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Bacterial Biofilm Formation on Biomaterials and Approaches to Its Treatment and Prevention

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Abstract: Bacterial biofilms can cause widespread infection. In addition to causing urinary tract infections and pulmonary infections in patients with cystic fibrosis, biofilms can help microorganisms adhere to the surfaces of various medical devices, causing biofilm-associated infections on the surfaces of biomaterials such as venous ducts, joint prostheses, mechanical heart valves, and catheters. Biofilms provide a protective barrier for bacteria and provide resistance to antimicrobial agents, which increases the morbidity and mortality of patients. This review introduces the formation process and drug resistance mechanism of biofilms in detail, and further summarizes the main characteristics of clinical persistent infection caused by biofilms and the many methods of treating biomaterial-related biofilm. This provides ideas and directions for the development of new biofilm infection strategies related to therapeutic materials.

Keywords: Biofilm formation; Biomaterials; Antibiotic resistance mechanisms; Biofilm infection; Antibiofilm strategies

1. Introduction

A biofilm is a population structure comprising a highly structured membrane complex formed by bacteria attached to various solid surfaces and their production of extracellular polysaccharides (EPSs), matrix proteins, and extracellular DNA (eDNA)[1]. The existence of biofilms provides many biological advantages for its internal bacteria, such as high infectivity, drug resistance, and strong viability[2]. It is estimated that more than 65% of nosocomial infections, about 80% of chronic infections, and 60% of human bacterial infections are caused by biofilms[3], including chronic biofilm diseases such as cystic fibrosis and periodontitis, as well as biofilm infections related to biomedical materials, such as medical device infections, implant-related infections, and others[4].

It is worth noting that once a biofilm is formed on medical equipment, conventional methods often cannot effectively eradicate the biofilm and usually require long-term antibacterial treatment[5]. As current antimicrobial therapy is expensive and will increase the drug resistance of pathogens, there is interest in the development of new antimicrobial therapies[6]. In this review, we introduced in detail the formation process and drug resistance mechanism of biomaterial-related biofilms and summarized their characteristics in clinical infections, such as persistent infections and inflammation. Two methods to prevent and treat biofilm infection related to biomaterials, including antibacterial coatings against biofilm infection and surface modifications of biomaterials, are also discussed in order to provide ideas for the development of new anti-biofilm therapies.

2. Biofilm formation on biomaterials

The formation of biomaterial-related biofilms mainly depends on the surface of medical devices and is a continuous, complex and multi-stage process that depends on many factors, such as the matrix, material surface medium, cell metabolism and internal characteristics, and signal molecules [7,8]. The biofilm formation includes three main steps: attachment, maturation, and dispersion[9,10] (Figure 1). In the first stage, microorganisms attach to the biomaterial surface, but the adhesion

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process is reversible because of the poor adhesion between the cells and the surface. Then, as the cells interact on the biomaterial surface of the material, the reversible attachment of bacteria cell becomes irreversible. Bacteria form microcolonies when their adhesion to the biomaterial surface is stable. However, when the external conditions change, such as temperature, nutrition, pH, and oxygen, the biofilm will disperse and the bacteria will return to the planktonic state[11,12].

2.1. Biofilm initial attachment

The initial attachment of a biofilm, as the name implies, refers to the attachment process of microorganisms on biomaterial surfaces[13]. However, due to the weak interaction between bacteria and the biomaterial surface, the initial adhesion stage of bacteria is not absolute, but instead a reversible process[8,14]. For example, when bacteria attach to a biomaterial surface, they are affected by the external environment, such as hydrodynamics and attraction or repulsion; or, if the composition, temperature, pressure, or pH of the biomaterial surface become unsuitable for bacteria to adhere, the bacteria will return to the planktonic state[8,15].

In this process, physical interactions such as polarity, London-van der Waals forces, electrostatic interactions, hydrophobic interactions, and protein adhesion can promote the adhesion of microorganisms to the biomaterial surface and contribute to the formation of biofilms[8,16,17]. A flagellum is a flagellate filamentous appendage that provides power for bacteria. It can attach to the surface of biomaterials to enhance the interaction between the bacteria and the attached surface and can also reduce the repulsive force between the cells and the surface, thus promoting cell attachment to the surface[18,19]. Interestingly, the reversible adhesion of bacteria can be transformed into irreversible adhesion under certain conditions. First, bacterial cells are usually negatively charged, and when the attraction between the microorganism and the implanted surface is greater than the repulsive force, the bacterial cells remain fixed and strongly adhere to the positively charged surface to form irreversible adhesion[16]. Second, bacterial adhesin plays an important role in the adhesion of microorganisms to the biomaterial surface. Fimbriae adhesin allows bacteria to adhere to each other and form an initial cell adhesion layer. For example, type 1 fimbriae is one of the most common adhesion organelles in *Enterobacteriaceae*, including *Salmonella*[20]. It can increase the initial surface adhesion of *Escherichia coli* and form irreversible adhesion[21]. In addition, in some bacteria, polysaccharide adhesin can regulate permanent cell adhesion, such as polysaccharide intercellular adhesin (PIA) that promotes intercellular adhesion and biofilm accumulation of *Staphylococcus epidermidis*[22,23]. Finally, EPSs can also promote the irreversible attachment of bacteria by interacting with surface materials and ligands[15].

