Chapter 1
Introduction to Antibacterial Surfaces

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Abstract Colonisation of surfaces by bacteria occurs commonly in the environment. When this colonisation occurs on materials that are used in modern civilization, it is most often a detrimental process. This can range from bacteria being responsible for the infection of medical implants or tissues causing infection to humans or animals, to biological layers being built-up on ships or in air-conditioning systems causing increased drag and fuel costs. In each case, the result is undesirable, and therefore it is highly desirable to identify ways by which the growth of bacteria on surfaces can be eliminated or controlled. This can be achieved through preventing the initial adhesion of cells, and/or by killing any cells that are able to attach to the surface. In this chapter, a brief overview is provided regarding some of the issues associated with the attachment of bacteria to surfaces, together with a description of the main strategies currently being employed for controlling the initial attachment processes. These strategies will be expanded upon in the subsequent chapters.

Keywords Antibacterial surfaces • Antibiofouling • Mechanobactericidal surfaces • Superhydrophobicity • Antibiotic resistance • Biofilm formation

1.1 The Definition of Antibacterial Surfaces

As a concept, the term ‘antibacterial surface’ is generally well understood. There are, however, one or two subtle clarifications to this term that should be addressed, for the sake of properly defining the term for its use in the subsequent chapters. The term ‘antibacterial surface’ applies to the surface of any material or agent that works to prevent or limit the growth and proliferation of bacteria (Hasan et al. 2013). This is an important distinction, as the word ‘antibacterial’ is commonly understood as being the effective of killing bacterial cells. Bactericidal surfaces certainly fall

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under the umbrella of being 'antibacterial', however 'bacteriostatic' and 'antibiofouling' surfaces can also be regarded as 'antibacterial' in their action. Each of these concepts will be addressed in greater detail in the following sections.

1.2 The Ancient Battle Against Bacteria

Bacteria are ubiquitous, being found in most places on the Earth and therefore it is necessary for all other organisms to develop strategies to coexist with them. These strategies vary greatly, from some organisms having developed self-cleaning surfaces that limit their need to interact with bacteria, the development of chemical defence mechanisms such as an immune system, and developing the ability to utilise bacteria as a food source. Mankind is not exempt from this requirement. Throughout history, humans have battled against the detrimental effects associated with bacteria, some of which have been, and are still responsible for some of the most deadly diseases known. The Bubonic Plague, also known as 'Black Death', resulted from humans being infected by *Yersinia pestis*, a bacterium from the *Enterobacteriaceae* family. This plague is famous for killing a third of the population of Europe (approximately 25 million people) in the fourteenth century (Haensch et al. 2010). It was suggested that the Austrian composer Mozart died as a result of a streptococcal infection at the age of 35 (Zegers et al. 2009). Of course, bacterial diseases continue to be a major concern in modern times. *Mycobacterium tuberculosis*, the bacterium responsible for tuberculosis, infected 8.6 million people in 2012, leading to 1.3 million deaths (World Health Organization 2015). In addition, post-operative infections caused by bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are a leading cause of death from complications arising after surgery (Boles and Horswill 2011; Lowy 2003; Klevens et al. 2007).

Pathogenesis and disease, while certainly being extremely important, are not the only undesirable bacteria-associated problems. Food spoilage by bacteria is commonplace, and many food preservation techniques such as salting and pasteurisation were developed in an effort to preserve food for greater periods of time. Adhesion of bacteria and their subsequent multiplication on the surfaces of substrate materials can lead to a wide range of problems, usually in the form of added mass, blockage or in the reduction of aero- or hydrodynamic properties. For example, the shipping industry incurs significant expenses in cleaning ships where bacteria and other organisms have attached to their hulls, causing decreased fuel efficiency (Schultz et al. 2011). Bacterial growth in water-cooled air conditioning systems can result in a loss of heat transfer efficiency and therefore results in an overall decreased effectiveness of the device (Ager and Tickner 1983; Gilbert et al. 2010). Many bacteria have also now been implicated in the phenomenon of biocorrosion, whereby cells that have attached to metallic surfaces (e.g. copper water pipes) lead to the corrosion of the material through the formation of 'pits' in the surface (Beech and Sunner 2004; Gu et al. 2009a, b). The problem of how to control bacterial adhesion and growth is one that has been posed for a long time, and has no simple solution has, as yet, been identified for a number of reasons, discussed as follows:

1.2.1 Extreme Adaptability of Bacteria

The first and most important factor that makes bacteria difficult to control is their high metabolic and physiological adaptability (Bos et al. 1999; Callow and Callow 2002). Bacteria have a high propensity for acquiring new genetic material. They generally can double their population in a short time, with each generation having the potential to develop new mutations, which may be beneficial, detrimental or neutral to the further proliferation of the bacteria. Horizontal gene transfer is also commonplace among bacteria, whereby genetic material is exchanged in the form of plasmids via pili linkages (Dalton and March 1998; Kjelleberg and Molin 2002; Daniels et al. 2004). Some bacteria are capable of taking up extracellular DNA and incorporating it into their transcriptomes and some viruses have the ability to introduce new genetic elements into a bacterial cell via transfection (Thomas and Nielsen 2005; Ochman et al. 2000). Together, these factors enable bacteria to tune their genetic makeup, and through natural selection they can exploit changes in their environment to establish an ecological niche. This has led to the ability of bacteria to colonise a wide variety of environments, including the harsh conditions present in deep sea vents, and their ability to utilise numerous sources of carbon (Baum et al. 2002; Hay 1996).

1.2.2 Antibiotic Resistance

Antibiotic resistance is a growing public health concern, which has arisen as a direct result of the high adaptability of bacteria. Through one of the mechanism listed in Sect. 1.2.1, bacterial cells may acquire the necessary properties to escape the actions of one or more antibiotics. Administration of antibiotics then eliminates the competition to the antibiotic resistant cells, which can then flourish (Fig. 1.1). Naturally, further doses of the same antibiotic no longer have an equivalent effect (Gilbert et al. 2010; Lowy 2003; Simões et al. 2009).

The World Health Organization considers antimicrobial resistance to be one of the three greatest threats to human health (World Health Organization 2014). In addition to this increased resistance, causing a decreased ability to treat established infections, rising antimicrobial resistance has flow-on effects to other treatments that rely on antibiotics to prevent infections. For example, any immunosuppressive therapies, such as chemotherapy, and implantation of biomedical devices become considerably more dangerous without antibiotics.

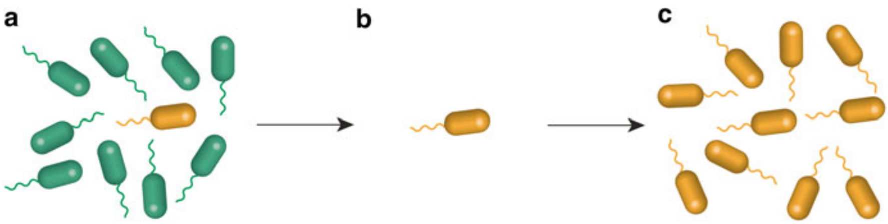

Fig. 1.1 (**a**) Within the natural microbiome, any given bacterium may acquire a gene resistance to a given antibiotic. (**b**) On administration of the antibiotic, all of the sensitive cells are eliminated, removing any nutritional competition for the resistant cell. (**c**) The resistant cell can then flourish, and the microbial community regenerates itself, this time with widespread antibiotic resistance

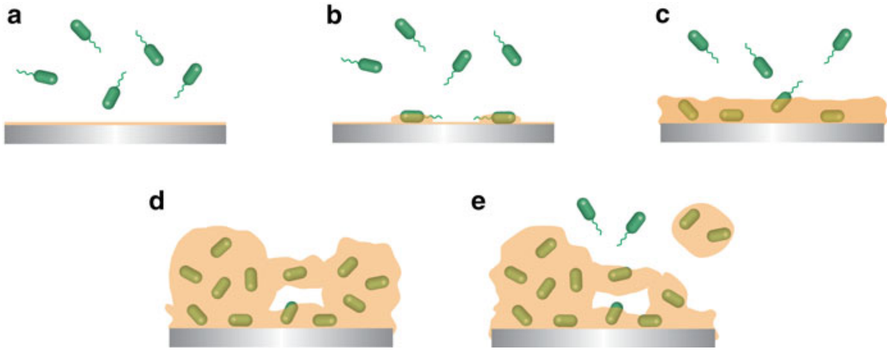

Fig. 1.2 (**a**) The lifecycle of a biofilm begins with the deposition of a conditioning layer composed of organic material, often produced by the cells in the surrounding suspension. (**b**) The conditioning film aids in the initial attachment of cells, even to surfaces that are otherwise unfavourable for bacterial attachment. These cells then begin to produce and excrete extracellular polymeric substances (EPS). (**c**) The attached cells proliferate and continue to produce EPS, forming a more continuous layer and attracting further cells to the surface. (**d**) The mature biofilm consists of numerous cells embedded within a matrix of EPS with a defined three dimensional structure. (**e**) Finally, pieces of the biofilm or individual cells are released into the surrounding environment and are able to colonise new areas

