Chapter 7
Cytotoxic Effects and Biocompatibility of Antimicrobial Materials

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Abstract The rising demand for medical implants for ageing populations and ongoing advancements in medical technology continue to drive the use of implantable devices. Higher implant usage has a consequent increased incidence of implant-related infections, and associated prolonged patient care, pain and loss of limb and other organ function. Numerous antibacterial surfaces have been designed that prevent the onset of biofilm formation, thus reducing or preventing implant-associated infections through inhibiting bacterial adhesion or by killing the organisms that successfully attach to the surface of the implant. Other surfaces have been designed to stimulate a local immune response, promoting the natural clearing of the invading pathogen. The desired antibacterial effects are typically achieved by modulating the surface chemistry and morphology of the implant material, by means of the controlled release of pharmacological agents and bioactive compounds from the surface of the material, or by a combination of both processes. An important issue for any type of antibacterial surface modification lies in balancing the non-fouling, bacteriostatic or bactericidal effects against local and systemic biocompatibility. In this chapter, we will first describe the concept of biocompatibility and its evolution, from devices that do not evoke a negative host response to those that actively drive host regeneration. We will then review the challenges associated with merging the need for an implant material to withstand a bacterial load with those associated with supporting function restoration and tissue healing.

Keywords Bacteriostatic • Bactericidal • Systemic biocompatibility • Cytotoxicity • Inflammation • Host regeneration • Tissue healing

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7.1 Introduction

Any surgical intervention carries a risk of infection. Whenever the skin, a natural barrier to infection, is perforated or incised, a significant opportunity is created for infective agents to enter the body. The likelihood of developing an infection is dependent on a multitude of procedure- and patient-related factors (Phillips et al. 2014). These include the type, invasiveness, duration and complexity of the surgery (Olsen et al. 2008; Neumayer et al. 2007). Minimally invasive surgery techniques have been found to notably reduce post-operative wound infections by as much as tenfold in the case of minimally invasive versus open spinal surgery (O'Toole et al. 2009).

The skill of the operating team, the cleanness of the surgical environment and the use of pre- and post-surgery antibiotics have also shown to vastly reduce, if not fully eliminate the patient's risk of being infected (Ridgeway et al. 2005; Sharaf et al. 2011). Indeed, it is very difficult to create a perfectly bacteria-free operating theatre and thus avoid at least low-cell-load bacterial contamination of the wound space. Whilst strategies can be used to lower the incidence of surgical wound infections that may occur though one process, this same strategy may inadvertently increase the risk of infection through another pathway. For example, intraoperative warming has been shown to reduce postoperative infections in patients undergoing major orthopedic, general, or urologic surgery by maintaining a cutaneous blood flow, increasing tissue viability and reducing the incidence of pressure sores. When intra-operative warming has been performed using forced-air devices, however, the moving air may act as a vector for the transfer of infectious agents, causing unwanted airflow disturbances (Weissman and Murray 2013; Augustine 2014).

Interestingly, in a large proportion of surgical site infections, the pathogens responsible are not exogenous, but emerge from the normal endogenous flora of the patient. *Staphylococcus aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa*, Enterococcus spp. and *Escherichia coli* are among most commonly implicated pathogens (Owens and Stoessel 2008). The act of surgery facilitates the transport of these microorganisms from their habitat, e.g. skin or gastrointestinal tract, to the sterile sites of the body, such as spinal cord or muscle tissue (Thakkar et al. 2014). An infection elsewhere in the body, whether being a pre-existing condition or having been acquired post-surgery, can also serve as an internal source of infection (Claridge et al. 2014).

An individual's risk of developing an infection depends greatly on the patient's state of health, and particularly their ability to resist infection. Immunocompromised patients, such as the elderly, pregnant women, those suffering from chronic illnesses, those undergoing cancer treatment, recovering from a recent illness or invasive intervention, or having certain genetic disorders, may succumb to significantly lower bacterial loads or develop infections caused by opportunistic bacteria (Neumayer et al. 2007; Ridgeway et al. 2005). For example, patients suffering from hypoalbuminemia prior to gastrointestinal surgery are more likely to develop deeper surgical site infections and recover more slowly compared to patients whose

pre-operative albumin levels were higher (Hennessey et al. 2010). According to a review study published by Pittet et al., patients being treated for breast cancer and undergoing a mastectomy experienced an increased risk of surgical site infection from an average rate of 2.5 % up to 53 %. This increase can be attributed to postoperative tissue ischemia and delayed wound healing (Pittet et al. 2005).

