectin [41].

Mechanisms of biofilm-induced resistance

The biofilm structure and its constituent bacteria contribute significantly to the emergence of antibiotic resistance. Treatments for biofilms and related diseases frequently include antibiotics, disinfecting agents, and germicidal chemicals. When compared to their planktonic stage, bacteria within biofilms show a significant rise (10–1000 fold) in drug resistance, especially antibiotic resistance [42]. Antimicrobial resistance is caused by several variables, including different rates of biofilm organism development, prolonged diffusion of drugs through biofilms, and functional changes. When bacteria are in a planktonic stage, they usually acquire resistance to drugs by using enzymes, efflux pumps, or mutations. However, research indicates that biofilm-associated antibiotic susceptibility is also influenced by traditional resistance mechanisms. The biofilm's components, including decreased drug penetration, modified chemical microenvironment, and differentiation in a subgroup of microorganisms, contribute to drug resistance. This phenomenon, known as recalcitrance, enables bacteria to survive high doses of drugs through various processes. Additionally, the EPS in the biofilm hinders antibiotic activity by inhibiting penetration, forming complexes, or undergoing enzymatic breakdown. The slower growth rate

of biofilm-associated bacteria and the presence of persisters further contribute to antibiotic resistance [43]. Horizontal gene transfer is another mechanism through which biofilm microorganisms acquire resistance genes. Studies have shown that mycobacterial biofilms can be resistant to antibiotics (amikacin and clarithromycin) and disinfectants. Antibiotic treatment is more effective during the early stages of biofilm development, suggesting that cells in these phases are less adapted to biofilm communities. Mycobacterial species can differ in the permeability of their anti-tuberculosis drugs, and resistance development is dependent on metabolism states and the induction of resistance-associated genes [44]. With a better knowledge of biofilm formations, more potent antimicrobial drugs and treatments may be developed that serve as barriers against bacteria. As an example, studies have demonstrated that planktonically examined *Staphylococcus epidermidi*s biofilm isolates were sensitive to vancomycin. *Pseudomonas aeruginosa* showed the traditional kind of antibiotic resistance when following its separation from a biofilm that has been subjected to ceftazidime on multiple instances [45].

Antibiotic resistance arises from inheritable genetic mutations, while antibiotic tolerance is a temporary and non-heritable physiological state in biofilm cell populations. Bacterial biofilms, known for their antibiotic tolerance, pose challenges in treating chronic infections. Biofilm-specific tolerance mechanisms differ from planktonic cells, with additional factors hindering treatment options and promoting persistent bacteria development. Antibiotic failure in biofilms is frequently caused by the following factors: a) The EPS barrier's inhibition of antibiotic penetration, b) The buildup of enzymes in the EPS that degrade antibiotics, c) The presence of eDNA promotes resistance to antibiofilm chemicals and aids in the horizontal gene transfer of genes that confer resistance to antibiotics, d) Biofilm quorum sensing affects channel development, volume, thickness, and roughness, e) Neutralization and modifications to the drug or target that result in drug inactivation and upregulation of efflux pumps, f) Microbial diversity and interactions between species, and g) Changes in growth rate, stress reaction, and persister cell presence. Persisters, dormant microorganisms within biofilms, contribute to antibiotic failure, bacterial infection relapse, and the emergence of resistant strains. Protection against alterations in concentrations of chemical substances, osmolality, and pH. The formation of concentration gradients by biofilms produces both aerobic and anaerobic microbial environments, which improve resistance to antibiotics and antiseptics [46]. The multicellular nature of biofilms contributes to antibiotic tolerance, emphasizing the importance of targeting their structure to enhance antibiotic effectiveness and host defenses.

Strategies to address biofilm challenges

Multidrug resistance is becoming more and more of a global concern to the health of individuals, plants, and animals. Pathogenic agents naturally develop multidrug resistance (MDR), which is becoming more and more prevalent and helps them survive. Apart from the primary factor of the misuse and overuse of antimicrobials, as identified by the World Health Organization (WHO), inadequate hygiene and sanitation, limited access to clean water, and deficiencies in the management and avoidance of disease also play roles in the surge of antibacterial susceptibility and resistance [47]. The extensive dissemination of resistance traits has led to a diminished efficacy of antibiotics and other antimicrobials. Plasmids, implicated in the horizontal gene transfer for evolution, are particularly responsible for the dissemination of antibiotic resistance characteristics as a last resort. Consequently, it is imperative to devise alternative strategies to manage the proliferation of such organisms. Given the association between biofilm formation and heightened cellular resistance to antimicrobials, compounds capable of inhibiting biofilms can offer effective treatment for infections. Biofilm-forming bacteria exhibit resistance levels up to 1000 times higher than planktonic-state bacteria. Bacteria in a biofilm are resistant to antibiotic doses up to 1000 times the minimum inhibitory concentration (MIC). This resistance is linked to the restricted spread of antibiotics, which is brought about by poor membrane permeability and fewer porins on external surfaces [48]. A range of strategies have been proposed to suppress the formation of biofilms, including the use of disinfectants, antiseptics, bacterial phage enzymes, essential oils, surface improvements, and quorum sensing (QS) inhibitors. Some of the anti-biofilm are discussed below.