2.2. Biofilm maturation

After the microorganisms adhere to the biomaterial surface and stabilize, the bacterial cells will continue to proliferate and produce intercellular adhesions to form microcolonies[1]. At this stage, as the bacterial density reaches a threshold level, the bacterial quorum sensing (QS) system is activated[16,24]. QS is a bacterial cell-to-cell communication system that relies on diffusible signal molecules. Bacteria use quorum-sensing signaling molecules to regulate their own behaviors, such as biofilm maturation, motility, and virulence factor expression[25,26]. Different types of bacteria secrete different signal molecules, such as acylated homoserine lactone (AHL) secreted by gram-negative bacteria, self-inducer peptide (AIP) secreted by gram-positive bacteria, and self-inducer-2 (AI-2) secreted by gram-negative and gram-positive bacteria[7,27]. Using *Pseudomonas aeruginosa* as an example, many studies have shown that *Pseudomonas* quinolone signal (PQS) molecules affect biofilm formation[28]. The deletion of the biosynthetic gene of a mutant PQS caused *P. aeruginosa* to form a defective biofilm, and it could not form the mushroom-like structure of the mature biofilm of the wild bacteria[28]. Extracellular DNA (eDNA) is essential for intercellular junction and biofilm stability[16]. The PQS also promotes the release of eDNA, which is beneficial to the maturation of *P. aeruginosa* biofilms[29]. In addition, QS not only regulates the entire process of biofilm formation but also promotes bacteria to secrete EPSs[30]. The secretion of EPSs helps to stabilize the biofilm structure and prevent itself from being attacked by antibacterial agents and immune cells[13]. The

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secretion of EPSs also contributes to the formation of three-dimensional structures of biofilm[31]. Voids and channels are formed in the three-dimensional structure of the biofilm, which are filled with water to distribute important nutrients, transmit molecular signals, remove waste from the biofilm, and act as a circulatory system[32,33]. After the formation of the first biofilm structure mediated by EPS, other bacteria of the same species and other species in the environment are also incorporated into the biofilm structure. In this manner, the biofilm structure develops from a thin layer to a mushroom-like structure[11].

2.3. Biofilm dispersion

As the biofilm matures, nutrient resources are consumed in large quantities, and toxic substances will continue to accumulate[34,35]. Therefore, to obtain more nutrients, the bacterial cells of the biofilm disperse from the biomaterial surface and migrate to other areas of the medical implant, thereby spreading the infection[8,16]. The dispersion mechanism in bacteria generally occurs in three stages: first, cells leave the microcolony; second, cells move to a new substrate; and third, cells attach to the new substrate and start a new biofilm formation process[11,36]. In fact, biofilm dispersion can be divided into active and passive modes[37]. When bacteria experience environmental stresses such as antimicrobial pressure and nutrient deficiency, they will actively dissociate from the implanted surface[38]. In the process of biofilm dispersion, some mechanisms are favorable for bacterial cell dispersion, such as the dissolution of EPSs[8]. Nutrient deprivation in biofilms stimulates small molecules of the fatty acid DSF (cis-11-methyl-2-dodecenoic acid) to trigger autophosphorylation and induce the degradation of c-di-GMP, leading to the dissolution of EPSs, which releases some of the planktonic cells[39,40]. In addition, in the process of active dispersion, the expression of genes related to bacterial motility caused by flagellum synthesis is upregulated, while the expression of genes related to bacterial attachment is downregulated, thus facilitating bacterial detachment[35].

