Chapter 2
Bacterial Infections: Few Concepts

Abstract A principal challenge defying current medicine in the twenty-first century is the large occurrence of antibiotic resistance, as well as, the risk posed by drug-resistant superbugs. In spite of this, progresses on the development of novel antibiotics to combat this problem are quite limited. It appears necessary to carry out a more concerted effort to advance in the discovery of novel therapeutic agents with excellent activity and unique mechanisms of action to overcome the problem of drug resistance. In this context, macromolecular antimicrobials with a different interaction with bacteria may offer an interesting alternative to current strategies in order to successfully prevent resistance. Furthermore, biofilm-forming bacteria are recognized to be gradually resistant to the action of antibiotics and are a leading cause of mortality or morbidity in nosocomial infections.

This chapter will, thus, describe the bacterial structure and summarize the mechanisms involved in the interaction between antibiotics and bacteria as well as the resistance mechanisms developed. In addition, the proposed models of interaction between macromolecular antimicrobials and bacteria will be analyzed.

The second part of this chapter is devoted to implant-associated infections produced by the formation of a biofilms at the surface of biomaterials. More precisely, the steps involved in biofilm formation and its particular properties that reduce the antimicrobial activity will be discussed. Finally, preliminary concepts on the use of polymers to overcome this limitation are depicted.

Keywords Bacterial structure • Antimicrobial mechanisms • Macromolecular antimicrobials • Pore-forming mechanism • Bacterial adhesion • Biofilm formation

2.1 Introduction

A principal challenge defying current medicine in the twenty-first century is the large occurrence of antibiotic resistance, as well as, the risk posed by drug-resistant superbugs. In spite of this, progresses on the development of novel antibiotics to combat this problem are quite limited. It appears necessary to carry out a more concerted effort to advance in the discovery of novel therapeutic agents with excellent activity and unique mechanisms of action to overcome the problem of drug

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resistance. In this context, macromolecular antimicrobials with a different interaction with bacteria may offer an interesting alternative to current strategies in order to successfully prevent resistance. Furthermore, biofilm-forming bacteria are recognized to be gradually resistant to the action of antibiotics and are a leading cause of mortality or morbidity in nosocomial infections [1].

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2.2 Bacterial Structure

Bacteria comprise a cytoplasm and a membrane and finally a cell wall. On the one hand, the cytoplasm does not have any organized organelles and is formed exclusively by ribosomes and DNA [2]. The membrane of bacterial cells share common features with those membranes found in mammalian cells, i.e., they are formed by a phospholipid bilayer. In both cases and in general biological membranes comprise five major biomolecules: phosphatidyl glycerol (PG), phosphatidyl ethanolamine (PhE), phosphatidylcholine (PhC), phosphatidyl serine (PhS), and sphingomyelin (SfgM). These biomolecules provide the surface charge present at the cell surface. More precisely, at physiological pH, whereas PhS and PhG are negatively charged, PhC, PhE and SfgM form zwitterionic species. In addition to these common biomolecules, bacterial cells present some structural differences that required to be analyzed in order to understand the antimicrobial properties of polymers [3].

The main differences between the plasma membranes in mammalian and microbial cells rely on their composition and their structure. Illustrative examples of a mammalian cell membrane and microbial membranes of Gram-negative bacteria, Gram-positive bacteria as well as Yeast are depicted in Fig. 2.1.

The first key difference between the two relies on the distribution of the negatively charged biomolecules. In mammalian cells, the outer monolayer of the membrane is often constructed from PhC and SfgM. Therefore, the negative charge provided by PS is concentrated in the inner part of the membrane. Microbial cell membranes possess, on the contrary, negative charges in both sides of the membrane as a result of a homogeneous distribution of PhS. As will be depicted later, this characteristic will be crucial for the design of selective antimicrobial polymers [4].

The second major difference concerns the additional components present in the cell wall of microbial membranes. The cell wall composition depends on the microbial strain. Therefore, antimicrobials would not behave equally to all bacteria but

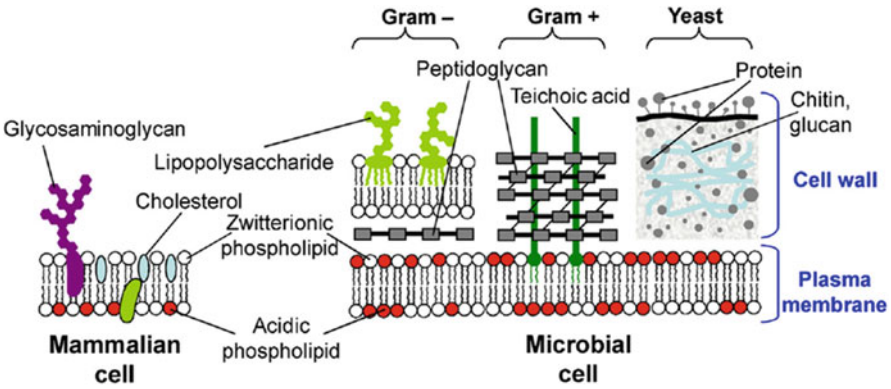

Fig. 2.1 Cell envelope structure and its effect on the antimicrobial selectivity. Cross-sectional illustration showing major changes between cell envelope of mammalian cells and various microbial families. Reproduced with permission from [3]

probably may exhibit larger activity of particular species. As depicted in Fig. 2.1, several differences can be observed between the cell wall of Gram positive, Gram-negative, and Yeast. First of all, Gram-positive bacteria have a thicker peptidoglycan layer in comparison to Gram-positive. Moreover, this layer is around 90 % of the cell wall components in Gram-negative while it supposes around 20 % in Gram negative. The yeast family does not possess peptidoglycans on their walls. In this case the membrane is formed by a layer of chitin and glucan cross-linked polymer network and an outer protein layer [5]. Other significant difference between Gram-positive and Gram-negative bacteria is that in Gram-positive bacteria teichoic acids are attached to the membranes and oriented outwardly. Gram-negative do not have teichoic acids and, in contrast to Gram-positive the peptidoglycan layer is embedded in an additional membrane known as outer membrane [6].

