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Review

Bacterial Communities and Their Role in Bacterial Infections

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Abstract

Since infections associated with microbial communities threaten human health, research is increasingly focusing on the development of biofilms and strategies to combat them. Bacterial communities may include bacteria of one or several species. Therefore, examining all the microbes and identifying individual community bacteria responsible for the infectious process is important. Rapid and accurate detection of bacterial pathogens is paramount in healthcare, food safety, and environmental monitoring. Here, we analyze biofilm composition and describe the main groups of pathogens whose presence in a microbial community leads to infection (*Staphylococcus aureus*, *Enterococcus spp*., *Cutibacterium spp*., bacteria of the HACEK, etc.). Particular attention is paid to bacterial communities that can lead to the development of device-associated infections, damage, and disruption of the normal functioning of medical devices, such as cardiovascular implants, biliary stents, neurological, orthopedic, urological and penile implants, etc. Special consideration is given to tissue-located bacterial biofilms in the oral cavity, lungs and lower respiratory tract, upper respiratory tract, middle ear, cardiovascular system, skeletal system, wound surface, and urogenital system. We also describe methods used to analyze the bacterial composition in biofilms, such as microbiologically testing, staining, microcolony formation, cellular and extracellular biofilm components, and other methods. Finally, we present ways to reduce the incidence of biofilm-caused infections.

Keywords: microbial community; biofilms; composition; biofilm-associated infection; therapy

1. Introduction

Bacteria can be planktonic (free-floating) or attached to a surface. Bacterial microcolonies increase the bacterial population size and form biofilms—complex communities of bacteria enclosed in an extracellular polymer matrix [1].

The attachment of bacteria to a surface leads to a rapid change in the expression of genes coding for the production of the extracellular polymer matrix (EPM) or the secretion and maturation of mucus. This transformation begins almost immediately after a colonization event and occurs on both biotic and abiotic surfaces. It results in a defense barrier to adverse conditions (antibiotics, external agents, etc.) [2]. Although the first observation of surface-associated bacteria was made in 1684, the term "biofilm" began to be used in 1978; nonetheless, it was not until 1993 that the American Society for Microbiology recognized the significance of biofilms [3].

One key contributing factor to the emergence of chronic hospital-acquired infections and the contamination of medical devices such as pacemakers, electric dialyzers, joint prostheses, and intravenous and urinary catheters is the formation of bacterial biofilms. Furthermore, biofilms can develop on drilling rigs, food processing equipment, paper manufacturing equipment, etc. [1–3].

Biofilm-caused infections may become a chronic problem, and research is underway to find alternative prevention and treatment strategies for such infections. However, a unified approach to combat biofilms has yet to be found.

Biofilm research is one of the most challenging areas in current medicine because classical tests cannot predict antibiotic susceptibility in biofilm bacteria. Here, we analyze biofilm composition and describe the main groups of pathogens whose presence in a microbial community leads to infection.

Despite the close attention of scientists to the study of biofilm formation, the problem of assessing the role of bacterial biofilms in various diseases, including those associated with multiple implants, needs to be sufficiently covered in the literature. Therefore, the work aims to address this gap by providing detailed information on biofilm formation, detection, and potential therapeutic strategies. Particular attention is paid to bacterial communities that can lead to the development of device-associated infections, damage, and disruption of the normal functioning of medical devices, such as cardiovascular implants, biliary stents, neurological, orthopedic, urological and penile implants, etc. Special consideration is given to tissue-located bacterial biofilms in the oral cavity, lungs and lower respiratory tract, upper respiratory tract, middle ear, cardiovascular system, skeletal system, wound surface, and urogenital system. We also describe methods used to determine bacteria in biofilms. Finally, we present ways to reduce the incidence of biofilm-caused infections.

2. Bacterial Biofilms

2.1 Composition and Architecture of Bacterial Biofilms

Bacterial biofilms are complex structures formed on both biotic and abiotic surfaces. The cells forming biofilms exist in various physiological and morphological states and are immersed in an EPM [4–6]. In most biofilms studied, the cellular biomass constitutes 10% of the dry matter, whereas the EPM accounts for more than 90% [7]. The 3D architecture of biofilms is formed by the EPM owing to the presence of extracellular organic compounds, including polysaccharides, proteins, lipids, nucleic acids, and other macromolecules [8,9]. The EPM also ensures the mechanical stability of biofilms, protects attached bacterial cells from environmental influences, and limits the flow of antibiotics and disinfectants [10]. Although the composition and structure of biofilms vary depending on their formation conditions and the type of bacteria, polysaccharides and proteins form the common structural components of the EPM [11]. Extracellular polysaccharides maintain the integrity of biofilms, ensuring the adhesion of bacteria to each other, firm attachment to surfaces, and the formation of three-dimensional architecture [12–14]. The matrix proteins are necessary for biofilm stability, and extracellular enzymes participate in the dispersion of bacterial communities, releasing persistent cells, which colonize new surfaces [15]. The EPM lipid content is much lower than that of polysaccharides and proteins; however, lipid molecules can bind to proteins to form lipoproteins, which are important for both the maturation of biofilms and their further full functioning [6,16]. Extracellular nucleic acids are also permanent components of the biofilm matrix and can be involved in self-organization and stabilization of bacterial communities through interaction with other macromolecules (exopolysaccharides, lipoproteins, amyloidogenic peptides, etc.) [17,18].

Bacterial cells within biofilms communicate through signaling molecules, whose operation is regulated and coordinated by complex quorum sensing (QS). This interaction leads to changes in the expression of genes and in the synthesis/induction of proteins necessary to perform specific functions within biofilms [19]. In microbial communities, horizontal gene transfer is also active, allowing favorable survival traits to be distributed throughout the cell population [4,20]. Through horizontal gene transfer, biofilm bacteria can acquire genetic antibiotic resistance, making biofilm-associated infections much more difficult to treat with standard antimicrobial agents [21–23].

Pathogens entering the body can form biofilms on organs and tissues, and the intensity of biofilm development depends on the immune system and the location of the infection. Biofilm bacteria can remain “invisible” to the immune system owing to the matrix coating of their antigens, which results in chronic subclinical infections [24]. These are well protected from adverse environments and can rearrange their metabolism to survive under limited nutrient supply and anoxia [25]. As biofilms mature and disperse, the pathogens may cause acute infections or spread to other body parts. Detection of foreign antigens in the matrix or on the surface of biofilm cells in its upper layers by the body’s immune system leads to prolonged inflammation accompanied by tissue damage and necrosis. At the same time, the biofilm community, as a source of the infectious process, remains almost unchanged, retaining its ability to spread throughout the body with the help of persister cells [26]. In general, biofilm-associated infections develop slowly and are important for the pathogenesis of chronic diseases in which the immune system fails to cope with its functions, and there is little or no response to antimicrobial therapy.

2.2 Biofilm Development

The formation of bacterial biofilms includes four main stages (Fig. 1, Ref. [27]): (1) attachment to the sub-

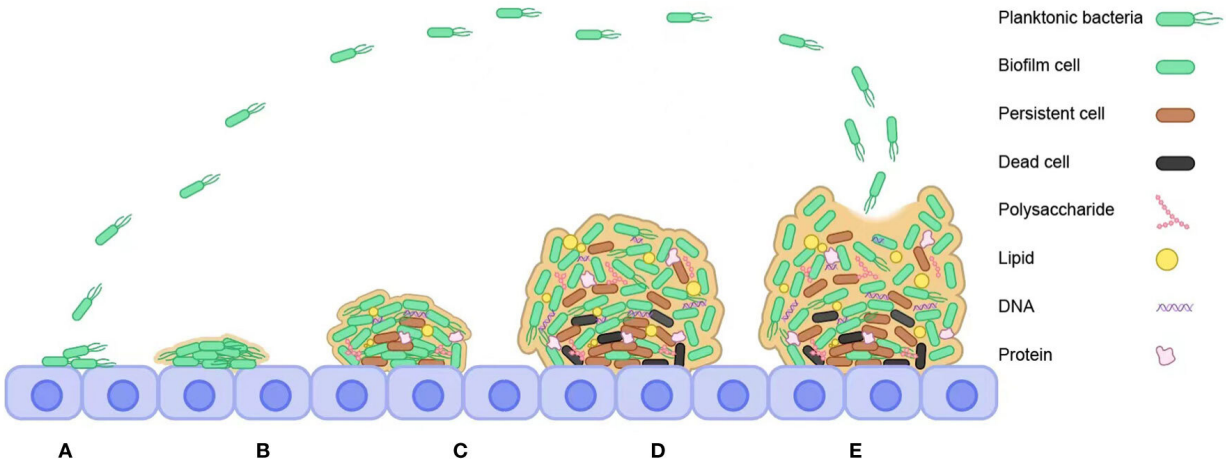

Fig. 1. Biofilm development. (A) Reversible attachment. (B) Irreversible attachment. (C) Elaboration and production of extracellular polysaccharides. (D) Maturation. (E) Dispersal. Reprinted under terms of the CC-BY license [27].

strate (reversible and irreversible); (2) formation of microcolonies; (3) biofilm maturation; (4) dispersion of biofilms [28–31].