7.1.1 Biomaterial-Associated Infections and Inflammation

The introduction of an implant into the body further predisposes the host to infection and inflammation. For one, tissue that has already been injured by the surgical intervention is subjected to further physical damage from implant insertion and positioning. Subsequent to the insertion, the implant may continue to inflict physical damage to the adjacent tissues, putting excessive pressure on the surrounding tissues, rubbing against the tissues or by breaching epithelial or mucosal barriers. This breach may provide a means for bacteria to enter host tissues, whereas the subsequent physical trauma may impair the ability of the tissue to recover and to resist bacterial invasion (Chandorkar et al. 2015).

A persistent sterile inflammatory response, in itself, presents a significant issue for all medical implants, irrespective of their type and method of implantation. For example, a chronic foreign body response to devices implanted into brain tissue affects the electrophysiological and neurobiological activity of nearby neurons and the neural circuitry by a variety of neuro-inflammatory mechanisms, including disruption of the blood brain barrier, demyelination and reduction in density of local nerve fibers and neuronal cell bodies, and changes in the local ionic milieu (Skousen et al. 2015). Continuing physical damage and chronic infection can lead to tissue necrosis and permanent changes in the tissue surrounding the implant.

The surface of the implant may serve as a vector for the introduction of the microorganisms into the body. Although in developed countries, this issue is rarely observed due to highly controlled sterilization practices, the sterilization compliance remains an issue in countries with limited-resources (Rosenthal 2008). It is important to note that implant sterilization does not automatically render it free from agents that can induce a pro-inflammatory response, namely the endotoxins and micro- and nano-sized particles. Endotoxins are the lipopolysaccharides in the cell membrane of Gram-negative bacteria (Vetten et al. 2014). Residual endotoxin contaminations can vary significantly, depending on the method used for sterilizing the biomaterial.

For example, microelectrodes sterilized by autoclave, dry heat, or ethylene oxide gas, retained residual endotoxins of 0.55 EU per mL, 0.22 EU per mL, and 0.11 EU per mL, respectively (Ravikumar et al. 2014). At early stages of implantation into neural tissue, the extent of microglia/macrophage activation is directly related to the level of endotoxins present on the implant surface, whereas the degree of astrogliosis, neuronal loss, and blood-brain barrier dysfunction exhibit a threshold-dependent response. The effect of endotoxin levels diminishes with longer implantation

periods, suggesting that the endotoxin contributes to the initial, but not chronic neuro-inflammation.

Just as initially sterile implant surfaces can induce a foreign body response, the abiotic surface of the implant provides excellent grounds for secondary colonization of the implant surface via the hematogenous dissemination from a remote focus of the infection. Indeed, conventional (non-antibacterial) biomaterials are not able to prevent bacteria from permanently attaching to their surfaces and forming a biofilm, nor activating a host immune response. Once colonized, the implant surface can not only lead to peri-implant infection but also serve as a reservoir from which bacteria can spread to other parts of the body (Claridge et al. 2014).

Implant-associated infections are difficult to treat with antibiotics alone and often necessitate the removal of the implant. The protective nature of a biofilm significantly reduces the efficacy of both pharmacological therapies and host defense mechanisms (Lisanti et al. 2015; Khan et al. 2014). In addition, as most biomaterials do not react to being colonized, the infection is often detected only after the biofilm has been formed and the pathogen activity begins to impact on the surrounding tissues. Although some progress has been made in the development of strategies for dissolution of biofilms that have already been established, targeting of the biofilm matrix for degradation, interference with the biofilm regulation (Hogan et al. 2015) and removal of biofilm-residing bacteria remains a challenge.