Passive dispersion of biofilm depends on external factors, such as enzyme degradation of the biofilm matrix and shear forces[37]. The microbial community within the biofilm produces different saccharolytic enzymes that digest the polysaccharides that stabilize the biofilm structure, helping to release the microbial surfaces to new colonization areas[16,31]. For example, in alginate, Pel and Psl polysaccharides are the skeleton components of the *P. aeruginosa* biofilm supporting structure, which can help bacteria to absorb nutrients and communicate signals between cells[41]. Alginate lyases have been shown to be used for the cleavage and subsequent separation of EPS substrates, while the extracellular polysaccharide hydrolases PelA and PslG induce biofilm dispersion and prevent biofilm formation[16,42,43]. PelA was more effective in dispersing biofilms with Pel as the main extracellular polysaccharide, while PslG was most effective in removing biofilms formed with Psl as the main extracellular polysaccharide[44]. Previously, our research group also overexpressed PelA and PslG enzymes in *P. aeruginosa* engineered bacteria through synthetic biology, and lysed the engineered bacteria in active and passive ways to release two types of extracellular polysaccharide hydrolases, PelA and PslG, thus destroying cytoskeletal components Psl and Pel. The result was destruction of the *P. aeruginosa* biofilm[45]. In addition to enzymatic degradation of biofilm matrixes, physical means such as shear force are important factors for passive dispersion of biofilms[46]. A sudden increase in shear forces causes the cells to be immediately released from the biofilm, resulting in the dispersion of the biofilm[47]. For example, during the formation of *Streptococcus* mutans biofilms, a 10-fold increase in shear forces resulted in an 85% reduction in the biomass of the biofilm[48]. In addition, ultrasonic waves, laser-induced shock waves, and other technologies have been found to passively disperse biofilms[37]. Ultrasonic treatment significantly reduced *E. coli* and *S. aureus* biofilms on a stainless steel surface[49,50]. Laser-induced shock waves destroyed biofilms grown on different types of medical devices by generating plasma, and although the shock waves themselves were not harmful to the bacteria, the biofilms exposed to the shock waves were more sensitive to antibiotics than those that were not exposed[51]. To summarize, both active and passive methods can facilitate the dispersion of biofilm. However, once biofilm cells that were dispersed and settled on a new surface are stimulated by adverse conditions, the isolated microbial cells upregulate the expression of flagellar proteins to help them move quickly and return to a floating state on the

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surface. The bacteria then reform the biofilm in the right environment, causing continuous infection[8,52].

3. Mechanisms of antibiotic-resistant biofilms

As one of the most successful life forms of bacteria, biofilms are not only widely distributed in various environments, but they also play an important role in promoting antibiotic resistance[53]. The sensitivity test of a biofilm model in vitro showed that the survival rate of bacteria covered by a biofilm was hundreds or even thousands of times higher than that of planktonic bacteria when using antibiotics for sterilization[54,55]. Many studies have shown that in biofilms, antibiotics do not exert their antibacterial function, but instead cause the emergence of superbugs, which increases the difficulty of treating biofilm infections[56,57]. In addition, the formation of a biofilm is conducive to the emergence of inflammation, which leads to persistent infection. For example, biofilm formation plays an important role in pulmonary infection, burn infection, and medical device infection in patients with cystic fibrosis caused by *P. aeruginosa*[58]. Therefore, revealing the main causes of drug resistance and the mechanism of drug resistance of biofilm bacteria is of great significance for the clinical treatment of biofilm infection[59].

3.1. Prevention of antibiotic penetration by biofilms

EPSs not only maintain the structural stability of a biofilm but also serve as a barrier for antimicrobial agents such as bleaching agents and antibiotics[60]. Aminoglycoside antibiotics cannot penetrate the biofilm because the positive charge of the aminoglycoside antibiotics is neutralized by the negatively charged matrix[61]. For example, cations are chelated by a subinhibitory level of negatively charged eDNA, which limits the penetration of antibiotics by inducing the PA3552–PA3559 operon to develop resistance to cationic antimicrobial peptides and aminoglycosides[59,62]. Furthermore, the presence of enzymes in the biofilm matrix also prevent the penetration of antibiotics[63]. For example, β-lactamases accumulated in extracellular polysaccharides promote the degradation and inactivation of antibiotics, and the role of β-lactamases is mainly against β-lactam antibiotics (carbapenem, imipenem), for which drug resistance is particularly important[64,65]. It is also believed that the poor permeability of antimicrobial agents is due to the decrease in diffusion and adsorption in the biofilm matrix[6], and there is no strong interaction between most antibiotics and biofilm matrix components[66,67].

3.2. Presence of slow-growing or persistent cells in biofilms

The formation of the biofilm matrix leads to slower penetration of antimicrobial agents that develops a resistant phenotype called persistent cells, resulting in antibiotic resistance[68,69]. An experiment of a biofilm in vitro showed that the oxygen concentration on the surface of a biofilm is high, and the metabolism of surface bacteria is also high, while the oxygen concentration in the center of the biofilm can be very low, and the bacteria growth relatively slow or halted[64,70]. These slow-growing bacteria contribute to the antibiotic resistance of the biofilm[69]. For example, fluoroquinolones and tetracycline can only effectively kill metabolically active cells in the upper layer of a *P. aeruginosa* biofilm[71]. Persistent cells are thought to be cells that differentiate into a dormant state, and they contribute to the persistence of biofilms[63]. Because the cell metabolism of persistent cells slows or ceases, the target of the antibiotic is not active, and the antibiotic cannot work[68].