Phytoextracts

Several plant extracts have undergone in vitro testing to assess their potential in treating biofilm-related infections. Research indicates that plant extracts with high concentrations of bioactive chemicals and secondary metabolites present promising therapeutic options against biofilms. Bacteria may exist in biofilms. Studies have been conducted to determine the effectiveness of antimicrobial and antifungal substances obtained from plant extracts against biofilm-forming bacteria and fungi. In the realm of oral health, fungal infections, particularly those caused by Candida species, are prevalent. Various *Candida* species are implicated in periodontal infections, and plant extracts were evaluated for their impact on these fungal biofilms. Essential oils extracted from *Allium sativum* L. bulbs, *Cinnamomum zeylanica* Blume leaves, and *Cymbopogon citratus* (DC. Stapf.) leaves have shown promise in combatting infections brought on by the opportunistic

pathogen *Candida*, particularly *Candida albicans*, which causes Candidemia and Candidiasis when it enters the bloodstream [35].

Bacillus gaemokensis biofilms have been reported to be effectively removed and new biofilms to be formed by the *Piper betle* chloroform extract [36]. Antimicrobial substances with bioactive properties, including phenolic compounds, flavonoids, terpenoids, and alkaloids, are present in phytoextracts. The effectiveness and range of action of phytoextracts may differ when compared to conventional antibiotic treatments, despite the possibility that some of them have antibacterial activity [49]. The study examined the antibiofilm properties of *Capsicum baccatum var. pendulum*, against *Pseudomonas aeruginosa and Staphylococcus epidermis*. Because it inhibits adhesion without harming planktonic bacteria, the residual aqueous extract from seeds (RaQS extract) was discovered to suppress the development of biofilms. The production of biofilms is known to be inhibited by phytoextracts and volatile essential oils through a variety of mechanisms, including membrane damage and membrane protein formation of leaky channels, inhibition of ATP production, signal interruption, and consequent cell-to-cell communication [50]. The root extract of *Panax quincuefolius* was shown to be useful in reducing the expression of virulence and reducing the motility of bacterial swarming and swimming, which led to a decrease in *P. aeruginosa* biofilm formation. Scanning electron microscopy (SEM) revealed that the essential oils (EOs) extracted from *Piper nigrum* and *Mentha suaveolens*, at 1% v/v concentration, reduced the development of *Staphylococcus aureus* biofilms by 40% [28]. However, the EOs did not prevent bacterial growth; instead, they primarily broke down EPS and disassembled the surface. It was discovered that the anti-biofilm effect was caused by eugenol and β-caryophyllene [51].

Quorum sensing (QS) system blockers

The microbial communication system known as QS is very distinctive and relies on distinct biochemicals generated by the microorganisms, which regulate many biological processes such as the development of factors associated with virulence and biofilm production. Consequently, QS system inhibitors can effectively suppress the genes and protein components that contribute to pathogenicity [52]. Research utilizing polypeptides, cephalosporins, aminoglycosides, and quinolones has shown that QS inhibitors are effective in conjunction to impede the formation of biofilms, enhancing the effectiveness of some drugs. An alternative to traditional antibiotics is the use of antibiotics in combination with QS system inhibitors. QS inhibitors are known to suppress *Pseudomonas aeruginosa's* QS system by downregulating the expression levels of the lasR and rhlR genes. Examples of these inhibitors are N-(4-butanoyl)-L-homoserine lactone (FABHL) and N-(4-[4-chloroanilno]butanoyl)-L-homoserine lactone (CABHL) [53].

Phage therapy

Phages are excellent candidates for progression as anti-biofilm compounds due to their restricted availability of bacteriophage sensors on eukaryotic organisms, their ability to infect both Gram-positive and Gram-negative bacteria, and their prevalence in nature [54]. Even though water pathways are made to make it easier for nutrients to enter cells, phages may readily enter cells through them. Additionally, bacteriophages generate lysins and exopolysaccharide-degrading enzymes, such as polysaccharide depolymerase, which are found at tail ends and are capable of breaking down biofilms. Advancement of phage therapies includes the impact of bacteria on the host's microbes and immune system, the mode of implementation, the required dosage, the increase in the incidence of resistance, the development of a phage mixture to combat multispecies biofilms, and the lack of adequate clinical studies. Phage treatment combined with antibiotics may have beneficial effects, according to assumption. The treatment of diabetic foot ulcers (DFU) caused by *Staphylococcus aureus* infection has shown promise when using the bacteriophage Sb-1. To combat food-borne pathogens like *Listeria* spp., the FDA (Food and Drug Administration) authorized the use of phages in packaged meat and cheese in 2006 [55].