The first stage in forming biofilm communities is the attachment of bacteria to a surface. Bacteria reach the surface of an object through convection, Brownian motion, or sedimentation. An important part in the attachment is played by chemotaxis, which helps free-floating cells move in liquids to chemical attractants or sources of nutritional compounds. The interaction of bacteria with an attractant-coated surface promotes the surface colonization and growth of planktonic bacteria [27,32]. Planktonic cells attach to surfaces through nonspecific physical forces such as van der Waals forces, electrostatic forces, and hydrophobic interactions [33]. The fixation of cells on the substrate is called reversible adhesion because, owing to their motility and the influence of external factors, the cells may leave the populated surface. The physicochemical properties of the substrate, including its roughness, hydrophobicity, and surface charge, can influence the efficacy of bacterial colonization and biofilm formation [31].

The next phase of attachment is irreversible adhesion, which occurs with the formation of hydrogen, covalent, and ionic bonds and dipole–dipole interactions between bacterial cells and the substrate surface [27,32]. The bacterial adhesion factors are flagella and fimbriae, which facilitate physical contact of planktonic cells with the substrate [33,34]. Flagella and fimbriae promote strong adhesion of bacteria to the surface and one another. As an intercellular signaling mechanism, the QS system also strongly facilitates biofilm development. Attached cells use QS to synthesize and excrete chemical attractants, which attract secondary colonizers and promote the growth of biofilms on the substrate [35]. This mechanism is manifested in both Gram-positive and Gram-negative bacteria. In particular, Gram-positive bacteria use oligopeptides as chemical stimuli for biofilm formation, and Gram-negative bacteria attract new bacteria using acyl homoserine lactones [1].

After irreversible attachment, bacteria actively divide and form small aggregates called microcolonies [6,36]. At this stage, the genes responsible for forming biofilms are activated and expressed, promoting the production of many EPM to strengthen adhesion further and protect microcolony clusters from external influences [37,38]. EPM components can form ion bridges, promoting bacteria condensation within biofilms. The interaction of EPM macromolecules determines the internal structure and framework of microcolonies and the mutual recognition of cells. It also facilitates signal transduction, obtaining nutrients, exchanging genetic information, and other processes [39].

The third stage of biofilm development is maturation. With the continuous proliferation of microcolonies and increased secretion of EPM, cell clusters eventually merge and form a three-dimensional biofilm structure in which nutrient transport and cellular activity are balanced. As EPM components accumulate, the expression of several genes changes in the cells of microcolonies, the products of which are used for the synthesis and excretion of new "building material" and the growth of the bacterial community [6,27,32]. When the biofilm matrix is fully developed, water channels are formed that act through a mechanism similar to the body's circulatory system, delivering available nutrients to the cellular community while rejecting metabolic waste products [31].

The final stage of the life cycle of biofilms is dispersion, an active process often triggered by the deterioration of living conditions. Biofilm dispersion may be influenced by temperature, pH, oxygen concentration, nutrient content, and other factors [40]. In particular, low-oxygen environments may promote biofilm dispersion by accelerating the degradation rate for the second messenger c-di-GMP, which is implicated in EPM production and the development of biofilm surface structures [41]. This process is critical for the proliferation and self-renewal of the cellular community, allowing bacterial cells to actively exit mature biofilms and spread to create new biofilms in distant locations [42]. The mechanism of spread of persister cells involves three standard steps: Separation of bacteria from biofilms or microcolonies remaining during dispersion, transfer of bacteria to other substrates, and colonization of new substrates by cells [43]. Thus, dispersion is not only the last stage of the development of one biofilm but also the beginning of the life cycle of another biofilm.

2.3 Multispecies Biofilms

Biofilms can be monospecies or multispecies [27,44]. The development of multispecies biofilms is a complex but strictly regulated process determined by the interaction between resident species [45]. Such interactions lead to various changes in the structure and the functioning of biofilms. Thus, compared to single-species biofilms, multispecies communities are characterized by spatial organization changes and increased biomass, cell numbers, metabolic activity, and resistance to antimicrobials [46]. These features contribute to increased pathogenicity of the species that comprise multispecies biofilms [47], resulting in traditional therapeutic approaches losing effectiveness [10].

There are four main types of interspecies interactions in multispecies biofilms. (1) Physicochemical intercellular interactions, which are mediated, among other things, by ligand–receptor binding, facilitate the exchange of genetic material between individual species. Such contacts promote synergistic relationships between species that enhance the stability and resistance of biofilms, making them less susceptible to antibiofilm agents [48]. (2) Genetic intercellular interactions are carried out through horizontal gene transfer by using mobile genetic elements [49]. (3) Metabolic cell–cell interactions are achieved through matrix components secreted by collaborating microorganisms

or through cross-feeding, where one species uses the by-products of another as a source of nutrients [50]. (4) Signaling intercellular interactions arise owing to the production of autoinducers, which are crucial for regulating the physiological aspects of biofilm activity and modulating interactions between species [51]. Owing to complex molecular processes, these between-species interactions can lead to so-called social communication, which can be competitive, cooperative, or neutral [52,53].

Multispecies biofilms are widespread in nature and have been found in the oral cavity, on implantable medical devices, and in the intestines of mammals [53,54]. Oral bacteria form multispecies biofilms through interspecies synergistic communication, with each species playing a specific part. For example, the bacterium *Enterococcus faecalis* is required to protect and promote the growth of the bacterium *Porphyromonas gingivalis* as part of a two-species biofilm, which leads to a noticeable increase in the thickness of the microbial community [55]. The ability to co-aggregate largely depends on the properties of the cell surface, with some species of oral bacteria able to recognize streptococcal cell wall polysaccharides. Such recognition subsequently gives rise to coaggregates, thereby playing a key part in the eventual development of biofilms [56].

When forming multispecies biofilms, bacteria can produce various compounds that facilitate the formation and functioning of communities but which are not found in free-living or monospecies forms [53]. During the development of rhinosinusitis, the bacterium *Haemophilus influenzae* intensely expresses type IV pili only when it coexists with the bacterium *Streptococcus pneumoniae* in a two-species biofilm. These pili participate in the cooperation of the two species, improve their adhesion to epithelial cells of the host organism, and contribute to forming a stable multispecies community [57]. The coexistence of *Pseudomonas fluorescens* and *Lactococcus lactis* in the same biofilm largely increases their joint adhesion. *P. fluorescens* bacteria produce polysaccharides and other macromolecules into the matrix, increasing the adhesive ability of pseudomonads and lactobacilli. Conversely, some metabolites excreted by *L. lactis* bacteria can serve as nutrients for *P. fluorescens* [50]. Similarly, the bacterium *Ralstonia insidiosa* created a favorable nutrient-rich environment within a multispecies biofilm, thereby benefiting *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica* as other consortium inhabitants [52].

Multispecies biofilms cause various diseases, such as cystic fibrosis, diabetic foot ulcers, chronic wounds, and otitis media [58,59]. Infections associated with multispecies biofilms tend to have more severe consequences than diseases caused by single-species biofilms [53,60]. Thus, deterioration of the condition in patients with cystic fibrosis was observed when the lung tissue was covered with two-species biofilms consisting of *Pseudomonas aeruginosa* and *Staphylococcus aureus* [61]. Often, within the same multispecies biofilm, one species can enhance the virulence of others. For example, the presence of the fungus *Candida albicans* in combination with *S. aureus* increases the infectivity of staphylococci. Peptidoglycan, present in the cell wall of Gram-positive bacteria, serves as a signaling molecule for *P. aeruginosa*, triggering the production of additional virulence factors that harm the body and regulate the composition of the microbial community. In addition, coinfection with *E. faecalis* aggravates pyelonephritis initially caused by *P. aeruginosa* [62].