7.1.2 Resistance and Persistence of Pathogenic Bacteria

When resident in a biofilm, certain species of bacteria, such as staphylococci, exhibit increased mutation ability and enhanced ability to acquire/disseminate plasmid-borne antibiotic resistance determinants by horizontal gene transfer (Savage et al. 2013; Ryder et al. 2012). Compared to planktonic cultures, the mutation ability of *S. aureus* and *S. epidermidis* biofilm cultures increased up to 60-fold and 4-fold, respectively. Such enhanced spontaneous mutation accelerates the emergence of heritable antibiotic resistance and horizontal gene transfer ensures that this resistance spreads throughout the biofilm community. Mixed-species biofilms can also be more resilient to the action of antibiotics due to phenomenon known as indirect pathogenicity, where an antibiotic-resistant microorganism of low intrinsic virulence shields an antibiotic-sensitive pathogen from eradication (O'Connell et al. 2006). For example, antibiotics such as penicillins and cephalosporins can be inactivated via hydrolysis of the cyclic amide bond by beta-lactamase-producing bacteria, thereby preventing these antimicrobials from acting upon susceptible microorganisms. With an increasing prevalence of multidrug resistant bacteria, identifying antibiotic agents that would be able to overcome multidrug resistance in biofilm-dwelling bacteria is very challenging.

In addition to enzymes, antibiotic-sensitive strains can also benefit from the action of multifunctional extracellular polymers produced by other bacteria within the community. For example, *P. aeruginosa* produces Psl polysaccharide, which

acts as molecular glue to enable bacterial attachment, self-organization of the attached cells into micro-colonies, and communal biofilm organization (Zhao et al. 2013a). As such, Psl provides a generic first line of defense against antibiotics with diverse biochemical properties during the initial stages of biofilm development. In mixed-species biofilms, Psl mediated matrix protection is extended to non-Psl-producing antibiotic-sensitive *E. coli* and *S. aureus* cells (Billings et al. 2013). Reducing surface adhesion and the production of bacterial adhesins are often a strategy of choice to prevent biofilm formation and implant-related infections.

Even in a population of genetically identical antibiotic-sensitive bacterial cells, not all cells will die as a result of an antibiotic treatment, even at a sufficiently high antimicrobial dose; the killing efficacy of penicillin depends on the physiological state of the cell, with rapidly growing bacteria being killed most efficiently, followed by slowly growing cells, whereas dormant (non-growing) bacteria are generally not affected by the drug (Tuomanen et al. 1986; Gerdes and Maisonneuve 2012). Even in an actively growing population, however, not all cells will succumb to penicillin treatment. While most of the population will be killed, a fraction of this genetically homogeneous microbial population may persist. In contrast to resistant mutants, persistent microorganisms do not acquire resistance to the said antibiotic (Balaban et al. 2004), which confirms that persistence is a phenotypic and not genotypic effect.

Persistence stems from inherent phenotypic heterogeneity within a microbial population, where distinct subpopulations with different growth rates co-exist: normal cells and persister (slow-growing) cells. Normal cells ensure population survival under normal conditions, whereas persisters provide fitness benefit under stressed conditions, e.g. antibiotic load (Patra and Klumpp 2013). The phenotype switching is reversible, with the population regrown from the persister cells possessing both normal and persister cells. Peptidoglycan plasticity is a key factor in drug sensing and signaling to mitigate antibiotic exposure, where peptidoglycan remodeling enzymes are intimately involved in dormancy and resuscitation phenomena (Cava and de Pedro 2014; Amoroso et al. 2012; Courvalin 2006). In a dormant organism, the presence of specific peptidoglycan fragments can trigger the resuscitation pathway directly; peptidoglycan can also function as a substrate for the endogenous resuscitation-promoting factors, whereby it is hydrolyzed to liberate stimulatory fragments of different size (Nikitushkin et al. 2013). In addition to diversifying the growth rate to ensure population survival, bacteria can gain survival advantages from differentiation into different shapes (i.e. from bacillary to coccoid morphology) and into a filamentous morphology (Justice et al. 2014; Friedlander et al. 2013).

At present, the molecular mechanisms underlying bacterial persistence remain undiscovered (Maisonneuve et al. 2011), however it is believed that gene pairs encoding cognate toxin-antitoxins present in virtually all bacteria control reversible bacterial growth arrest (Holden 2015; Germain et al.). Equally, little is understood about the relationship between the recurring use of antibiotics, persistence and development of antibiotic resistance. In the future, the persistence-enabling toxin-antitoxin and associated signaling mechanisms may be targeted to either prevent

persisters from arising or to transition them into a normal and thus antibiotic-susceptible state (Holden 2015).