3.3. Increased expression of efflux pumps in biofilms

Increasingly more studies have shown that the presence of efflux pumps is a key factor in the development of antibiotic resistance in biofilms[72,73]. Efflux pumps, which are membrane proteins necessary to maintain bacterial homeostasis by expelling toxic substances, are present in all types of bacteria[74]. Bacteria present in the biofilms also use efflux pumps to maintain cytoplasmic concentrations of certain antibacterial compounds below critical thresholds or to expel antibiotics, and the antibiotic resistance of the biofilm is caused by the increased expression or activity of the

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efflux pumps[75,76]. The ATP binding box (ABC) family, the small multi-drug resistant (SMR) family, the multi-drug and toxic compound extrusion (MATE) family, the drug-resistant nodular cell division (RND) family, and the large promoter superfamily (MFS) are the five major families of efflux pumps, which play an important role in biofilm antibiotic resistance[77,78]. For example, in *Acinetobacter baumannii*, TetA and TetB efflux pumps of the MFS family cause resistance of its biofilm to tetracycline and minocycline, in which the Tet efflux pump uses proton exchange as an energy source to extrude tetracycline[79]. The physiological function of these pumps is to expel toxic molecules that denature cell membranes and confer resistance to bacteria that are present in the biofilms[80]. MacB is a member of ABC family, and it helps bacteria excrete macrolides and endows biofilms with antibiotic resistance[81]. In addition, *P. aeruginosa* MexAB-OprM and MexCD-OprJ, which belong to the RND family, play important roles in antibiotic resistance in biofilms, and MexAB-OprM pumps contribute to biofilm resistance to ofloxacin[82,83]. *S. aureus* responds to antibiotics by upregulating various ABC transporters, thereby increasing antibiotic resistance in biofilms. For example, GraRS may confer vancomycin resistance to a biofilm by upregulating the VraFGABC transporter[84,85]. BraRS can also activate BraDE and VraDEABC transporters and increase biofilm resistance to nisin and bacitracin[86,87].

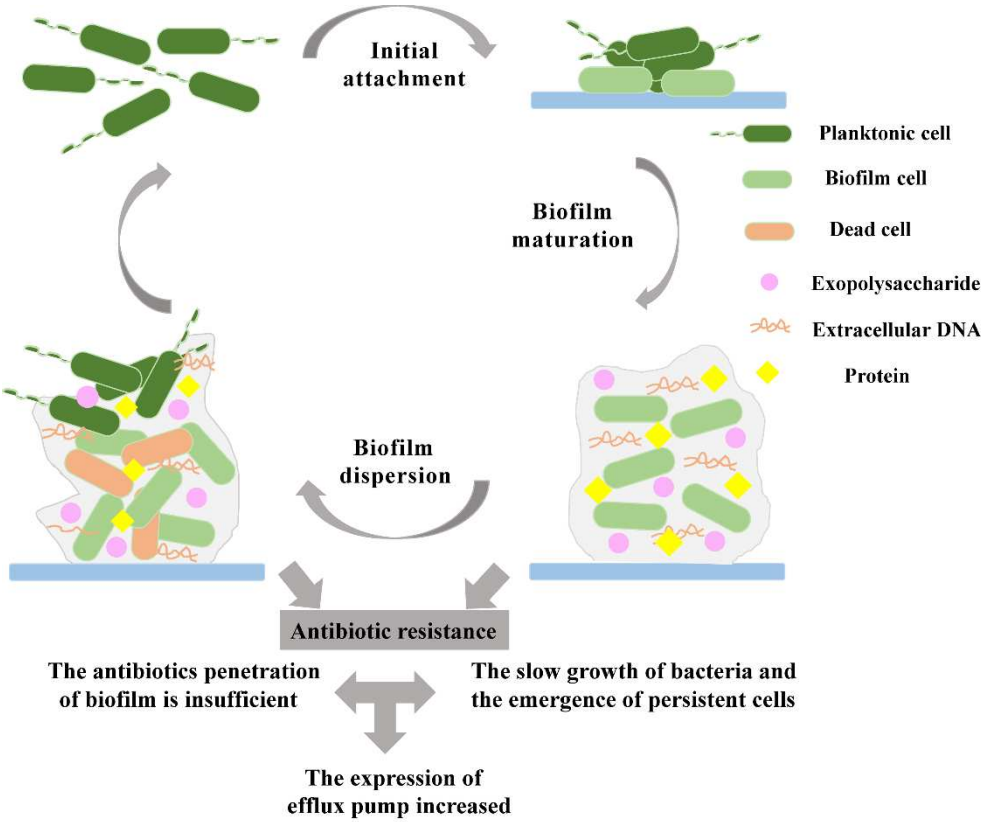

Figure 1. The process of biofilm formation and antibiotic resistance mechanisms. Biofilm formation is divided into three steps. (1) Initial attachment of the biofilm. (2) Biofilm maturation. (3) Biofilm dispersion. The mechanisms of antibiotic resistance in biofilms are also divided into three factors. (1) The antibiotic penetration of the biofilm is insufficient. (2) The bacteria grow slowly and persistent cells emerge in biofilms (3) The expression of efflux pumps increases.