3. Biofilm-Associated Infection

According to modern estimates, 65 to 80% of bacterial infections are due to biofilm communities [63,64]. Biofilms can colonize a variety of medical devices, causing device-associated infections, or they can directly colonize organs and tissues, displacing normal commensal microbiota [27,64,65]. This section will discuss both types of infections and will list the main groups of biofilm-forming pathogens (Table 1, Ref. [27,66–133]).

3.1 Surface-Located Biofilms

The use of implants and other medical devices improves the quality of life of patients and strongly increases life expectancy. However, the surface of medical devices can often be colonized by biofilm-forming bacteria (Fig. 2). Such bacterial communities can damage devices, disrupt their optimal functioning, and lead to infections, including generalized ones with fatal outcomes. In this regard, device-associated infections seriously threaten modern healthcare with significant clinical and economic consequences [27,66].

3.1.1 Cardiovascular Implants

Implantable cardiac devices are vital for patients with cardiovascular disease. The annual use of pacemakers, implantable cardioverter defibrillators (ICDs), cardiovascular implantable electronic devices (CIEDs), and prosthetic heart valves is constantly increasing [66,67,134,135]. As the service life of these devices increases, many of them require replacement owing to malfunction or bacterial contamination. According to recent estimates, the proportion of contaminated cardiac devices currently reaches 2.5% of the total, whereas the infection rate increases 10 times after the device is replaced or upgraded. Infections occurring during the first year after surgery are predominantly associated with infection during surgery, and 25% of them are diagnosed within the first month after implantation [66,67]. The isolates obtained from the surface of contaminated devices are dominated by coagulase-negative *Staphylococcus* spp. (42–77%) as the causative agent of most CIED infections and by *S. aureus* (10–30%), including a methicillin-resistant strain [2,67,69,136]. In fewer clinical cases, researchers have identified *Streptococcus* spp. (3–10%); *Enterococcus* spp. (0.4–10%); *Cutibacterium* spp. (0.8–8%);

Table 1. Main groups of microorganisms that cause biofilm-associated infections.

Microorganism	Biofilm-associated infection			
	Device-associated infection	References	Tissue-associated infection	References
		Gram-negative bacteria		
Acinetobacter spp.	Respiratory tract infection and pneumonia	[90]	Wound infection	[123]
Aggregatibacter spp.	Infective endocarditis	[67]	Diabetes mellitus and rheumatoid arthritis, osteomyelitis	[95,96,114]
Burkholderia spp.	–		Cystic fibrosis	[101]
Campylobacter spp.	Dental caries and root canal infection	[83,85]	–	
Cardiobacterium spp.	Infective endocarditis	[67]	Osteomyelitis	[114]
Eikenella spp.	Dental caries and root canal infection, infective endocarditis	[67,83]	Osteomyelitis	[114]
Enterobacter spp.	–		Osteomyelitis	[117]
Escherichia spp.	Bacterial prostatitis, mastitis, respiratory tract infection and pneumonia, urinary tract infection	[66]	Kidney stones, urinary tract infection, wound infection	[93,127–130]
Fusobacterium spp.	Dental caries and root canal infection	[83,85]	Periodontitis, osteomyelitis	[94,118]
Haemophilus spp.	Infective endocarditis	[67]	Bacterial prostatitis, bronchiectasis and persistent bacterial bronchitis, chronic otitis media, osteomyelitis	[103,108,114,126]
Kingella spp.	Infective endocarditis	[67]	Osteomyelitis	[114]
Klebsiella spp.	Bloodstream infections, prosthetic joint infections, respiratory tract infection and pneumonia	[66,75,79,89]	Chronic otitis media, kidney stones, osteomyelitis	[106,108,117,127]
Moraxella spp.	–		Bronchiectasis and persistent bacterial bronchitis, chronic otitis media	[103,108]
Neisseria spp.	Respiratory tract infection and pneumonia	[66]	–	
Porphyromonas spp.	Dental caries and root canal infection	[83–85]	Diabetes mellitus and rheumatoid arthritis, osteomyelitis	[95,96,115]
Prevotella spp.	Dental caries and root canal infection, respiratory tract infection and pneumonia	[66,83,85]	Bacterial vaginitis, diabetes mellitus and rheumatoid arthritis, periodontitis	[94–96]
Proteus spp.	Bacterial prostatitis, urinary tract infection	[66]	Chronic otitis media, kidney stones, urinary tract infection, wound infection	[106,108,124,127,131]
Pseudomonas spp.	Bacterial keratitis, bacterial prostatitis, bloodstream infections, infections from neurosurgical implants, otitis media, prosthetic joint infections, respiratory tract infection and pneumonia, urinary tract infection	[66,75,78,79,86,91,92]	Bacterial prostatitis, bronchiectasis and persistent bacterial bronchitis, chronic otitis media, cystic fibrosis, kidney stones, osteomyelitis, wound infection	[97,98,103,106–109,114,115,117,124–127]
Serratia spp.	–		Osteomyelitis, wound infection	[93,115]
Sneathia spp.	–		Bacterial prostatitis	[126]
Tannerella spp.	–		Diabetes mellitus and rheumatoid arthritis, periodontitis	[94–96]
Treponema spp.	Dental caries and root canal infection	[83,85]	Diabetes mellitus and rheumatoid arthritis, periodontitis	[94–96]
		Gram-positive bacteria		
Actinomyces spp.	Dental caries and root canal infection	[83]	Osteomyelitis	[93,118]
Allobaculum spp.	–		Bacterial prostatitis	[126]
Anaerococcus spp.	–		Osteomyelitis	[115]

Table 1. Continued.

Microorganism	Biofilm-associated infection			
	Device-associated infection	References	Tissue-associated infection	References
Bacillus spp.	Mastitis	[66]	–	
Corynebacterium spp.	Bacterial keratitis, mastitis	[66]	Osteomyelitis	[115]
Cryptobacterium spp.	–		Diabetes mellitus and rheumatoid arthritis	[95]
Cutibacterium spp.	Infective endocarditis, infections from neurosurgical implants, mastitis	[67,78,82]	Osteomyelitis	[114]
Enterococcus spp.	Bacterial prostatitis, bloodstream infections, infective endocarditis, gallbladder and biliary tract infections, prosthetic joint infections, urinary tract infection	[66,67,70,71,75,79]	Bacterial prostatitis, infective endocarditis, kidney stones, wound infection	[111,124,126,127]
Fannyhessea spp.	–		Bacterial vaginitis	[133]
Finegoldia spp.	–		Osteomyelitis	[115]
Gardnerella spp.	–		Bacterial vaginitis	[132]
Lactobacillus spp.	Mastitis	[66]	–	
Mycobacterium spp.	Mastitis	[66]	Cystic fibrosis, infective endocarditis, lung abscess	[27,93,102,104,105]
Peptococcus spp.	Dental caries and root canal infection	[83]	–	
Rothia spp.	–		Infective endocarditis	[112]
Staphylococcus spp.	Bacterial keratitis, bacterial prostatitis, bloodstream infections, dental caries and root canal infection, infective endocarditis, infections from neurosurgical implants, mastitis, otitis media, prosthetic joint infections, respiratory tract infection and pneumonia, urinary tract infection	[66–69,72–74,76–79,81,83–86,88,91,92]	Bronchiectasis and persistent bacterial bronchitis, chronic otitis media, cystic fibrosis, infective endocarditis, kidney stones, osteomyelitis, wound infection	[99,100,103,106–110,113–117,119,120,122,124,127]
Streptococcus spp.	Infective endocarditis, mastitis, otitis media, periodontitis	[66,67,91]	Bronchiectasis and persistent bacterial bronchitis, chronic otitis media, cystic fibrosis, infective endocarditis, osteomyelitis, periodontitis, wound infection	[27,93,94,99,100,103,106,108–110,114,115,118,120–122]
		Fungus		
Aspergillus spp.	Bacterial keratitis	[87]	–	
Candida spp.	Bacterial keratitis, bacterial prostatitis, bloodstream infections, dental caries and root canal infection, infective endocarditis, gallbladder and biliary tract infections, otitis media, urinary tract infection	[66,67,70,79,80,83,87,91]	–	
Fusarium spp.	Bacterial keratitis	[87]	–	