Finally, a third difference between mammalian and bacterial cells is associated to the mechanical stability of the membrane. Microbial cell exhibit, in comparison to mammalian cells membranes, enhanced mechanical stability. This characteristic has to be considered in the design of antimicrobial polymers since, while providing excellent antibacterial activity these may result toxic to mammalian cells.

2.3 Interactions Mechanisms of Antimicrobials with Bacteria in Solution

2.3.1 Bacterial Targets of Antibiotics

Antibiotics are usually antibacterial drugs that interfere with one or more of the bacterial crucial routes such as growth or survival. A strong development during the 60–70s leads to the discovery of many diverse classes of antibiotics such as

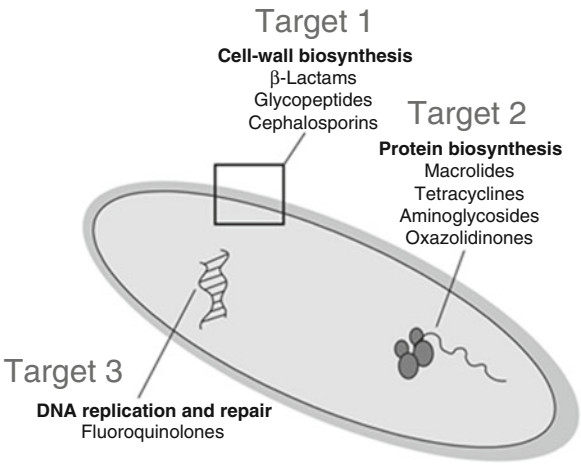

Fig. 2.2 Main targets of antibacterial drugs: Target 1: interaction with the cell wall biosynthesis preventing the cross-linking of peptidoglycan peptide strands. Target 2: blocking the protein biosynthesis at the ribosome in particular those steps involving rRNA and the proteins of the ribosome at the peptidyl transferase center. Target 3: interfering DNA replication. Figure adapted from [7]

vancomycins penicillins, or cephalosporins. As reported in an excellent review by Walsh [7], the antibiotics currently developed have three main targets (Fig. 2.2):

Cell wall biosynthesis: the peptidoglycan layer at the bacterial cell wall confers the stability and strength. This layer is formed by a mesh of peptides and glycans that can be covalent cross-linked. In this context, several antibiotics have been developed to target this layer. Penicillins and cephalosporins inhibit the peptidoglycan to form cross-links thus leading to a weaker wall that predisposes the treated bacteria to a killing lysis of the cell wall layer. An additional family of glycopeptide antibiotics is vancomycin. This antibiotic ties up the peptide substrate [8] and thereby prevents it from reacting with either the transpeptidases or the transglycosylases.

Protein synthesis: many inhibitors of protein synthesis target different steps in ribosome action selective for bacteria. The selectivity is simply reached since the prokaryotic ribosomes are considerably different to those existing in eukaryotic cells. Moreover, taking into account the amount of steps involved in the protein assembly by the ribosome (initiation, elongation, and termination), there are many different processes that can be changed using protein synthesis inhibitors.

DNA replication and repair: ciprofloxacin that belongs to the fluoroquinolones type are antibiotic molecules that target the enzyme responsible for uncoiling the intertwined circles of double-stranded bacterial DNA, i.e., DNA gyrase [9].

2.3.2 Antibiotic Resistance Developed by Bacteria

In spite of the different targets that can be focused to reduce bacterial contamination, bacteria have the ability to find alternatives to overcome the effects of antibiotics. This phenomenon, known as bacterial resistance, appears typically in periods of months for many of the currently available antibiotics thus limiting their use.

Mc Manus [2], Walsh [7], and more recently Blair et al. [10] have reported comprehensive reviews dealing with this aspect. Our discussion herein will be thus limited to mention the most important mechanisms developed by bacteria to survive antibiotics.

2.3.2.1 Mechanism 1: Pump Out the Antibiotic

Taking into account that for antibiotics in order to be active require both enough concentrations but also to approach the selected target and effective way to overcome antibiotic treatments involves the active pumping out of the cell by the so-called efflux pumps. In this case, the drug is pumped out faster than it can diffuse in, so antibiotic concentrations are maintained low and do not affect the protein synthesis [11, 12].

Similar efflux pumps have been observed in different bacterial strains. For instance, efflux pumps have been employed by *staphylococci* to become resistant to the erythromycin class of macrolide antibiotics [11, 13]. Other examples of efflux pumps include FuaABC in *S. maltophilia* [14], KexD in *K. pneumonia* [15], and LmrS in *S. aureus* [16, 17]. It is worth mentioning that some efflux pumps have revealed narrow specificity while others are capable of transporting different substrates, multidrug resistance (MDR) efflux pumps [10].

2.3.2.2 Mechanism 2: Reduce the Permeability of the Cell Membrane

Tamber and Hancock [18] reviewed this alternative mechanism developed by bacteria and concluded that the permeability of the outer membrane can be reduced in order to limit the amount of antibiotic that may enter into the cytoplasm. This can be achieved either by the downregulation of porins or by the replacement of porins with more-selective channels.

2.3.2.3 Mechanism 3: Modification of the Antibiotic Structure

Bacteria are able to develop synthetic routes to chemically modify the chemical structure of the antibiotic employed. Today, a large variety of enzymes have been identified that can damage and alter antibiotics of different classes, comprising β-lactams, aminoglycosides, phenicols, and macrolides [10]. One of these alternatives involves the inactivation of the antibiotic by *hydrolysis*. This is the case of the hydrolytic deactivation of β-lactam rings present both in penicillins and cephalosporins. Bacteria generate a hydrolytic enzyme known as β-lactamase. The closed β-lactam rings participate in the acylation and irreversible modification of the cell membrane cross-linking. The hydrolysis reaction resulted in an aperture of the ring inactivating the antibiotic [19].