4. Clinical characteristics of biomaterial-associated biofilms

Colonization of biomaterials by pathogens leads to the formation of biofilms, which greatly increases the potential for clinical infection[88]. This includes devices such as mechanical heart valves, prosthetic joints, endotracheal tubes, and contact lens with biofilm-related infection[89]. Once biofilms are formed on medical devices, eradicating microbes becomes extremely difficult and can be costly due to the lengthy hospital stays, surgery, and long-term antibacterial treatments that are often

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required[90]. The development of these biomaterials has saved millions of lives, but at the same time, the resulting biofilm infections are endangering people's health. The above-mentioned associated biofilm infections caused by biomaterials share the same clinical features[91,92]:

(1) Persistent infection (more than 7 days). For example, central venous catheter-associated biofilms and almost all indwelling central venous catheters are colonized by microorganisms that can produce biofilms. Common bacteria isolated from catheter biofilms are coagulase-negative *Staphylococcus*[93]. Colonization and biofilm formation on catheter surfaces by these microorganisms can occur within 24 hours of insertion and are pervasive and persistent[94]. In one study, short-term (<10 days) catheters had greater biofilm formation on the external surface; long-term catheters (>30 days) had more biofilm formation on the catheter inner lumen[31].

(2) Inflammation. An example is biofilm infection associated with mechanical heart valves. Infectious endocarditis (IE) is a disease with substantial morbidity and mortality today due to the increasing use of implantable devices, such as prosthetic heart valves[95]. Microbes initially adhere to the prosthetic valve surface via fibronectin and polysaccharides and grow in a platelet-fibrin matrix to form an intact biofilm[96]. Because biofilms formed on artificial surfaces promote inflammatory responses and hypercoagulability, the presence of foreign tissue needs to be maintained through inflammatory and thrombotic processes[97]. In addition, prosthetic joint infection (PJI), a serious complication after joint replacement, causes inflammation[98]. According to the symptoms that appear after implantation, PJIs can be divided into three categories, early infection, delayed infection, and late infection[99]. Both early and delayed infections are often the result of surgical contamination and are considered the most common cause of biomaterial-related biofilm infections. These infections are often associated with local and systemic symptoms, in addition to triggering an inflammatory response that is accompanied by increases in laboratory markers of inflammation, such as C-reactive protein, erythrocyte sedimentation rate, and white blood cell count levels[100-102]. Therefore, in addition to routine methods for detecting biofilm-associated infections, blood and tissue cultures can be used to detect PJI infections at an early stage[101,103].

(3) Failure of antibiotic treatment and recurrence of infection. It is the formation of biofilms on the surface of biomaterials that leads to the development of drug resistance in the treatment of related infections[104]. The formation of a biofilm matrix can maintain temporary dormancy of cells, which may lead to repeated infections[105]. For example, the treatment of PJI requires complex therapeutic strategies, and it is the long-term infection problems that lead to multiple surgical revisions and long-term antimicrobial therapy[98].

5. Treatment of biofilms on the surface of biomaterials

The biofilm that causes infection is progressive. Current treatments for implant-associated infections involve administration of high doses of antibiotics and surgical replacement if symptoms persist[106]. The increase in antibiotic resistance has introduced problems in the treatment of patients using medical devices[107]. Therefore, several new biomaterials have been developed to treat clinical medical device-related biofilm infection, from the addition of antimicrobials to surface modifications of the material itself, which have been used as alternative strategies for the prevention and treatment of biofilm infections (Table 1).

5.1. Antibacterial coatings

Antibacterial materials are widely used in the biomedical field, especially in the treatment of biofilm. The main pathogens of medical device-related infections are methicillin-resistant *S. aureus* (MRSA) and *E. coli*[108]. The common strategy for the treatment of this type of bacterial infection is antibiotic treatment. However, traditional antibiotic therapy will not only cause a variety of side effects but is also more likely to cause the emergence of multi-drug-resistant bacteria[109]. As a result, new methods different from antibiotic therapy have emerged to solve the problem of infection caused by medical devices, such as the use of antibacterial coatings on the surface of implants[110]. Implant coatings against biofilm-based infections can be divided into two categories[110]: (1) Active coatings, which can fight infection by releasing pre-incorporated antimicrobial agents. Coatings are prepared

by soaking a porous material or coating a surface with the desired antimicrobial compound, frequently using carriers such as hydroxyapatite (HA) and polycaprolactone (PCL)[111]. (2) Passive coatings, which prevent bacterial attachment through the use of hydrophilic materials[101].