1.2.3 Biofilm Formation

Biofilms are three-dimensional communities of bacteria embedded within an extracellular matrix that is usually composed primarily of polysaccharides. Formation of biofilms is an old and very effective method by which bacteria grow and proliferate. The initial formation begins with the colonisation of a surface by individual cells, which may be the same or different species. The cells that initially adhere to the surface start to grow and divide, and once the cell density reaches a certain level, the cells secrete extracellular polymeric substances (EPS) that form the biofilm matrix. As the biofilm matures, it develops channels to allow the intake of nutrients and the expulsion of wastes. At the final stage of the biofilm cycle, small pieces or individual cells either break off or are released from the biofilm, and may travel to and colonise new regions (Fig. 1.2). Biofilms provide exceptional mechanical and chemical resistance to the cells that they contain, and therefore once biofilms have been formed, they are exceedingly difficult to remove (O'Toole et al. 2000; Schmidt et al. 2012).

1.3 Strategies for Coping with Bacteria

Much progress has been made in preventing or mitigating the detrimental impact of bacterial contamination over a long period of time. The ancient Egyptians, Greeks and Aztecs all used copper-containing compounds and alloys as methods for sterilising wounds and drinking water (Michels et al. 2005). Silver has also proven to be highly effective at killing bacterial cells (Ivanova et al. 2011; Rai et al. 2009). The discovery of penicillin, in particular, was a huge step forward in fighting bacterial infections (Chain et al. 1940). This opened the door for the production of several generations of antibiotic compounds, subsequently revolutionising medicine.

The production of surfaces that are specifically antibacterial in their action is a somewhat more contemporary approach, and in recent times the field has enjoyed increased interest from researchers, with numerous advances made in this technology. With the recognition of the fact that once established, biofilms are extremely difficult to eradicate came the trend of developing methods for the prevention of bacterial attachment, rather than identifying ways to remedy surfaces that contained attached biofilms. Inspired by the work of Bartholott and Neinhuis in 1997 on the lotus leaf, many researchers have focussed on the investigation and production of surfaces that are able to remain clean in a similar way to the lotus (Barthlott and Neinhuis 1997). Such self-cleaning surfaces typically have special properties with regard to their wettability, i.e. superhydrophobicity, which aids in the removal of contaminants. Several other natural surfaces that possess similar properties have since been discovered (Gao and Jiang 2004; Watson et al. 2011; Bixler et al. 2014; Sun et al. 2009), and many more have been artificially fabricated (Zhang et al. 2006; Lee et al. 2004; Guo et al. 2012). Surfaces such as these, that prevent the adhesion and attachment of microbes, are termed 'antibiofouling surfaces', or often simply 'antifouling surfaces'.

Prior to the research being carried out on the development of antibiofouling surfaces, the common approach for developing antibacterial surfaces was to evoke bactericidal effects, i.e. to modify or functionalise the surface so that it had the capability to quickly kill bacterial cells that came into contact or close proximity to the surface, preventing them from proliferating and initiating the formation of a biofilm. Coating or functionalising surfaces with compounds known to be bactericidal, e.g. silver or antibiotics (Gazit 2007; Qi et al. 2004; In et al. 2007; Maness et al. 1999), was an early effective approach, however leaching and releasing bioactive components often occurs, which may present biosafety challenges and reduction in the longevity of the material. Polycationic polymers have also been shown to be effective bactericidal materials that possess some degree of durability (Haldar et al. 2008; Yang et al. 2014). Bactericidal surfaces have again come into focus with the discovery of a class of materials that have a novel mechanism for killing cells. The wing of the *Psaltoda claripennis* cicada was the first material reported to kill bacterial cells as a result of the physical nanostructure of the surface, with no apparent influence arising from the chemistry of the surface (Hasan et al. 2012; Ivanova et al. 2012; Pogodin et al. 2013). This mechanism by which this bactericidal activity was achieved was referred to as a 'mechanobactericidal' effect. Other insect wings have since been determined to be bactericidal through using a similar mechanism, and a bioinspired silicon analogue has also been reported (Ivanova et al. 2013).

1.4 Summary

The bacterial colonisation of surfaces can lead to many detrimental effects on human health, industry and the environment. Therefore, a technological need exists for the development of materials whose surfaces resist the build-up of bacteria. A range of approaches are available for the design of such surfaces, and in the following chapters a wide variety of antibacterial surfaces will be described. The prevailing mechanisms that determine their effectiveness, methods of fabrication in the case of synthetic surfaces, and an analysis of the applications in which these types of surfaces are likely to find use, will be discussed.

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