A second alternative to modify the antibiotic structure and therefore deactivate them involves the incorporation of chemical groups. This is the case of antibiotics that are not affected by β-lactams such as aminoglycosides. The principle of this

strategy relies on the fact that the addition of chemical groups to particular positions on the antibiotic molecule by bacterial enzymes prevents the antibiotic from binding to the target protein. In particular, aminoglycosides with chemical substituents are unable to bind to the RNA targets in the ribosome [20]. Chemical groups that have been transferred, include acyl, phosphate, nucleotidyl, and ribitoyl groups [21].

2.3.2.4 Mechanism 4: Changing the Target Structure

According to Blair et al. [10], two different alternatives can be employed to alter the structure of the antibiotic target thus conferring bacterial resistance. On the one hand, bacteria can react to the antibiotic by modifying the structure of the antibiotic targets for instance by mutation. On the other hand, it can chemically modify the targets to protect them from the antibiotic (for instance, by methylation processes).

Typically, antibiotics exhibit a high specific binding for a particular target. As a result, the antibiotic modifies and reduces the normal activity of the target. Bacteria react to this situation by introducing changes on target structure in order to prevent the antibiotic binding. Moreover, the changes introduced by bacteria still allow these targets to carry out its normal function. These processes generally involve genetic modifications, i.e., mutations. A single point mutation in the gene encoding may allow bacteria to provide resistance. Thus, the strains with this new genetic information can then proliferate. For instance, *S. aureus* able to incorporate the *mecA* (gene that encodes a PBP2′ protein with low affinity for all β-lactam antibiotics) offers the molecular base for the Methicillin-resistant *S. aureus* (MRSA) phenotype [22, 23] that is now widely disseminated.

In addition to the above-mentioned strategy, targets can be modified to protect them from antibiotics without the use of genetic mutation processes. For instance, Long et al. [24] identified that the chloramphenicol–florfenicol resistance (cfr) methyltransferase, which precisely methylates A2503 in the 23S rRNA; as a result, this provides resistance to a widespread variety of drugs that have targets nearby this position, including phenicols, streptogramins, pleuromutilins, lincosamides, and oxazolidonones (including linezolid).

In Table 2.1 are summarized few examples of the most extended antibiotics employed nowadays, their target and mode of action and finally the resistance mechanism developed by bacteria.

2.3.3 Macromolecular Antimicrobials

In view of all the mechanisms developed by bacteria to overcome the effect of antibiotics, there is an urgent need of novel antimicrobials [25]. In this context, as will be depicted throughout this book, synthetic polymers are currently being investigated as new molecular platforms to create alternative antimicrobial agents that could be active against drug-resistant bacteria [3, 26–28]. The versatility of the polymer chemistry allows for the fabrication of a variety of polymers with variable

Table 2.1 Targets, mode of action, and mechanisms of resistance of the main classes of antibacterial drugs

Antibiotic	Target	Mode of action	Resistance mechanism
Cell wall			
β-Lactams	Transpeptidases/ transglycosylases (PBPs)	Blockade of cross-linking enzymes in peptidoglycan layer of cell walls	β-Lactamases, PBP mutants
Vancomycin	D-Ala-D-Ala termini of peptidoglycan and of lipid II	Sequestration of substrate required for cross-linking	Reprogramming of D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser
Protein synthesis			
Macrolides of the erythromycin class	Peptidyl transferase, center of the ribosome	Blockade of protein synthesis	rRNA methylation, drug efflux
Tetracyclines	Peptidyl transferase	Blockade of protein synthesis	Drug efflux
Aminoglycosides	Peptidyl transferase	Blockade of protein synthesis	Blockade of protein synthesis
Oxazolidinones	Peptidyl transferase	Blockade of protein synthesis	Unknown
DNA replication/repair			
Fluoroquinolones	DNA gyrase	Blockade of DNA replication	Gyrase mutations to drug resistance

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backbones and functionalities that have been utilized to prepare antimicrobial polymers. Interestingly, some polymers, in particular bearing cationic groups, with high efficacy have been reported [29–32]. In addition, to the excellent activity against a broad spectrum of bacteria these polymers have shown low propensity for resistance development in bacteria [33]. This is, at least partially, due to the interaction mechanism of polymers with bacterial cells.

The main strategy for designing antimicrobial polymers has been determined taking into account the structural features of the cell membrane of bacterial cells. As has been depicted, the most important characteristic of the outer envelope of the cells is a net negative charge. As a result, considering as the target site the cytoplasmic membrane (so-called membrane active agents) antimicrobial polymers have been mainly designed as cationic hydrophilic–hydrophobic macromolecular systems [34, 35]. It is expected that macromolecular antimicrobials reduce the tendency of microbes developing resistance since they act on the microbial cell membrane and physically damage the membrane structure.

One of the pioneer works in attempting to correlate the structure of the antimicrobial polymer and the mechanism of interaction with bacteria was reported by Gilbert and coworkers [36–38] These groups investigated the mechanism of interaction

between a cationic polyelectrolyte salt polyhexamethylene biguanide chloride (PHMB) with *Escherichia coli*. In these pioneer works, the authors proposed a sequence of events during PHMB interaction with the cell envelope of *E. coli* was proposed as follows: (1) fast attraction of PHMB toward the negatively charged bacterial cell surface, with strong and specific adsorption to phosphate-containing molecules; (2) the structure of the outer membrane is impaired, and PHMB is attracted to the inner part of the membrane; (3) interaction between PHMB and phospholipids occurs, with an increase in inner membrane permeability to K^+ loss together with bacteriostasis; and (4) complete loss of membrane function. This fourth step occurs by precipitation of intracellular elements and finally a bactericidal effect [36–39].

More recently, Wimley [40] and Chan et al. [41] proposed different AMP-induced membrane disruption mechanisms. Wimley described the interaction macromolecule—bacteria by two main possibilities, i.e., **pore-forming** and **non-pore-forming** mechanisms. In the **pore-forming** mechanism, also known as transmembrane mechanism, the AMPs are inserted into the bacterial membrane thus forming aqueous pores across the membrane. The pore-formers AMPs induce the formation of stable pores in the outer envelope of the cells and disturb the homeostasis of the cell metabolism, eventually resulting in cell death. As depicted in Fig. 2.3, there are two main models, i.e., *barrel-stave* pore and toroidal pore model. The difference between the two relies on the fact that in the barrel-stave model, specific peptide–peptide interactions form the original approach for pore formation, which renders small nanopores (1–2 nm in diameter) [42]. On the other hand, the toroidal model does not involve specific peptide–peptide interactions and the role of the AMPs is to alter the curvature of the membrane. In this case, the diameter of the pores formed are larger (3–10 nm) in comparison with the barrel-stave model [43–45].