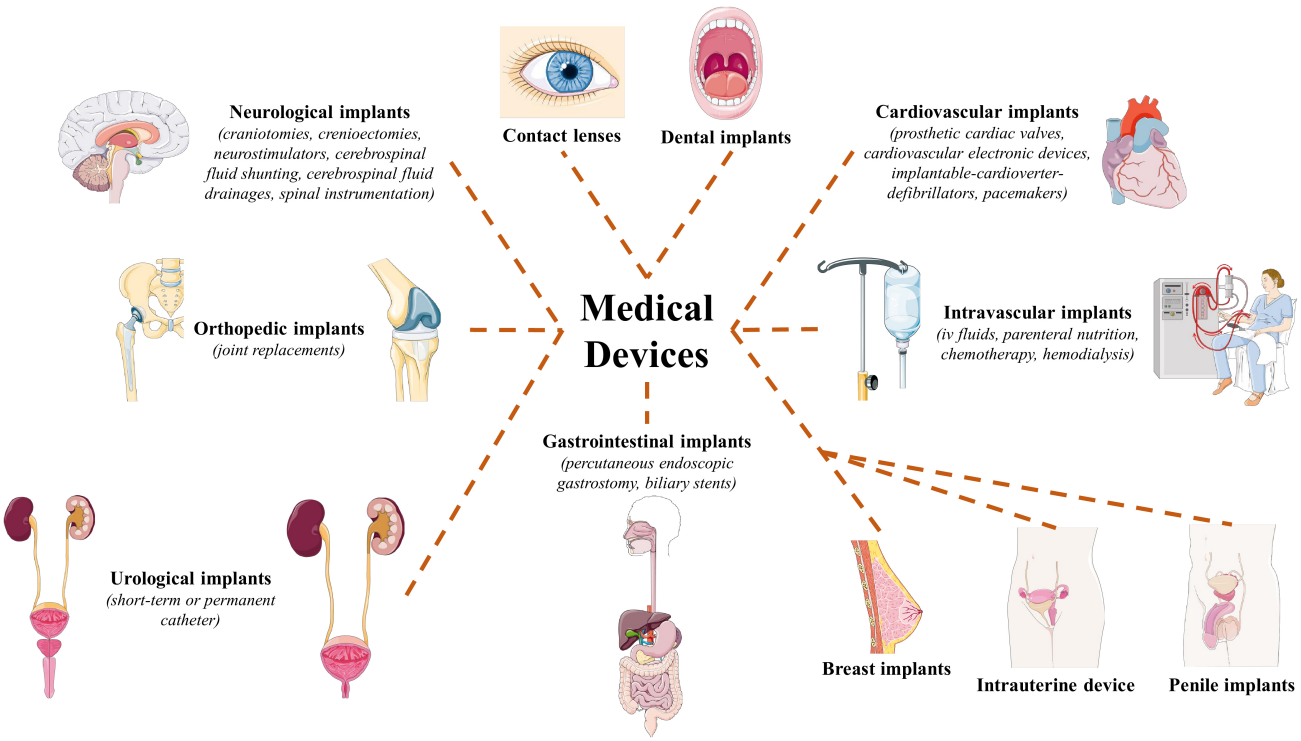

Fig. 2. Medical devices most colonized by pathogenic bacteria. This figure was created using Smart Servier Medical Art.

bacteria of the HACEK group, including *Haemophilus* spp., *Aggregatibacter* spp., *Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp. (6–11%); fungi, in particular *C. albicans* (0.4–1.4%) [66,67]. Multispecies communities were found on contaminated implants in 1–7% of cases, although their composition could not be determined [66]. Of note is that the pathogen *Streptococcus mutans*, which causes periodontitis, can reach the heart through the bloodstream and cause infection of implanted devices [137,138].

3.1.2 Biliary Stents

In more than 50% of cases, patients who have obstructive jaundice undergo biliary stenting to decompress the bile ducts and restore their patency. However, when a biliary stent is installed, duodenal bacteria can attach to its surface and form biofilms, which, when grown, lead to secondary blockage of the bile ducts. In the vast majority of isolates (95.8%), polymicrobial communities were obtained from the surface of biliary stents, consisting mainly of duodenal microbiota, including bacteria *Enterococcus faecium* (79.3%), members of the *Enterobacteriaceae* (73.7%), and fungi of the genus *Candida* (55.9%) [27,70,71].

3.1.3 Orthopedic Implants

Joint replacement is an effective method of restoring the function of the hip and knee joints in patients with arthritis. Despite the relative safety of the prosthetic procedure, patients may experience postoperative complications, the most common of which are prosthetic joint infections (PJIs), accompanied by purulent-necrotic reactions, osteomyelitis, and sepsis in severe cases [72,139]. PJIs are often caused by *S. aureus*, *Staphylococcus epidermidis*, and *Staphylococcus lugdunensis* [72–74]. Recent work has identified three main mechanisms in developing chronic staphylococcal PJIs: Forming small colony variants (SCVs), internalizing bacteria directly into osteoblasts, and forming biofilm communities [66,140,141]. In most cases, the severe, acute form of a PJI is associated with biofilm formations by *S. aureus*, predominantly the methicillin-resistant strain. Biofilms of *S. aureus* have been detected in bone cement samples obtained from prosthesis removal and in synovial fluid samples in the form of free-floating cellular aggregates [72,142]. Multispecies communities containing *P. aeruginosa*, *E. faecalis*, and *Klebsiella pneumoniae* are also involved in the development of PJIs of varying severity; MRSA (methicillin-resistant strain of *S. aureus*)–MSSA (methicillin-sensitive strain of *S. aureus*); MRSE (methicillin-resistant strain of *S. epidermidis*)–*E. faecalis* [66,75].

3.1.4 Neurological Implants

Neurosurgical implants include shunts, extraventricular and lumbar drainages for cerebrospinal fluid, and neurostimulators. The infection frequency of these devices varies from 3 to 15%, and under extraventricular drainage and craniotomy conditions, the number of infected implants increases strongly [27,66,143]. Such infections seriously complicate the course of the underlying disease and are often accompanied by death. The bacteria most often detected are *S. epidermidis* and *S. aureus* in lumbar drainages

[76], *S. aureus* and *Cutibacterium acnes* in extraventricular drainages [77], and *S. aureus*, *P. aeruginosa*, and *C. acnes* in neurostimulators [78].

3.1.5 Urological and Penile Implants

Bacterial biofilms can form on various medical devices used to treat diseases of the urogenital tract, including permanent or temporary urinary catheters, nephrostomy tubes, and penile implants. In modern clinical practice, indwelling catheters are widely used to relieve upper urinary tract obstruction, prevent the formation of scarring strictures in the ureters, and also for drainage of the urinary tract [66,144]. With permanent stents, which in most cases are colonized by various pathogens, the most common complications are urinary tract infections and bacteremia, which can be fatal [27,145]. The most common microorganisms that form biofilms on permanent and temporary urinary catheters are *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *E. faecalis*, *Staphylococcus* species, and *Candida tropicalis* [27,66]. Infectious complications after the installation of penile implants typically occur in 3% of clinical cases from primary implantation surgeries. During revision or reoperation, microorganisms are found on such implants in 70% of clinical cases, most of which are members of the genus *Staphylococcus*, particularly *S. epidermidis* [146–148].

3.1.6 Intravascular Devices

Intravascular devices are commonly used in healthcare settings for intravenous infusion, hemodialysis, hemodilution, and parenteral nutrition. Needleless intravascular devices reduce the risk of puncture injuries because of their ease of installation and rapid maintenance during use [27,66]. However, the lack of a universal disinfection procedure and improper cleaning methods increase the risk of infection, with subsequent development of bloodstream infections [149,150]. More than 50% of clinical cases of catheter-associated infections are associated with needleless infusion devices. Pathogens implicated in such infections include the bacteria *S. aureus*, *S. epidermis*, *E. faecalis*, *P. aeruginosa*, and *K. pneumoniae* and the fungus *C. albicans* [79,80]. These microorganisms cause bloodstream infections, which are a serious problem for public health throughout the world, as they lead to prolonged hospitalization of patients, require protracted antimicrobial therapy, and almost double the risk of death [81,151].