5.2. Active coatings

The most common active coatings are antibiotic or silver compound coatings, which are applied to implant surfaces as antimicrobial or anti-biofilm agents for the prevention of medical device-related infections[112-114]. For example, the antibiotic hydroxyapatite coating, which is widely used in the medical field, can be used with antibiotic solutions and modified by surface-adsorbed antibiotics, resulting in anti-biofilm effects[40]. Polymers usually have no intrinsic antibacterial properties, and polymer antibacterial materials can be prepared by using antibacterial additives to modify a polymer matrix in different ways[115]. A study incorporated antibacterial Ag nanoparticles into a hydrophobic polymer in situ in a polycaprolactone matrix and achieved long-term release of active silver. The Ag-containing composite material had strong antibacterial and anti-biofilm properties and therefore could be used in medical antibacterial equipment[116]. Some zwitterionic polymers, such as hydrogels and sulfobetaine polymers, can delay or even prevent microorganisms from attaching to the surface because the tight hydration layer around the ion surface prevents the adsorption of non-specific proteins[117,118]. However, zwitterionic polymers cannot inactivate bacterial cells and therefore can be used as carriers to achieve synergistic antibacterial effects with fungicides[118]. Impregnating ultrasmall silver nanoparticles (AgNPs) into biocompatible thermosensitive hydrogels can not only control the delivery of AgNPs at an appropriate concentration but also confer long-term storage stability and highly potent antibacterial activity[119]. AgNP hydrogels show excellent biofilm dispersion properties and are safe and non-toxic to mammalian cells in a wound environment, which provides a promising new strategy for hydrogels as an effective antibacterial platform[119]. In addition to traditional antibacterial agents, the release of other molecules (such as enzymes and antimicrobial peptides) can be used to treat infections. One study found that the acylase enzyme coating on the surface of a silicone catheter significantly inhibited the formation of a biofilm on its surface[120]. The negatively charged acylase enzymes were immobilized on the silicone catheter through alternate deposition, and the acylase enzymes on the surface reduced the biofilm by inducing the degradation of QS signals[120]. Antimicrobial peptides have been shown to effectively kill bacterial cells without toxic effects[121,122]. For example, a thin polymer multilayer film composed of chitosan and hyaluronic acid covered a catheter, and then a β-peptide coating was applied to the surface of the membrane. The film containing the peptide inhibited bacterial biofilm formation by releasing the β-peptide[123]. These β-peptide-containing films offer a new and promising localized delivery method for preventing orthopedic implant infections.

5.3. Passive coatings

Passive coatings are used to prevent bacteria from attaching. The development of coatings that prevent bacterial attachment is a promising approach to prevent biofilm formation using non-cytotoxic mechanisms[110]. The attachment of bacteria to the surface of biomaterials mainly consists of two key stages, the initial reversible attachment through physicochemical interactions, and the irreversible attachment mediated by bacterial adhesion proteins[8]. Therefore, blocking the adhesion of bacteria on the surface of biomaterials provides a good theoretical target for the development of new therapeutic methods. Strategies to reduce bacterial adhesion to biomaterial surfaces include the use of hydrophilic polymers, such as hyaluronic acid, hydrogel coatings, and heparin coatings[8]. Medical-related devices made of hydrophobic materials are more prone to biofilm infection due to the commonly observed preferable attachment of bacteria on hydrophobic surfaces[111,124]. In contrast, hydrophilic surfaces are covered by water molecules and prevent the attachment of cells and bacteria, which play a role as a physical and energy barrier[125]. Previous studies found that hyaluronic acid has notable antiadhesive properties and shows moderate activity against bacterial biofilms[126]. A recent study combined quaternized chitosan with bactericidal properties, acylase

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with anti-quorum sensing properties, and hyaluronic acid with anti-adhesion properties. This multifunctional coating inhibited most bacteria from initially attaching, killed the attached bacteria, and interfered with their quorum-sensing systems involved in biofilm formation[127]. In addition, chitosan can be used to prepare thermosensitive hydrogels for the treatment of bone defects. These hydrogels have attracted wide attention because they can successfully regenerate bone tissue without surgical intervention, thus reducing the invasiveness of treatment[128,129].

Due to the enormous burden of biomaterial-related infections (those associated with implants and catheters), the effectiveness of traditional antibiotic therapy is diminished, and bacterial resistance is increasing. At the same time, the transfer of antibacterial compounds onto the surface of biomaterials is also limited, and therefore, the use of antibacterial coatings has become a new approach to prevent infection. The polymer-based composite materials composed of antibacterial coatings and polymer materials are easy to manufacture and have high biocompatibility and are therefore widely used[111]. However, there are still challenges, such as the lack of long-term performance, and many antibacterial materials have only been studied in vitro without entering clinical research[110]. Therefore, follow-up studies should address these important challenges and enable these materials to make real progress in the biomedical field.