The second alternative for macromolecules (herein AMPs) to interact with bacteria is based on the **non-pore-forming** mechanism (Fig. 2.3c, d). In this case, AMPs interact in a parallel manner on the surface of microbial cells. Also two alternative models have been described for this mechanisms, i.e., the carpet model and the detergent model. Shai et al. [46] proposed that AMPs are active only on the bacterial membrane by forming a *carpet* on the bilayer surface that finally leads to large defects (larger than 10 nm pore size) on the bacterial membrane. Finally, the *detergent* model in which the AMPs induce a massive collapse of membrane integrity has also been employed to explain the antimicrobial mechanism of AMPs [41].

According to Chan et al. [41] in addition to the above depicted mechanisms, two other lesser known models, the molecular electroporation [47] or the sinking raft model [48, 49], can be important to explain the interaction mechanisms of antimicrobial peptides with bacteria. As shown in Fig. 2.4a, on the one hand, in the molecular electroporation model, the cationic peptides establish interactions with the membrane of the bacteria and generate an electrical potential difference across the membrane that finally forms upon reaching a critical potential value [47, 50]. On the other hand, the sinking raft model (Fig. 2.4b) suggests that an imbalance produced upon binding of the peptides to the membrane leads to an increase in the membrane curvature. Moreover, peptides are able to associate and penetrate inside the membrane producing transient pores [49].

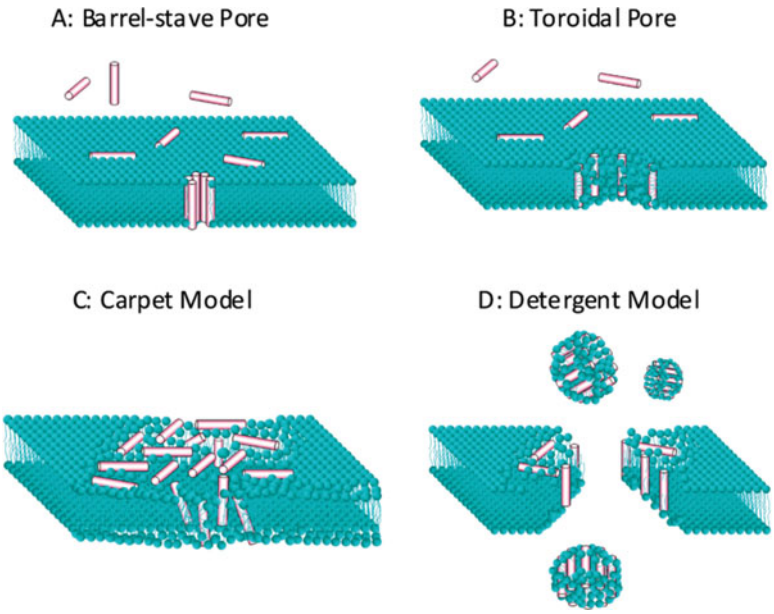

Fig. 2.3 Commonly cited models for antimicrobial peptide activity. Barrel-stave and toroidal pores are membrane-spanning aqueous channels. Antimicrobial peptides are described with the carpet model. Such peptides permeabilize membranes by "carpeting" the bilayer with peptides. At high concentrations, carpet model peptides can behave more like detergents. Reproduced with permission from [40]

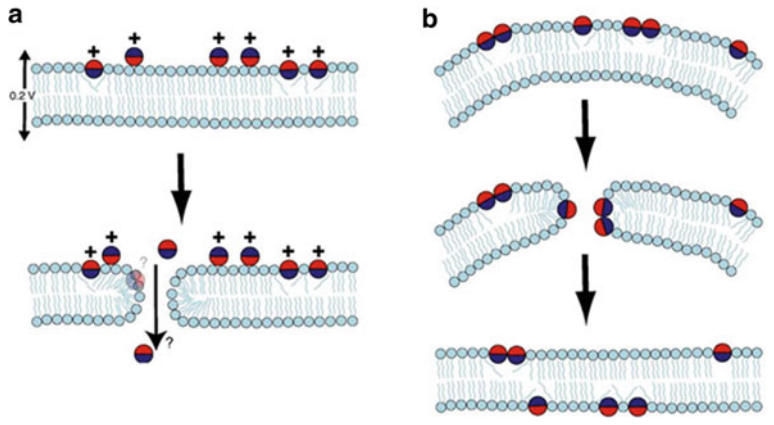

Fig. 2.4 (**a**) The molecular electroporation model and (**b**) the sinking raft model (adapted with permission from [41])

2.4 Biomaterials Surface: Device-Associated Infections

Microorganisms normally attach to both living and inert surfaces, including those of indwelling medical devices, finally leading to biofilm formation made up of extracellular polymers. In this state, microorganisms are highly resistant to antimicrobial cure and are strongly bonded to the surface. Therefore, a today's crucial issue in polymeric materials uses for biorelated applications involves the contamination by microorganisms and in particular bacteria. This problem affects many different areas ranging from such as medical devices, healthcare products, water purification structures, clinics, dental office tools, food storage, household sanitation, or food packaging just to mention a few of them [28, 51]. Moreover, applications free of bacteria surfaces include: dentistry (surface of acrylic resins) [52], implants [53], intraoral materials [54].