3.1.7 Breast Implants

Breast implants are used for both post-mastectomy breast reconstruction and cosmetic procedures. Often, the installation of breast implants leads to serious complications, including postoperative seroma, changes in nipple sensitivity, scarring, capsular contracture, and infections [66,152]. Studies have shown that 85% of breast implants with periosteal contracture had bacterial contamination, with microbial biofilms found in more than half of the cases [27]. Various species of *Staphylococcus* spp. often colonize breast implants. In particular, *S. epidermidis* and *S. aureus* have been identified in isolates from the surface of removed implants with capsular contracture [27,66]. In addition to staphylococci, biofilms on implants can be formed by *Bacillus* spp., *Mycobacterium* spp., *Corynebacterium* spp., various strains of *E. coli* and *C. acnes*, and streptococci and lactobacilli [66,82,153].

3.1.8 Dental Implants

The oral cavity is a favorable habitat for microorganisms. Its microbiome is extremely diverse and contains both beneficial and pathogenic strains. In dysbiosis, it becomes dangerous to human health, causing tooth decay and inflammatory processes in the gums. In addition, pathogenic inhabitants of the oral cavity can strongly aggravate chronic diseases of the heart and lungs, diabetes mellitus, and many systemic autoimmune diseases [27,154,155]. Microorganisms that form monospecies or poly-species biofilms can colonize dental implants, which restore the normal functioning of the teeth. This colonization often leads to periimplantitis or mucositis [156]. The consequences of active colonization of implanted devices are the destruction of dental resin composites, demineralizing tooth enamel, extensive bacterial invasion, etc. [157]. Initially, implants are colonized by primary colonizers, including Gram-positive coccoid and rod-shaped bacteria. These microorganisms create the basis for secondary colonization, which involves the bacteria *Actinomyces* spp., *Fusobacterium* spp., *P. gingivalis*, *Treponema denticola*, *E. corrodens*, *Prevotella intermedia*, *Staphylococcus warneri*, *Campylobacter* spp., and *Peptococcus* spp. and the fungus *C. albicans* [83–85].

3.1.9 Other Devices

Contact lenses are a major risk factor for developing bacterial keratitis, an infection of the cornea that, if not treated promptly, can cause partial or complete vision loss. Microbial biofilms form on contact lenses, which, when interacting with the surface of the eye, transfer persistent pathogens to the cornea, causing inflammation [27,158]. In most cases, bacterial keratitis is caused by biofilms of coagulase-negative staphylococci, *Cutibacterium* spp., *Corynebacterium* spp., *S. aureus*, and *P. aeruginosa* [66,86]. In tropical and subtropical climates, keratitis can be caused by the fungal species *Fusarium*, *Aspergillus*, and *Candida* [87]. Polybacterial biofilms consisting of *S. aureus*, *S. epidermidis*, and *Fusarium solani* have been reported to colonize human corneal tissue culture *ex vivo* [159].

Patients on mechanical ventilation are susceptible to ventilator-associated pneumonia, which occurs due to bacterial contamination of the breathing tubes and the formation of biofilms on their surface. The main species that

colonize the ventilation apparatus of intubated patients are *Streptococcus*, *Neisseria*, and *Prevotella*. [66]. In addition, polybacterial communities, including *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *Acinetobacter baumannii* have been found in isolates obtained from the surface of contaminated endotracheal tubes [88–90].

Cochlear implants, used by patients with hearing loss of varying severity, are also susceptible to bacterial (*S. aureus*, *S. epidermidis*, *Streptococcus pyogenes*, *P. aeruginosa*) and fungal (*C. albicans*) infections, with the rate of infectious complications ranging from 1.7 to 4.1% [66,91,92].

Various device-associated infections increase the morbidity and mortality of patients, making it difficult to provide qualified medical care. In modern realities, more attention should be paid to studying the composition of microbial communities on specific medical products and devices, studying the characteristics of biofilm formation on various surfaces *in vitro* and *in vivo*, and analyzing the effect of the implant environment on microbial colonization [27].

3.2 Tissue-Located Bacterial Biofilms

In the human body, bacterial biofilms as components of the normal microbiota were first described by Antonie van Leeuwenhoek as microorganisms in fibrous structures on teeth, which are now known as dental plaque. Since the 1970s, both pathogenic and commensal biofilms have been detected on various organs and tissues by electron microscopy, fluorescence *in situ* hybridization combined with confocal laser scanning microscopy, and other methods [93]. This subsection is devoted to the main human infectious diseases caused by monospecies or mixed microbial communities on the surface of tissues and organs (Fig. 3, Ref. [93]).

3.2.1 Oral Cavity

Oral microorganisms maintain mutually beneficial relationships with one another and the host without causing serious disease. However, any imbalance in the oral microbiota can provoke pathological processes, leading to periodontitis, gingivitis, and other diseases. Periodontitis is a very common gum infection, accompanied by plaque and damage to soft tissues with swelling of the arteries and the bones that support the teeth [27,160,161]. Periodontitis is caused by the bacteria *P. intermedia*, *Fusobacterium nucleatum*, *T. denticola*, *Tannerella forsythia*, *S. mutans*, and *Streptococcus gordonii*, among others [64,94], which develop biofilms on various surfaces, including the mucous membranes of the oral cavity. In addition, microbial communities can penetrate the mucosa, redistributing calcium fluxes in epithelial cells while simultaneously releasing toxins that induce inflammation [64]. Recent studies have shown that *P. gingivalis*, *T. denticola*, *T. forsythia*, *Cryptobacterium curtum*, *P. intermedia*, and *Aggregatibacter actinomycetemcomitans*, which form biofilms on teeth, may be involved in many systemic diseases such as diabetes mellitus and rheumatoid arthritis [95,96].

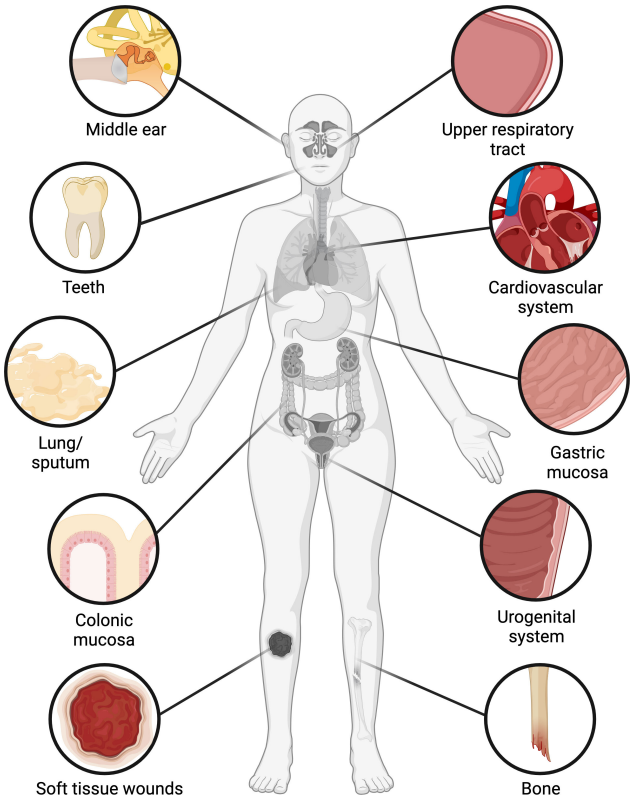

Fig. 3. Location of biofilms in the human body. The figure was made using BioRender.com (https://www.biorender.com/). Reprinted under terms of the CC-BY license [93].

3.2.2 Lungs and Lower Respiratory Tract

One disease associated with the development of bacterial biofilms directly on organs and tissues is cystic fibrosis (CF) [162]. The most common pathogens that form biofilms in the lower respiratory tract and sputum of CF patients include *P. aeruginosa* [97,98], *Staphylococcus* spp., and *Streptococcus* spp. [99,100,163] in both monospecies and mixed communities [164,165]. *Burkholderia cepacia*, a bacterium capable of forming biofilms *in vitro* and *in vivo*, has been identified in sputum samples from CF patients; however, it has not yet been possible to identify them as part of biofilms in clinical isolates [101]. Qvist *et al.* [102] obtained samples of *Mycobacterium abscessus* biofilms formed in the interstitial spaces of the alveolar wall of CF patients, yet they did not detect the biofilms in sputum samples. Since chronic infections in cystic fibrosis are hard to treat, including those owing to the increased resistance of biofilms to antibiotics, complications often develop in the form of irreversible damage to the epithelium, airway obstruction, or respiratory dysfunction, which leads to premature death from respiratory insufficiency.