5.4. Modification of the surface of medical implants

The physicochemical properties of the material surface of implants affect the adhesion behavior of cells on the material surface and the subsequent biofilm formation process[130]. These characteristics include electrostatic interactions and van der Waals forces between the material surface and cells, surface energy and hydrophobicity of the material, morphological characteristics of the material surface, such as roughness, surface composition, and topography, and functional chemical group modifications of the material surface[131]. The surface modification of biomaterials is a potential strategy to prevent bacteria from attaching and forming biofilms on the surface of materials[132]. According to the modification process, the surface modification of polymers can be divided into physical modifications and chemical modifications.

5.5. Physical modifications

Surface-treated polymers have been widely used with biomedical materials, including optimized modified hydrogels, nanomaterials, and phage materials[133,134]. The surface modification of polymers by physics is relatively simple, economical, and effective. The surface of the polymer is modified by physical methods to change its wettability, but the modification does not change the inherent chemical properties of the polymer[135]. Common modification methods include changing the surface roughness of materials and preparing hydrophobic or superhydrophobic surfaces, which affect cell adhesion[132,133,136].

The superhydrophobic surfaces have antibacterial adhesion properties as they naturally have nano- or microscale structures in addition to the repulsion of water, which limits the access of bacteria to the superhydrophobic surface[137,138]. A femtosecond laser-induced surface structure is a large-scale nano- and microstructure formation technology, which can effectively modify the optical, electrical, mechanical, and tribological properties of materials[139]. A surface treated by a femtosecond laser shows different surface structure on scales of the order of nano- and micrometers. Jalil et al. produced superhydrophobic surfaces on gold with femtosecond laser pulses, in which the original hydrophilic Au was transformed into a superhydrophobic surface and reduced the adhesion of *Escherichia coli*. The physical inhibition of bacterial colonies and biofilm formation by this technique is a crucial step in reducing antibiotic resistant infections[140]. In addition, studies have designed flexible superhydrophobic surfaces decorated with copper hydroxide nanowires that lead to dual-scale roughness and superhydrophobicity. These nanowires are grown separately and transferred onto polydimethylsiloxane (PDMS) surfaces by mechanical peeling, and thus, non-planar 3D surfaces can be fabricated[141]. These surfaces have shown blood repellence, antibacterial activity, and hemocompatibility, indicating that they are suitable for medical and health care applications.

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A change of roughness will change the surface energy and hydrophobicity of a material[142], which will affect cell adhesion. It is generally believed that a lower surface roughness reduces bacterial adhesion to commonly used implant materials and prevents the formation of biofilms on the biomaterial surfaces[143-145]. For example, etching technology is used to design micro- or nano-scale surface structures to increase the surface roughness of biomaterials[146,147]. Studies have used a simple etching method to produce multi-scale roughness on the surface of Al alloys. This method not only rapidly etched large-size substrates but also showed a high bactericidal effect on rod-shaped and coccoid-shaped bacterial cells[148]. The latest advances in surface technology and materials have led to the development of surface patterns, some of which can affect bacterial colonization and biofilm formation without added antimicrobial agents, such as sharklet micropatterns that have been shown to control the bioadhesion of pathogenic bacteria[149,150]. Studies have incorporated nano and shark micropatterns into the surface of central venous catheters. This surface modification reduced microbial colonization and biofilm formation and can be applied clinically to reduce catheter-related infection[151]. In addition, some studies have combined chemical and topographic properties to obtain a sharkskin-mimicking graphene oxide (GO)-modified chitosan membrane with antibacterial properties and cytocompatibility. GO-coated sharkskin mimicking membranes can significantly reduce the formation of bacterial biofilms while promoting cytocompatibility and therefore can be used as a convenient biomaterial in many biomedical applications, such as those requiring a biodegradable, highly biocompatible, and antibacterial implantable medical equipment coating[152].

Unlike other surface treatment methods, physical modifications are performed as part of the surface manufacturing itself. In addition, polymers modified by physical methods are strong and do not age or deteriorate, as the resulting surface roughness rarely affects the surface chemistry of the polymers[153,154].

5.6. Chemical modification

The initial adhesion of microorganisms and the formation of biofilms can be regulated by changing the chemical properties of the material surface[155]. The main methods of surface chemical modifications include covalent modification of materials, non-covalent modifications, controlled release of small molecules, and degradation of the polymerized surface [156,157].