Bacterial contamination is still a common unresolved problem present in the major cases in which a biomaterial is required. While this is a general problem present independently of the biomaterial considered, it is even more serious in those cases in which durable implants are used. For instance, long-term catheters can produce implant-associated infections. Particularly critical are those cases in which the infections become resistant to antibiotics (those cases in which biofilm is already produced), and the implant need to be removed. Depending on the implant and the infection created by the microorganism can be even critical since the antibiotics cannot be effectively delivered. The impact of implant failures on the entire population and on the costs for the national health systems is enormous. This effect is above all significant for septic failures, when microbial infections grow on biomaterial surfaces. Subsequently to an initial occupation, bacterial biofilms establish on contaminated surfaces, critically compromising the performance of the implant itself, recruiting inflammatory cells, affecting the integration in the neighboring tissues, but in addition exposing the patient to a serious risk of general infections, septicemia, and in some cases, decease. Moreover, once the bacterial biofilm has been formed, conventional medical therapies based on universal antibiotics are not efficient and implant removal often represents the only chance to eradicate the infection. Thus, to better know and control biofilms in the case of indwelling medical devices, researchers should develop consistent sampling and measurement methods, study the role of biofilms in antimicrobial drug resistance, and establish the relationship between biofilm infection and patient contamination [55].

While this is true, biomedical devices are a vital part of the human healthcare system. For instance, the quantity of artificial hip and knee implants has improved significantly during the last decades, and heart valves, stents, vascular grafts, and other implants devices have been used widely to protect lives and to reestablish the quality of life for many people. For instance, according to the Freedonia Group, the demand in the USA for implantable medical devices is projected to rise 7.7 % annually to $52B in 2015 [56]. Polymers are expected to be the fastest increasing class of materials between 2013 and 2019, mainly due to the rising applications of these biomaterials and numerous advantages over metals that include longevity, elasticity,

flexibility, biocompatibility, and bio-inertness. The use of polymers, for instance, polyurethanes (PUR) and polytetrafluoroethylene (PTFE), is being popular for synthetic vascular grafts, whereas the extended use of polymers in ophthalmology is estimated to grow accordingly with the increasing amounts of ophthalmic illnesses and continuous demand from geriatrics.

2.4.1 Adhesion, Adherence, and Attachment

Before proceeding to analyze the factors involved in the bacterial adhesion to solid substrates (such as living tissues or biomaterials) it is worth analyzing few concepts that will be later employed and have in ambiguously employed in the literature. As described by An and Friedman [57] bacterial adhesion refers to a situation in which bacteria are strongly adhered to the biomaterial surface by physicochemical interactions. These are the result of an initial reversible physical contact and a subsequent irreversible chemical and cellular adherence. Therefore, an energy has been employed to form interactions between the bacteria and surfaces.

According to these authors, adherence should be applied to describe the initial process of bacterial attachment directly to a surface. This term has been employed in a less scientific environment to refer bacterial adhesion. Finally, attachment is associated to the initial stage of bacterial adhesion which are reversible and, thus, refers more to physical contact than to chemical and/or cellular interactions.

2.4.2 Bacterial Adhesion to Biomaterials Surfaces

The first step in the pathogenesis of foreign-body-related infections is the bacterial adhesion that, in general, leads to colonization. Moreover, the early phases of microbial adhesion on biomaterial surfaces that will lead to biofilm formation depend on the contamination route followed by the microorganism. On the one hand, contamination may occur in a dry state by direct transfer from a contaminated material. On the other hand, contamination is produced by either airborne bacteria or by the contact with physiological fluids in wet conditions. As reported by Campoccia et al. [58] contamination by airborne bacteria or by contamination transfer can be reduced or completely avoided by implementing aseptic procedures and by precisely controlling the manipulation protocols of sterile devices [59].

More complicated to prevent are those infections produced by contaminations transferred from liquid carriers. These include physiological fluids, such as blood and serum, or artificial low protein content solutions including saliva or urine. As will be depicted, in this case, bacterial adhesion cannot be prevented by using aseptic protocols. However, there are a number of variables that are involved in the bacterial adhesion that can be identified and applied to reduce contamination. These

parameters are the type of pathogen the physiological fluid involved but also several parameters related to the biomaterial interface [60].

In one of the first reviews devoted to this topic, An et al. [57] reported that the bacterial adhesion phenomenon is a two-phase process. The phase one concerns an initial, instantaneous, and reversible physical adhesion of bacteria to biomaterial surfaces. In phase two, a time-dependent and irreversible molecular and cellular phase are formed. These two phase approach was first proposed by Marshall and colleagues [61, 62] but has been accepted by the majority of researchers [63]. The most prominent results of the analysis of the process leading to bacterial adhesion and biofilm formation on biomaterial surfaces have been recently reviewed among others by Arciola et al. [64, 65], describing the possible implications for the development of biofilm-resistant materials.

These reports indicated that bacterial adhesion on biomaterial surfaces take place through multiple mechanisms, were certain are affect all microbial species, while others are species-specific or even strain-type specific [58]. Mechanisms that involve different bacterial species without any specificity finally leads to passive adsorption of the bacterial cells at the surface of the polymeric material by means of physicochemical surface interactions and are usually observed in the initial adhesion stages. On the other hand, strain-specific adhesion, also known as active mechanisms of adhesion are mediated by bacterial structures termed bacterial adhesins [66, 67].

2.4.2.1 Phase One in Bacterial Adhesion

As mentioned above, the initial interactions between bacteria and a solid surface are nonspecific in nature. In this phase, bacteria are, therefore, passively adsorbed onto the material surfaces [65]. These bacteria–surface interactions are established by different forces including hydrophobic, electrostatic, Van der Waals forces as well as hydrogen bonding [58]. In particular, bacterial behavior is strongly influenced by surface hydrophobicity as well as the electrostatic charge. As a result, both functional groups and chemicophysical properties displayed by the biomaterial surface that will interact with those of the bacterial cells determine the kinetics of microbial adhesion.

A large amount of different factors such as surface morphometry or environmental conditions play additionally a key role on these initial stages (Table 2.2). Even fluid flow rate has however been observed to have a direct influence on the bacterial adhesion kinetics [11].