Nanoparticles against biofilms

Nanotechnology can be used to address microbial illnesses associated with biofilms. By functioning as a channel for the distribution of medications in the ideal quantity to specific areas and boosting their antibacterial activity, it offers a way to eradicate a variety of diseases. Nanoparticles possess several characteristics that render them appropriate for infringing down biofilms, including their small dimension, high susceptibility, and enormous surface area-to-volume ratio [56]. Additionally, they shield the medications from enzymatic reactions. The biofilm EPS disruptive forces are transported via the nanoparticles (NPs). NPs are considered a viable alternative to antibiotics in addressing infections associated with multidrug resistance and biofilm formation. Biofilm pore sizes, charges, hydrophobicity, and the EPS chemical gradient may all have an impact on the three stages of the biofilm-NP interaction: the movement of NPs within the biofilm, the binding of NPs to the EPS of the biofilm, and infiltration of NPs into the EPS, enabling their diffusion-based movement inside the biofilm. Challenges associated with antibiotic therapy, such as limited diffusion into biofilms, can be effectively addressed through the utilization of nano-formulations, which can infringe the

biological barrier [57]. The research additionally suggested that calcium fluoride nanoparticles (CaF_2-NPs) may disrupt the enzymatic processes linked to glucan synthesis, cell adhesion, acid production, and tolerance, as well as quorum sensing [58]. These actions collectively contribute to the inhibition of biofilm formation. Nanoparticles made of metals (like iron oxide and zinc oxide) and metal oxides (like copper, zinc, and silver) are often utilized [59]. By interacting with the functional group and surface charges of biofilms, these nanoparticles transform into harmful ions that can break down both bacterial cells and EPS if they connect with their outermost layer. Hordenine-AuNPs showed stronger antibiofilm characteristics on *P. aeruginosa* PAO1, indicating that natural chemicals supplied by NPs can be effectively used in biofilm-related infections [60]. While antibiofilm tactics are still in their infancy, there are some clinically available instances of antimicrobial catheters, implants, and wound dressings incorporating AgNPs. Therefore, additional in vivo research on the utilization of nanoparticles for medicinal purposes is needed.

Future direction and prospects

The visualization of biofilm structures has traditionally been challenging due to their complex three-dimensional nature. However, future directions in microbial biofilm research involve cutting-edge imaging techniques that provide unprecedented insights. High-resolution imaging technologies, such as super-resolution microscopy and advanced confocal laser scanning microscopy, enable researchers to observe biofilms at the microscale with enhanced clarity. Additionally, emerging techniques like atomic force microscopy and cryo-electron microscopy contribute to a deeper understanding of biofilm architecture, composition, and interactions. Real-time imaging approaches will likely play a pivotal role in capturing the dynamic processes of biofilm formation, maturation, and dispersion.

The development of innovative biomaterials is a key area for future exploration in preventing biofilm formation. Researchers are investigating surface modifications and coatings that can inhibit bacterial adhesion and disrupt biofilm formation on medical devices, implants, and other surfaces. Nanostructured materials with inherent antimicrobial properties, such as nanoparticles embedded in coatings, hold promise for preventing biofilm attachment. Smart materials that respond to environmental cues or exhibit controlled release of antimicrobial agents can offer targeted and sustained biofilm prevention. Advancements in biomaterial science will likely lead to the design of surfaces that resist bacterial colonization and biofilm development without compromising the functionality of medical devices.

CONCLUSION

Microbial biofilms significantly impact the pharmaceutical industry, necessitating extensive research and innovative solutions. These biofilms provide strong barriers to antimicrobial drugs, leading to prolonged infections and decreased medication efficacy. They are characterized by varied communities and protective extracellular matrices. The complex process of biofilm development poses a barrier to conventional antibacterial methods, spanning from the initial microbial attachment to maturity and removal. Biofilms are a major problem in the pharmaceutical industry because they can contaminate manufacturing plants and medical equipment, endangering the quality and safety of drugs, as discussed. Novel testing procedures and medicines that particularly target biofilms are required because traditional methods focusing on planktonic cells are insufficient for evaluating the efficacy of new drugs against infections associated with biofilms. Innovative biomaterials are also being developed to prevent biofilm formation. Researchers must explore surface modifications and coatings that inhibit bacterial adhesion and disrupt biofilm development on medical devices and implants. Nanostructured materials with antimicrobial properties, along with smart materials that respond to environmental stimuli or control the release of antimicrobial agents, offer targeted and sustained biofilm prevention. Addressing the challenges of microbial biofilms in the pharmaceutical industry requires a multidisciplinary approach, integrating advanced imaging techniques, innovative biomaterial design, and novel antimicrobial strategies. Ongoing research and collaboration between scientists and industry professionals are essential to develop effective solutions for preventing, managing, and treating biofilm-associated challenges that enhance drug efficacy and ensure patient safety.

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