In the lower respiratory tract, biofilms are also found in bronchiectasis and persistent bacterial bronchitis. The

causative agents of these diseases include *H. influenzae*, *Moraxella cataralis*, *S. pneumoniae*, *P. aeruginosa*, and members of the genus *Staphylococcus* [93,103]. Most biofilm-forming pathogens that cause pulmonary infections are detected as suspended aggregates in sputum [166]. However, biofilms lining the resected part of the lung tissue have been described in obstructive pulmonary disease [167]. *Mycobacterium tuberculosis* can also form biofilms in necrotic lesions and lung cavities in tuberculosis patients [104,105].

3.2.3 Upper Respiratory Tract and Middle Ear

Infections associated with biofilm formation often affect the middle ear and the upper respiratory tract. In patients with chronic otitis media, monospecies biofilms of *H. influenzae*, *M. cataralis*, *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *P. aeruginosa*, and *Proteus* were found in aspirates and biopsy samples of the mucous membrane. The size of these biofilms varied from microcolonies to large cell aggregates [106–108]. The listed pathogens are also common colonizers of upper respiratory tract tissues [109,110]. In particular, they form biofilms in the mucous membranes of the sinuses, adenoids, and tonsils [93].

3.2.4 Cardiovascular System

Microbial biofilms have also been found in cardiovascular diseases [168]. Infective endocarditis is a dangerous disease whose mortality rate may exceed 20% [27]. With the development of infective endocarditis, the ability to form biofilms is key to the virulence of pathogens, which include strains of *S. aureus*, *S. epidermidis*, *Mycobacterium fortuitum*, *Streptococcus* spp., *Enterococcus* spp., and *Rothia* [27,93,111–113]. These bacteria enter the bloodstream and attach to the heart valves or the lining of the heart chambers, often in patients with congenital valve abnormalities or damaged heart tissue [169]. As the disease progresses, extensive biofilm structures appear on the cardiac mucosa, identified in biopsies of patients with endocarditis [93]. Serious complications of infective endocarditis develop when persistent colonies from biofilms enter the bloodstream, resulting in bacteremia and subsequent sepsis in the body. Although control of bacteremia is possible through combination antimicrobial therapy, deep layers of biofilm communities can become a source of new infections and can, in more severe cases, cause septic embolism [27,170]. Biofilms have also been documented in atherosclerotic arteries both within arterial tissue and between vascular smooth muscle and cholesterol plaques [93,171].

3.2.5 Skeletal System

In bone tissue infections or osteomyelitis, which may occur because of injury or vascular damage, bacteria also form biofilms. Using scanning and transmission electron microscopy of tissue samples from patients with osteomyelitis of the femur, tibia, or fibula, researchers identified either large multispecies biofilms covering the bone surface or microcolonies attached to osteocytes [93]. Most of these biofilms contained *S. aureus*, *P. aeruginosa*, *S. marcescens*, *C. acnes*, *Streptococcus* spp., and bacteria of the HACEK group [114]. Using scanning electron and confocal microscopy, bacterial clumps were detected on the bone surface in osteomyelitis patients with diabetic foot syndrome. The vast majority of such biofilm samples contain members of the genera *Corynebacterium*, *Finegoldia*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Enterobacter*, *Porphyromonas*, and *Anaerococcus* [115–117]. Biofilms are also formed in osteomyelitis or osteonecrosis of the jawbone, covering large areas of both the internal and the external surface of the bone. In jawbone osteomyelitis, biofilms are dominated by *Actinomyces* bacteria, also found in the oral cavity microbiota, and osteonecrosis, biofilms contain mostly members of the genera *Fusobacterium* and *Streptococcus* [93,118].

3.2.6 Wound Surface

Wound infection occurs as a postoperative complication or as a traumatic violation of tissue integrity. The development of infection and its spread depends on the presence of foreign bodies, blood clots, necrotic tissue in the wound, insufficient blood supply to the damaged area, bacterial load, and the general condition of the body [172]. Wound infections may be caused by biofilms of both Gram-positive (*S. aureus* and *S. pyogenes*) and Gram-negative (*P. aeruginosa*, *E. coli*, and *Serratia marcescens*) bacteria [119,120]. The pathogens *A. baumannii*, *S. pyogenes*, and *S. aureus* are the most common causative agents of necrotizing fasciitis. Biofilms of these bacteria are resistant to antibiotics and physical and biochemical influences, which can slow wound healing, provoke cell death, and lead to amputations [121–123]. The biofilms found in trophic ulcers of the lower extremities include the Gram-positive bacteria *S. aureus* and *E. faecalis*, coagulase-negative staphylococci, the Gram-negative bacterium *P. aeruginosa*, and members of the genus *Proteus* [124,125].

3.2.7 Urogenital System

Pathogenic bacteria can penetrate the kidney tissue, form biofilm structures, and, as a result, cause chronic pyelonephritis and bacterial prostatitis. In the genitourinary system, bacterial aggregates surrounded by an extracellular polymer matrix were found in biopsies of patients with chronic prostatitis [173]. Usually, bacterial prostatitis is caused by biofilm-forming bacteria of the genera *Pseudomonas*, *Haemophilus*, *Sneathia*, *Allobaculum*, and *Enterococcus* [126]. In addition, microbial communities can form on the surface of kidney stones, with typical bacteria being *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Staphylococcus* spp., and *Enterococcus* spp. [127,128,174]. Some of these bacteria have urease activity, accelerating the

formation of specific minerals necessary to frame kidney stones. Many biofilm extracellular polymer matrix components may be key in cementing nascent crystals during kidney stone formation [93].

Disturbances in the composition of the vaginal microbiota increase the risk of biofilm-associated urinary tract infections in women [175]. The most common bacteria implicated in urinary tract infections in women are uropathogenic *E. coli* (UPEC), which evade the body's immune response by promoting proinflammatory responses and masking endotoxins within host biomolecules. UPEC bacteria form biofilms on both the bladder mucosa and the vaginal wall [129,130]. The genus *Proteus* members also belong to the causative agents of urinary tract infections. *P. mirabilis* can attach to the surface of the female genitourinary system, actively multiply, and form biofilms that protect the bacteria from the host immune system [131]. Bacterial vaginitis is a very common vaginal infection worldwide, which seriously complicates the daily lives of women. On the surface of the vaginal epithelium, various biofilms may be present, in particular consisting of the bacteria *Gardnerella vaginalis*, *Fannyhessea vaginae*, and *Prevotella bivia*, which actively colonize the vaginal tissue and produce virulence factors [132,133].

Bacterial infections associated with biofilm formation are currently extremely common and affect almost all organ systems and tissues. Owing to the increased resistance to negative environments and antimicrobial agents, biofilms pose a global threat to human health and life. Studying the exact composition of biofilm communities concerning a specific type of infection and an active search for possible mechanisms of their eradication will allow us to develop effective treatment regimens that will selectively destroy pathogens in biofilms while maintaining the normal microflora of our body.

4. Analysis of the Bacterial Composition in Biofilms

Microbial cells and extracellular polymeric substances make up biofilms. EPS can be thought of as the main matrix material of the biofilm and may make up 50% to 90% of the total organic carbon in biofilms. Although EPS's chemical and physical characteristics might vary, polysaccharides comprise most of its composition [7]. Owing to the widespread distribution of biofilm bacteria, it is vital to develop methods not only to monitor the formation of biofilms but also to analyze their composition (Fig. 4).

4.1 Traditional Microbiological Methods

Traditional microbiological methods of biofilm examination include sample collection, culturing, identification, and antibiotic susceptibility testing, if necessary. For example, prostheses, catheters, stents, and other foreign bodies removed from patients with suspected biofilm infections should be microbiologically tested. The isolated biofilm method is widely used to examine biofilm composition [176].

The ability of crystal violet dye to bind to the cells and matrix of biofilms is used to obtain information about the relative densities of the entire biofilm on the substrate surface [177]. When examining biofilm composition, one must consider the optimal conditions for testing microorganisms [176,178].