A commonly used chemical modification technique is the self-assembled monolayer (SAM), which is a thermodynamically stable and regularly arranged monolayer formed spontaneously by a physicochemical interaction between molecules and substrate materials[158,159]. SAM technology can effectively control the types of microbial-oriented functional groups and the concentration of ligands loaded on the material surface, and then purposefully change the surface energy and surface charge density[160]. For example, chitosan inhibits bacterial growth through the interaction between positively charged amino groups and negatively charged outer membranes[161]. It was shown that chitosan hydrogel films were covalently attached to thiol-modified substrates via a thiol one-click reaction. After that, heparin-mimicking polymer chains were grafted onto the hydrogel thin film layer via surface-initiated atom transfer radical polymerization, and covalent adhesion of the hydrogel was realized[162]. This self-defense double-layer hydrogel coating can switch from a cell adhesion surface to an antibacterial adhesion surface and provides a new approach for the antibacterial protection of biomedical equipment.

There are also several functional chemical modifications designed against the formation mechanism of bacterial biofilm, such as quorum sensing inhibitors (QSIs) to inhibit the key regulatory mechanism of bacterial biofilm formation[163,164]. Surface-immobilized QSIs block bacterial communication and affect the formation of biofilms on the surface of biomaterials[165,166]. It has been found that furanone is a substance with a similar structure to N-acyl homoserine lactone (AHL), a signal molecule of gram-negative bacterial QS, which competes with AHLs for receptors, thus interfering with QS to inhibit biofilms[167]. The intergeneric quorum sensing signal molecule autoinducer-2 (AI-2) of *Fusobacterium nucleatum* is very important for the development of pathogenicity and biofilm formation[168]. A new brominated furanone was recently found that

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inhibited biofilm formation through AI-2, and thus inhibits biofilms caused by periodontopathogens, including *F. nucleatum*, *Porphyromonasgingivalis*, and *Tannerella forsythia*[169].

The physicochemical interaction between the material surface and cell provides a design idea for the preparation of materials that regulate microbial film formation. Through a series of complex physical, chemical, or biological mechanisms, the surface of the material has an impact on bacterial adhesion and the formation of biofilms. There are various methods for influencing these mechanisms, including the addition of an antibacterial coating on the material surface and the surface modification of biological materials. The surface properties of materials are very important to the research and development of inhibitory biofilm-forming materials, and the method of regulating biofilms through material modification provides a more comprehensive scientific reference for related research.

Table 1. Different approaches to treat infectious biofilms on biomaterials.

Types		Mechanisms of action	Materials	References
Antibacterial coatings	Active coatings	Fight infection by releasing antimicrobial agents	Antibiotic or silver compound coatings, enzymes, antimicrobial peptides	[40,116,119], [120], [123]
	Passive coatings	Prevent bacteria from attaching using hydrophilic materials	Hyaluronic acid, hydrogel coatings	[127], [128,129]
Modification of the surface of medical implants	Physical modification	Affect cell adhesion by changing the surface roughness and generating hydrophobic surfaces	Femtosecond laser-induced surfaces, superhydrophobic non-planar 3D surfaces, nano and shark micropattern surfaces	[140], [141], [148-152]
	Chemical modification	Regulate the formation of biofilms by changing the chemical properties of the material surface	Chitosan hydrogel films, surface-immobilized brominated furanones	[162], [169]

6. Conclusion

Biofilms are the most common growth state of microorganisms. For bacteria, the formation of a biofilm is a simple, multi-stage process. Bacteria must go through three steps: initial adhesion, maturation, and dispersion. Once the biofilm is formed, a biofilm community with an ordered structure and functional differentiation can provide protection for bacteria in adverse environments, thus causing infection. Particularly in clinical practice, bacterial colonization forms biofilms on various medical instruments, causing serious nosocomial infections. At present, conventional antibiotic therapy has been unable to effectively eradicate biofilms. In contrast, with the use of antibiotics, bacteria have evolved multiple resistance mechanisms, increasing the difficulty of treating biomaterial-associated biofilm infections. Therefore, it is of great practical significance to purposefully regulate the adhesion of microorganisms on material surfaces and the formation of biofilms. The use of antibacterial coatings or surface modifications of medical materials not only prevents bacterial adhesion but also directly fights biofilm infections by releasing antibacterial drugs. In addition, appropriate antibacterial therapy can save money and shorten the duration of treatment.

With the increasing number of biomaterial studies of biofilm regulation, most of which are in vitro studies, more mechanistic studies are needed in the future to overcome these shortcomings and enable these therapies to enter clinical trials. To better solve the problem of biofilm resistance, it is necessary to conduct in-depth research and optimize preventive measures, such as developing new antibacterial coatings (biodegradable, biocompatible) and optimizing medical materials. In conclusion, the research in the field of biofilms involves the intersection and integration of multidisciplinary methods such as microbiology, molecular biology, surface chemistry, and material

science, which is not only of academic significance but also of extremely important application value for clinical treatment, which still needs further research.

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