2.4.2.2 Phase Two in Bacterial Adhesion

In addition to passive bacterial adsorption that spontaneously occurs on almost all biomaterial surfaces, active stable anchorage of the bacterial cells can be established by adhesins [65]. Adhesins are able to bind of host proteins previously adsorbed onto the biomaterial surface. As depicted by Montanaro et al. [68] and

Table 2.2 Variables influencing bacterial adhesion and colonization on biomaterial surfaces

Surface morphometry	Macroporosity
	Microporosity
	Micro-roughness
	Nano-roughness
Physicochemical properties	Surface energy
	Hydrophylicity/superhydrophylicity
	Hydrophobicity/superhydrophobicity
	Hydrophobic functional groups
	Polar functional groups
	Charged functional groups
	Functional groups with specific activities
	Degree of hydration
Environmental conditions	Electrolytes
	pH
	Temperature
	Host proteins/host adhesins
	Shear rate/fluid viscosity
	Fluid flow rate
Pathogen	Gram-positive/Gram-negative
	Genus/species
	Bacterial shape
	Surface energy
	Strain type and specific set of expressed adhesins

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Patti et al. [69], host proteins are usually represented by receptorial proteins named "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs). These host proteins, also named "host adhesins" for their function, include elastin, fibronectin, collagen, fibrinogen, vitronectin, laminin, clumping factor A and B, bone-sialoprotein, IgG. Nevertheless, other still unknown components of the extracellular matrix may also participate in this process.

One of the pioneer studies evidencing that specific proteins mediate the binding to abiotic surfaces was reported by Heilmann et al. [70]. These authors reported that autolysins (enzymes present at the bacterial surface) possess a double function: enzymatic and adhesive and their structure depends on the bacterial strain. For instance, in *S. aureus*, Foster [71] found that the autolysin/adhesin is AtlA, a 137 kDa protein, highly homologous to AtlE. Similarly, in *S. epidermidis*, Heilmann et al. [70] reported that the major autolysin/adhesin is AtlE, a 148 kDa protein, which mediates attachment to polystyrene.

Finally, it is worth mentioning that adhesins can also intervene in the process of bacterial internalization into host cells [58]. The adhesins mentioned above, i.e., AtlA and AtlE, due to the glycine-tryptophane dipeptide repeats, participate both in the surface association and biofilm formation but also they play a key role on staphylococcal internalization by host cells [72].

2.4.3 *Biofilm Formation*

The term biofilm has been defined differently. According to Taraszkiewicz et al. [73] microbial biofilms can be defined as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface." Donlan [74] defined biofilm as an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface. Thus, bacterial biofilms are formed when single organisms come together to generate a larger cell community that will be, in turn attached to a surface and covered by polysaccharide membrane.

Biofilms are usually formed on the surface of synthetic materials such as medical devices, catheters, artificial hips, or contact lenses. However, they can be equally built on living tissues. Examples of living tissues that can be covered by biofilms include endocardium, wounds, and the epithelium of the lungs, particularly in cystic fibrosis patients [75, 76]. The biofilm comprises a matrix (in charge of the structural stability but also protection against adverse environmental conditions) mainly formed by polysaccharides, but also by proteins and extracellular microbial DNA. Moreover, in the same biofilm several microbial species bacterial or fungal can simultaneously coexist. This highly organized structure causes a multitude of problems in the medical field, particularly in those cases related to prosthetic devices such as endotracheal tubes or indwelling catheters [77]. Moreover, these infections are very difficult to be eradicated by conventional antibiotic therapy.

The role of the biofilms can be summarized in three different aspects [78]. First of all, biofilms provide intercellular signaling and communications pathways. In addition, biofilms assist bacteria to evade and deceive the immune system, one of the roles of the latter being detecting and eliminating pathogens. Finally, and most importantly, biofilms protect bacteria from antibiotics and other toxins. According to Chandra et al. [79], biofilm formation happens in three main stages:

(a) Biofilms at the early stage
In the first stage, bacterial cells approximate the surface using their flagella or directed by body fluids [80]. The contact is established and a monolayer of cells is positioned at the surface (Fig. 2.5a, b) [81–83]. In this situation, the bacterial cells can be reversibly detached, and more importantly they are susceptible to antibiotics. As will be depicted, biofilm formation limits the success of generally employed antibiotics.

(b) Intermediate stage
The initial reversible interactions between the bacteria and the surface are irreversible in the next step. This stable situation allows the bacterial cells to grow and multiplicate forming small, micrometer size colonies (Fig. 2.5c, d). According to Stephens [80], the biomaterial surface promote physiological adaptations, including secretion of exopolysaccharides (EPSs) to create a protective matrix surrounding the cells [84]. As a result, the colonies are composed

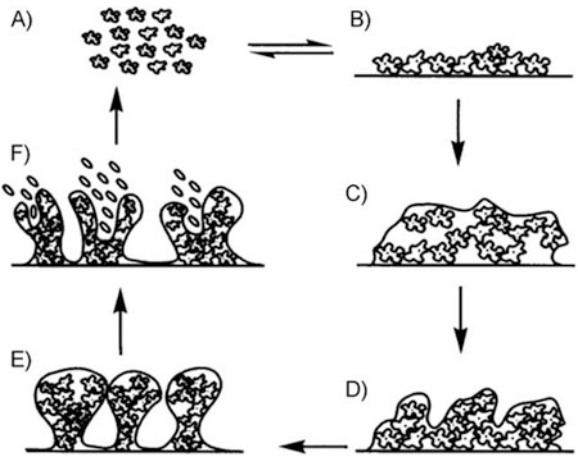

Fig. 2.5 Biofilm growth cycle: (**a**) Planktonic bacteria, (**b**) reversibly attached to a surface suitable for growth, (**c**) bacteria begin secretion of the EPS and attachment becomes irreversible, (**d**) the maturing biofilm begins to take a three-dimensional shape, (**e**) the biofilm fully matures, and a complex architecture is observed, (**f**) bacteria disperse from the biofilm to reinitiate biofilm colonization of a distal surface. Reproduced with permission from [78]

of a mixture of polymeric compounds, mainly polysaccharides (the matrix gives 50–90 % of the organic matter in biofilms) [85]. Nevertheless, the biofilm matrix is a rather complex material [86, 87] formed by:

- Polymers secreted by microorganisms within the biofilm.
- Cell lysis products, i.e., macromolecules including nucleic acids polysaccharides and proteins.
- Absorbed nutrients and metabolites.
- Peptidoglycan, lipids, phospholipids, and other cell components.