4.2 Staining

Various methods are available for the fluorescent staining of biofilms, such as using specific dyes and antibodies that carry a fluorescent label. The matrix proteins and polysaccharides are visualized by immunofluorescence. The methods used to visualize biofilms do not ensure differentiated coloring of its components because it is

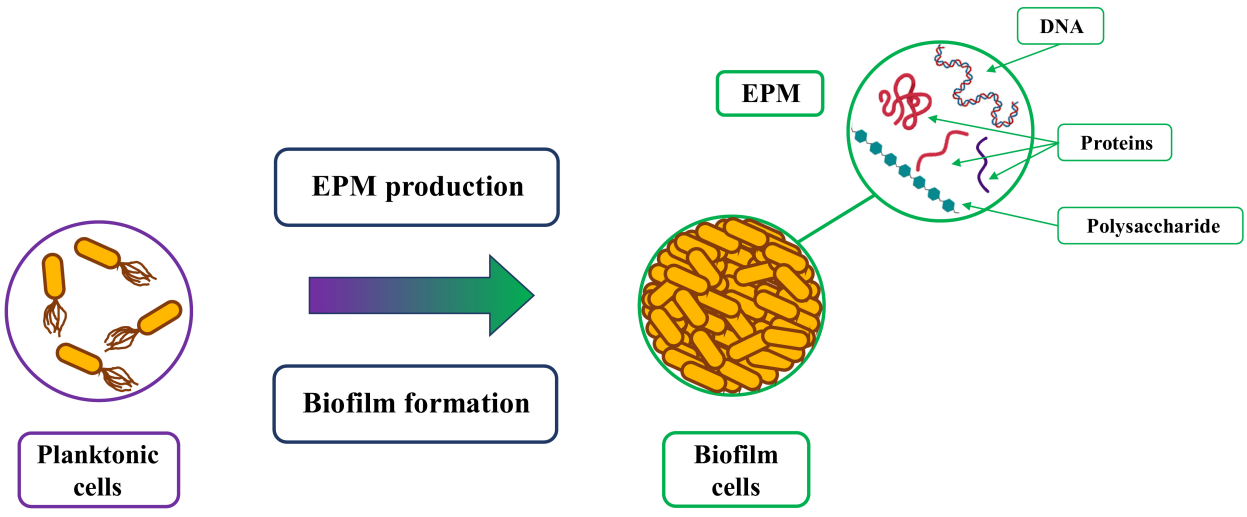

Fig. 4. There are variations amongst matrix regions due to the non-homogeneous distribution of the main matrix constituents, polysaccharides, proteins, and DNA throughout the cells. EPM, extracellular polymer matrix.

not possible to establish a substrate stained with gentian violet (the dye that can form complexes with both intracellular and extracellular structures). This approach does not allow adequate examination of the antibiofilm effects of drugs, whereas the results of studying the interaction of drugs with biofilm components may ensure the correct choice of drugs. A method was described to investigate biofilm formation in mono- and mixed cultures of the nitrile-hydrolyzing bacteria *Alcaligenes faecalis* 2, and *Rhodococcus ruber* gt 1, and a quantitative method was developed to evaluate the yield of the polymer matrix using the fluorescence of biofilms stained with concanavalin A—tetramethylrhodamine. The formation of biofilms was examined by staining them with crystal violet, utilizing the energy status of the biofilm cells, and using the yield of the polymer matrix [179].

Of particular note is the indirect identification of amyloid fibers using Congo red dye [180]. It is based on the ability of Congo red to bind to amyloid groups of proteins. The best-known microbial amyloid protein is the *E. coli* curli protein, which plays an important structural role in the biofilm. With this technique, biofilms are not examined directly, although it can be concluded that hypothetical amyloid-like protein structures are present.

4.3 Microcolonies Formation and Cellular and Extracellular Biofilms Components Examined

Much can be learned about the behavior of biofilm bacteria by simply observing the early stages of biofilm development, including adhesion and the formation of early microcolonies. This stage may be especially important for studying mutants, for example, when assessing the contribution of motility [176].

Another study examined the composition of biofilms using an approach that first evaluated the proportion of the matrix and then examined its cellular and extracellular components in the aggregate. Such a differentiated analysis makes it possible to determine the points of action of drugs and increase the therapeutic effect through a combination of drugs with different action targets. The method allows one to differentiate between the cellular component and the biofilm matrix [181].

4.4 Metabolic Activity Detection

The tetrazolium salt 2,3-bis-[2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT)/menadione assay allows one to estimate the metabolically active cells in biofilms. XTT is a tetrazolium salt whose transparent solutions are nontoxic to cells. Singh *et al*. [182] showed its use to determine the viability of *M. tuberculosis, M. bovis* BCG, and *M. smegmatis* in a microplate format. With the addition of metabolic activators, viable cells converted XTT to a formazan with a clearly detectable color. The method is quite simple, has a high throughput, does not violate the integrity of cells, and gives numerical indicators.

Many modifications to this method exist, including commercial ones, with all of them based on determining the metabolic activity of prokaryotic and eukaryotic cells.

4.5 Microscopy Analysis

Biofilm detection methods include scanning electron microscopy, confocal laser scanning microscopy [183–186], surface-enhanced Raman spectroscopy [187], magnetic resonance imaging [188], and field emission scanning electron microscopy [189]. Separately, sensor technologies are being developed to detect biofilm bacteria [190–192].

Three-dimensional imaging of live/dead *P. acnes* biofilms with different biochemical properties using confocal laser microscopy showed different distributions and ratios between live and dead cells. It was suggested that extracellular DNA is implicated in forming biofilms containing living cells, and it was revealed that the biochemical properties and structure of biofilms varied among *P. acnes* isolates [193].

Differences between biofilms formed by Gram-positive methicillin-resistant *S. aureus*, Gram-negative *P. aeruginosa*, and the yeast-type *C. albicans* were investigated by synchrotron macro attenuated total reflectance–Fourier transform infrared (ATR–FTIR) microspectroscopy. The study showed that the polysaccharides area accounted for the most significant difference between biofilm samples; other spectral regions corresponding to amides, lipids, and polysaccharides contributed to sample variation. Multivariate analysis separated the three biofilms based on their spectra and provided probable chemical functional groups contributing to the significant differences between and within them [194].

4.6 Taxonomic Characterization and Metaproteomics

The method used most widely owing to its reliability is the taxonomic characterization and assessment of biomass contributions to biofilm composition based on 16S/18S rRNA amplification of isolated DNA and sequencing. A different approach to tandem mass spectrometry proteotyping, consisting of obtaining data on peptides and their interpretation against a common database without any *a priori* data, is presented in [195]. Peptide sequence information is converted into useful taxonomic information that permits one to obtain different biomass contributions at different taxonomic ranks. This method is the first to analyze the composition of biofilms from minute amounts of material collected from a pool used to store radioactive sources at a nuclear facility. For these biofilms, the authors reported the identification of three genera (*Sphingomonas*, *Caulobacter*, and *Acidovorax*) and their functional characterization with metaproteomics, which shows that these organisms are metabolically active [195].

The lack of reliable information about the underlying microbiome leads to conflicting statements regarding the degradative potential of many microorganisms within

the biofilm. Metagenomics and traditional microbiological methods are used to solve this problem [196].

Metaproteomics has been used to obtain new information about natural microbial biofilm. The most common strategy for a metaproteomic study is to first collect metagenomic data on the same sample to obtain a database of protein sequences specific to that sample. Subsequently, the spectra recorded for the peptides obtained from proteolysis of extracted proteins are used for metagenomic information [197].

Sonication of permanent devices (implants, prostheses, or catheters) from patients with a suspected infection largely improves the detection rate for biofilm bacteria. Peptide nucleic acid fluorescence *in situ* hybridization is promising for detecting biofilm infections in cystic fibrosis and chronic wounds. In addition to detecting bacteria, fluorescence *in situ* hybridization is very sensitive for invasive yeast infections but is less sensitive for molds [198].

Although DNA extraction, amplification of 16S/18S rRNA gene amplicons, sequencing, and taxonomic sequence analysis are the primary detection methods, bulk DNA extraction and PCR amplification of rRNA genes can be species-dependent [199]. Moreover, using rRNA information alone is generally insufficient for taxonomy determination because a relatively high percentage (at least 5%) of 16S rRNA sequence records contains significant anomalies [200]. Another option is to sequence all the DNA extracted from the sample using metagenomics. However, this approach may require amplification steps to obtain enough material for library construction, possibly introducing some bias into the results. The experimental workflow used to identify taxa in various samples consists of protein extraction, peptide analysis by tandem mass spectrometry coupled with reversed-phase chromatography, and metaproteomic analysis of the resulting data. For proteotyping biofilm composition, spectra were interpreted against the NCBInr database (a general database containing annotated proteomes of several tens of thousands of reference organisms). Then, the identified peptide sequences were mapped to the taxa whose proteomes contained them [195].