A crucial mechanism, critical at this stage, that regulates the biofilm formation is the quorum sensing (QS) [28–31]. The QS mechanism is related to the communication between microbial cells. More precisely, QS mechanism is a process that regulates back and forth the gene expression of those genes required for the formation and maturation of the biofilm. Some studies have evidenced that gene production is activated when a particular bacterial density is achieved and is retarded when the density is low. Hooshangi and Bentley [88] demonstrated that this process is regulated by signaling molecules and identified three well-defined groups in bacteria oligopeptides, acyl homoserine lactones (AHLs), and autoinducer-2 (AI-2).

(c) Mature stage

The final stage involves the formation of a mature biofilm clearly distinguished by the formation of mushroom-shaped colonies (Fig. 2.5e). The mature biofilm can be partially disrupted in order to promote the delivery of microbial cells.

The latter are able to swim to other surface areas and promote the biofilm formation in a non-contaminated zone (Fig. 2.5f).

As a result, in order to fabricate antibacterial/antifouling surfaces one of the key steps is the prevention of bacterial adhesion and thus biofilm formation. These two objectives have been typically pursued using different strategies to modify the surface and render the polymeric material antimicrobial.

2.4.4 Antibiotic Resistance of Bacteria in Biofilms

In the previous paragraph, it has been anticipated that antibiotics are only effective on the initial stages of bacterial adhesion but do not exhibit any influence in mature biofilms [89]. For example, Anderl et al. [90] estimated that a β-lactamase-negative strain (obtained from *K. pneumonia*) had a minimum inhibitory concentration of 2 μg/mL ampicillin in aqueous suspension. The same strain, when grown as a biofilm, was poorly affected (66 % survival) by 4 h treatment with 5000 μg/mL ampicillin, a quantity that eliminated free floating bacteria. Moreover, when bacteria are dispersed from a biofilm they quickly become vulnerable to antibiotics [91, 92]. This fact evidenced that resistance of bacteria in biofilms is not only acquired via mutations or mobile genetic elements [93–95] nor is due to efflux pumps [96]. Therefore, in contrast to the antibiotic resistance mechanisms depicted above such as efflux pumps, modifying enzymes, or target mutations [7] biofilm resistance should additionally be influenced by other processes.

According to Mah and O'Toole [76], three main hypothesis can explain the mechanisms of resistance to antibiotics in bacterial biofilms.

The first hypothesis is related to the slower or incomplete penetration of the antibiotic into the biofilm. While it is true that measurements on the antibiotic penetration revealed that there is no generic barrier to the diffusion of solutes through the biofilm matrix [97, 98], it is also true that, in some cases, if the antibiotic is neutralized in the biofilm, infiltration can be deeply retarded. For example, Anderl et al. [90] demonstrated that ampicillin is able to infiltrate through a biofilm made by a β-lactamase-negative strain of K pneumonia but not a biofilm formed by the β-lactamase-positive wild-type strain of the same microorganism. In the wild strain biofilm, the antibiotic is deactivated in the surface layers more rapidly than it diffuses [99–102].

The second hypothesis is related to the altered chemical microenvironment within the biofilm that can, in some cases change an aerobic environment into anaerobic. Debeer et al. [103] demonstrated that oxygen can be totally consumed in the superficial layers of a biofilm, leading to anaerobic areas in the deep layers of the biofilm. In this context, aminoglycoside antibiotics are clearly less effective against the same microorganism in anaerobic than in aerobic conditions [104]. In addition to the oxygen content, local accumulation of acidic waste products might lead to important pH differences between the bulk fluid and the biofilm interior, which could directly antagonize the action of an antibiotic [105]. Equally, the deple-

tion of a substrate or accumulation of an inhibitive waste product that might cause some bacteria to enter a non-growing state [106]. Finally, variations on the osmotic environment within a biofilm may induce an osmotic stress response. Such a response could contribute to antibiotic resistance by altering the relation of porins in a way that reduces cell envelope permeability to antibiotics [107].

A third and still controversial mechanism of antibiotic resistance is related to the unique characteristics of biofilms that form a highly protected, phenotypic state. This is true for some cases while other findings contradict this model. For instance, newly formed biofilms can show resistance even if their barriers to penetration are too thin to either an antimicrobial agent or metabolic substrates [108, 109] (Fig. 2.6).

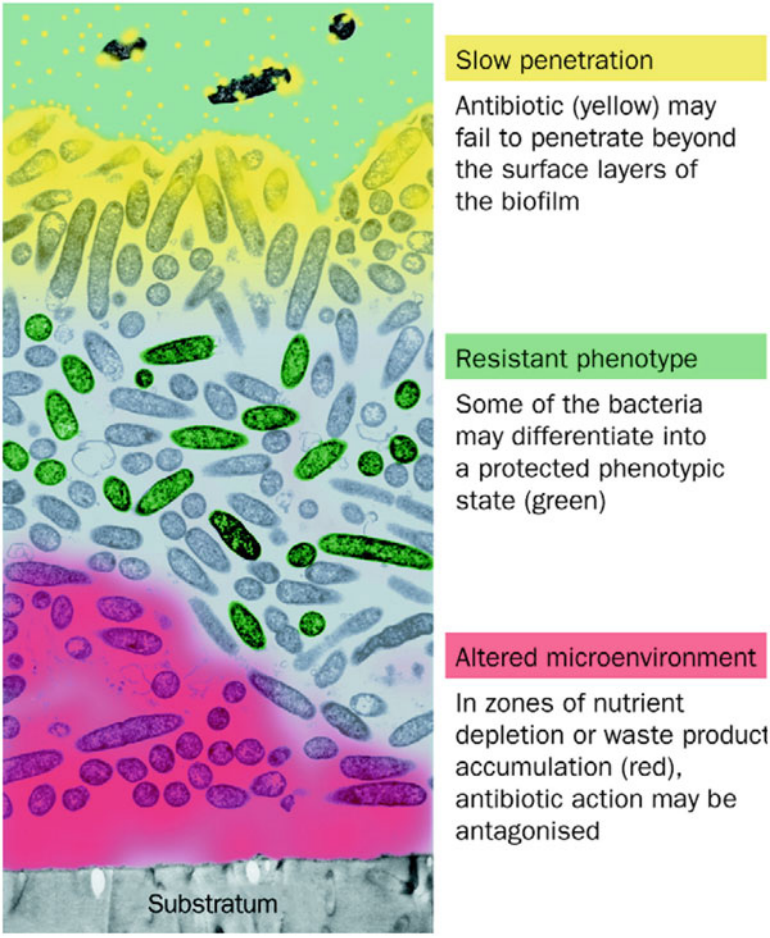

Fig. 2.6 Currently proposed hypotheses for mechanisms of antibiotic resistance in biofilms The attachment surface is shown at the bottom and the aqueous phase containing the antibiotic at the top. Reproduced with permission from [89]

2.4.5 *Approaches Developed to Achieve Polymeric Biomaterials with Antibacterial Properties*

The large amount of requirements that antibacterial biomaterials need to fulfill are very broad. In particular, these depend on the final application and, of course, they should resist microorganism and mainly bacterial infections. The strategies developed to produce antibacterial surfaces and interfaces that will be thoroughly described in Chaps. 5 and 6 are summarized in Fig. 2.7. These include the fabrication of surfaces with low adhesion or bacterial repulsion, the incorporation of compounds with bactericidal activity or the attack to bacterial surviving mechanism (quorum sensing existing between bacteria, the modulation of the host immune system, or the interference with bacteria).

2.4.5.1 Bacteria Repelling and Antiadhesive Surfaces

The first strategy to prevent biofilm formation involves the development of alternatives to prevent bacteria to adhere to the material surface. In this case, a thorough analysis of the contamination route, i.e., whether contamination occurs in a dry state by direct mechanical transfer through contaminated objects and by airborne bacteria or in wet conditions, if contamination occurs by the contact with physiological fluids. Whereas, direct airborne bacteria or mechanical deposition of bacteria can be reduced to a large extent by following strict aseptic procedures during manipulation contamination by the contact with physiological fluids that cannot be completely removed [59].

2.4.5.2 Bioactive Materials with Intrinsically Antibacterial Properties

Surface functionalization with materials exhibiting antibacterial properties is also an extended alternative to reduce bacterial contamination. This functionalization can be achieved by immobilizing antibacterial compounds or delivering biocides.

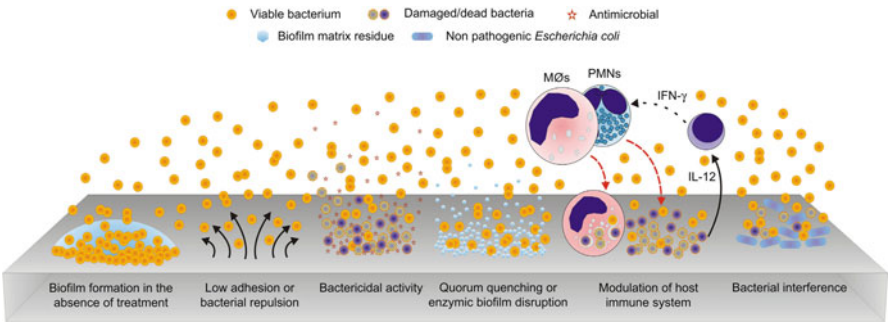

Fig. 2.7 Strategies designated to contrast the establishment of infections on medical devices. Reproduced with permission from [58]

For instance, silver has been described as one of the earliest materials to be intentionally used in surgery for its bactericidal properties. In addition to the material functionalization, bulk materials are described as intrinsically antibacterial when they exhibit an antibacterial action in the absence of modifications, such as loading with bactericidal molecules or coating with active biocides.

2.4.5.3 Materials Incorporating Bioactive Molecules Interfering with the Production of Bacterial Biofilm

Taking advantage of the continuous advances in the understanding of the molecular mechanisms underlying biofilm formation of different bacterial species has opened new alternatives to reduce the contamination on biomaterials surfaces [110, 111].

These approaches rely on the grafting or release of active substances conferring the surface antibiofilm activity [112]. As reviewed by Campoccia et al. [58], these surfaces may be decorated with active substances involved in different mechanisms:

(a) enzymes capable of selectively degrading extracellular polymeric substances of the biofilm (e.g,. Dispersin B, rhDNase I)
(b) bactericidal molecules capable of killing even metabolically quiescent bacterial cells inside biofilms (e.g., lysostaphin, AMPs)
(c) molecules interfering with the Quorum sensing system and inducing biofilm dispersion (e.g., furanones)
(d) molecules downregulating the expression of biofilm extracellular polymeric substances (e.g., *N*-acetylcysteine) or nevertheless reducing the biofilm metabolism (e.g., hamamelitannin)

For a detailed description of the alternatives to reduce bacterial contamination using bioactive compounds, the reader is referred to the following references [113–120].

2.5 Conclusions

In this chapter, we have revised the mechanisms involved in the antiobiotics–bacteria interactions. In contrast to traditional antibiotics to which bacteria can easily develop resistance, macromolecular antimicrobials have emerged as an interesting alternative to overcome this issue. Macromolecules, due to their large molecular weight, associated to particular functional groups (in general positively charged) establish interactions and alter the processes occurring in the cell membrane. As a result, by different mechanisms (following pore or non-pore-forming models) the permeability of the membrane is altered and finally provokes cell apoptosis.

The biofilm formation on the surface of polymeric biomaterials, also a major remaining problem among others in implant-associated infections, has also been considered in this chapter. Resistance in biofilms occurs, in addition to those

depicted for single bacteria, by other mechanisms such as changes on the environment, limiting diffusion or modifying bacteria. As a result, the fabrication of antibacterial surfaces is focusing on reducing or completely avoiding the initial bacterial adhesion.

The following chapters will be devoted to the different methodologies and strategies developed to fabricate polymeric materials with antimicrobial activity in solution and at surfaces of, for instance, planar rigid polymers or soft membranes but also on fibers or topographically structured surfaces.

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