Biofilm composition may be directly analyzed using metaproteomics with 0.02 mg of biological material—an innovative method for processing spectra obtained after protein extraction was developed to prototype microorganisms present in biofilms. The developed strategy can be used to identify microorganisms present in any samples and to decipher their respective contribution to the biomass. The final advantage of proteomic analysis is that it provides direct access to the functioning of microorganisms present in microbial communities [201].

4.7 Phylopeptidomics

Phylopeptidomics is a new direction that can help determine the composition of microbial communities present in biofilms and evaluate their contribution to biomass directly through their peptide signal. Metaproteomics is more accurate for biomass estimation than DNA sequencing methods, as recently found with protein-centric [202] or peptide-centric [203] methods. The peptidome signal is directly mapped to taxonomic information, whereas more classical metaproteomic approaches rely on protein inference and subsequent assignment of detected proteins to taxonomic information. Protein inference is a procedure that assembles identified peptide sequences into the most suitable set of proteins. Since it bypasses the protein inference problem, the peptide-centric approach provides a more direct and objective view of the taxonomic units present in a sample and, thus, a better assessment of their contribution to biomass. However, this method has the limitation that the NCBInr database does not provide a comprehensive representation of biodiversity on Earth, meaning the technique may not be applicable to identify new microorganisms whose genomes have yet to be sequenced or to accurately determine the most accurate taxonomic rank (species, subspecies, or rank strain).

Current efforts by the scientific community to sequence the entire genome of pathogens, emerging pathogens, and environmental samples are helping to fill this gap over time. Therefore, the peptido-centric method can be expected to become more powerful in the future with further updates to the database.

Christensen *et al*. [204] described one of the first attempts to study biofilm formation by spectrophotometry. Using *S. epidermidis*, they assessed the dependence of changes in the absorbance of colored biofilms on their biomass sorbed on polystyrene tablets. The measurements were correlated with a visual assessment of bacterial adhesion on culture tubes and plates. The absorbance of the biofilms formed on the surface of plastic tissue culture equipment serves as a quantitative model for studying the adhesion of staphylococci to medical devices.

Biofilms contain so-called persisters (dormant cells capable of carrying very high levels of antimicrobial agents). Even if most bacteria in a biofilm are killed by antimicrobial therapy, persister cells can resume the infection after the threat is eliminated. The mechanism of persister cell dormancy has yet to be fully understood, but developing biofilm composition techniques that consider persister cells is important for successful biofilm eradication [205].

Routine microbiological testing is important and reliable for diagnosing infections but is less sensitive for detecting biofilms. Therefore, new methods to analyze bacteria in biofilms should be introduced as an effective addition to the existing standard methods. Despite the many existing methods for analyzing biofilm composition, research in this direction continues.

5. Ways to Reduce Biofilm-Associated Infections

Currently, no tools exist to directly and completely eradicate biofilms, but there is an understanding of how to prevent the formation of biofilms, control their growth, and eventually destroy them. Analysis of the literature [2,206,207] permits the following main strategies for combating biofilms to be identified:

- Development and creation of antiadhesive materials and substances with prolonged properties.

- Inhibition of the attachment of microorganisms to the substrate by using special compounds and destruction of biofilms early in their formation.

- The use of compounds that interfere with QS and the production of functional bacterial adhesins causes the detachment of biofilms and disrupts the mechanisms regulating their vital activity.

- Use of physical destruction means (lasers, cold plasma, etc.).

- Development of drugs that destroy the biofilm matrix, facilitating cell access.

- Genetic engineering of phages.

- Use of antibacterials together with matrix-destroying factors.

- Use of probiotic substances [206].

Fig. 5 (Ref. [2]) presents the main strategies for combating bacterial infections, including prevention, diagnosis, and treatment [2].

6. Conclusion and Prospects

Most environmental, industrial, and medicinal issues and processes that microbiologists are interested in are dominated by sessile organisms [208]. Bacterial biofilms cause serious health problems worldwide and are responsible for many intractable wound infections caused by pathogenic multidrug-resistant and biofilm-forming bacteria. Biofilms are of major importance in clinical medicine. When the immune system is weakened during a certain disease and medical intervention is carried out in the human body, secondary infections (i.e., concurrent) may occur caused by environmental bacteria or the normal human microflora.

In this case, biofilms may serve both as a factor directly related to infection and pathogenesis and as a reservoir for pathogenic microorganisms during colonization of an organ or organ system. Biofilms have several features that distinguish them from free-floating bacteria under conditions of persistent infection. These changes have significant therapeutic implications, including increased antibiotic resistance, chronic inflammation, tissue damage, and diagnostic difficulties. Bacteria within a biofilm are protected from antibiotic exposure by a protective biofilm matrix, making them highly resistant to antibiotics. Biofilms also include increased resistance to the immune system and drugs. These differences highlight the importance of individualized treatment plans and the development of new methods to understand and treat the complexities of

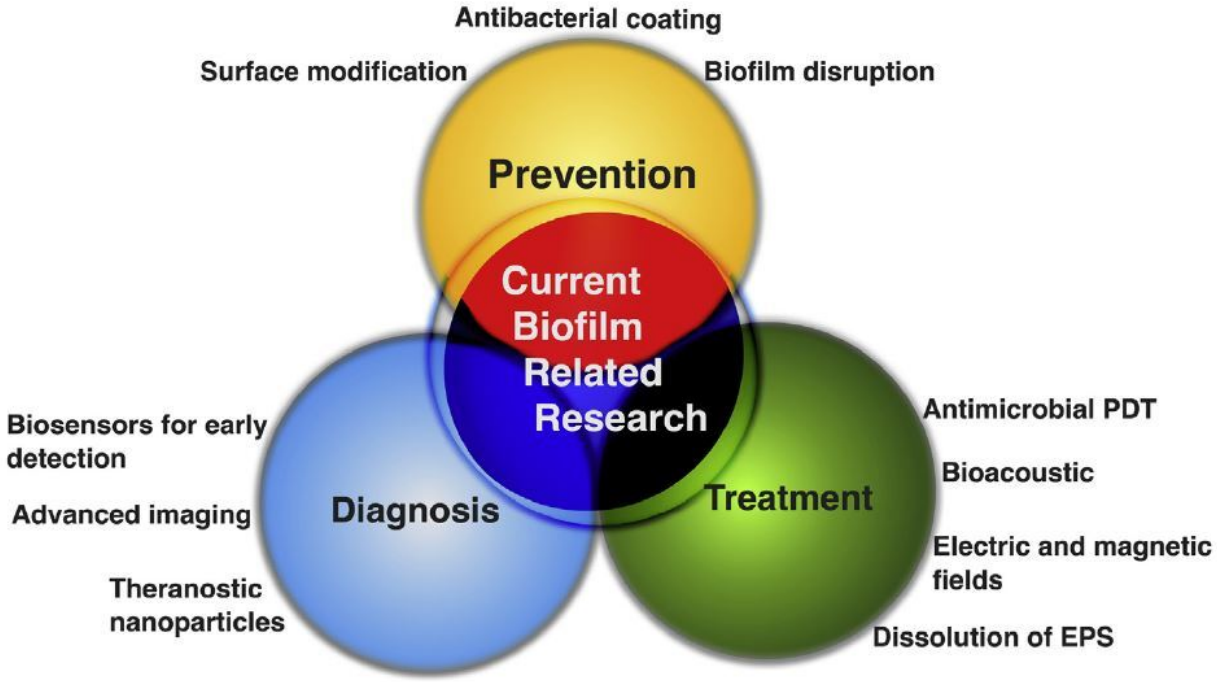

Fig. 5. Strategies to combat bacterial colonization of implantable devices. PDT, photodynamic therapy; EPS, extracellular polymeric substances.

biofilm-associated infections and analyze biofilm composition [209]. Treatment of biofilm infections is challenging and requires collaboration in clinical microbiology, surgery, internal medicine, pharmacology, and basic science.

Author Contributions

OIG — Conceptualization and Design, Project Administration, Formal Analysis, Visualization, Writing—original draft, Writing—review & editing. SSE — Formal Analysis, Software, Resources, Visualization, Writing—original draft. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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