7.1.3 Preventing Implant-Associated Infections via Implant Modification

It is evident that antibiotic prophylaxis and tightly controlled operating environment does not prevent an implant-associated infection from occurring. Furthermore, once established on the surface of the implant, a biofilm is difficult to eradicate with systemic antibiotics alone. The efficacy of these antibiotics is also diminishing, with antibiotic resistance spreading faster than the rate at which new antimicrobial compounds, such as teixobactin, can be discovered, evaluated and clinically introduced (Ling et al. 2015). Besides, persistent cells may remain on the surface of the implant, giving rise to chronic infections, and therefore the most logical step would be to localize the antibacterial activity to the surface of the implantable material itself.

Over the years, numerous strategies have been devised to prevent implant-associated infections by targeting different stages of biofilm formation and molecular and cellular mechanisms of bacterial pathogenesis (Grainger et al. 2013). These include various specific and non-specific anti-adhesive surfaces, and controlled systemic or localized drug delivery to interrupt cell-cell communication, inhibit bacterial aggregation and biofilm formation, or eliminate bacteria directly.

There are several important aspects that need to be considered when imparting antibacterial properties, whether they are physical or chemical features, onto the surface of the implant. An obvious factor is that the bacteria-repelling, bacteriostatic or bactericidal property should be maintained for the intended period of time under *in vivo* conditions, considering that *in vivo* environment may vary significantly over time and between patients. For example, if the anti-infective effect is due to the specific surface topographical features of the implants, these features should remain available and not be masked by protein fouling or cell debris.

The selected processing methodology should be compatible with the type of the implant material/s and not undermine the physico-chemical and mechanical integrity of the modified device. Neither should this modification destabilize the interactions between the individual components and/or different materials that may constitute a device or be a part of the fixative system, e.g. the adhesion of cement to the metallic component of the bone implant should be maintained. The modification itself should be mechanically robust to withstand normal pre-, intra-, and post-operative handling and operation. For example, an antibacterial coating on a load-bearing implant will experience a wide range of mechanical stresses and strains.

Critically, the modified implant should remain biocompatible and retain its ability to perform its intended function with an appropriate host response. Host response is triggered by the trauma of the surgical and implantation process, and then maintained by the physical, chemical, biological and mechanical interactions between

the resident implant and the surrounding tissues. Depending on the nature of the implant and the stage of implantation, host response will include inflammatory and wound healing responses, foreign body reactions, or fibrous encapsulation (Anderson 2001).

7.1.4 Host Response and Implant Biocompatibility

Initiated by the action of implantation of a foreign body into a host, the tissue response continuum comprises a diverse ensemble of biological responses, including early events, such as injury, blood-implant interactions, provisional matrix formation, and acute inflammation, and later events, e.g. chronic inflammation, tissue granulation, foreign body reaction and fibrosis (Anderson 2001; Karlsson et al. 2014).

The extent of implantation injury is associated with the placement of the implant, its dimensions, weight and configuration, as well as its mechanical (e.g. flexible vs rigid) and surface (e.g. low-friction vs. abrasive) properties. Most implant-related anti-infective strategies are confined to the surface of the material, potentially altering the surface properties and three-dimensional configuration of the implant.

The injury incites a wound healing cascade, beginning with initial platelet adhesion to the injury site, followed by platelet activation and aggregation. In turn, this initiates a coagulation cascade, leading to clot formation as a measure to manage blood loss and minimise the risk of microbial infection. Clot formation is followed by highly mobile neutrophil granulocytes infiltrating the injury site as part of innate immune responses. Phagocytosis ensues clearing the debris of damaged tissues and pathogens that may have been introduced into the peri-implant space. Concurrently, phagocytic cells release growth factors to stimulate tissue regeneration, promoting the formation of granulation tissue.

Where an injury results in only exudative inflammation, inflammation resolution and restoration of the original tissue structure proceeds relatively quickly. On the other hand, dominance of pro-inflammatory over generative-type macrophages at the injury sites with significant tissue necrosis and/or loss of basement membrane structures may lead granulation tissue to grow into the inflammatory exudate. As a result, instead of restoration of original tissue structure, organisation and development of fibrous tissue takes place. It is important that even in the case of tissue restoration, re-grown cells may differ in their growth and differentiation behaviour from the original (pre-surgery) tissue. Regenerated cells can differ in their type, size, number, and function level; their ability to produce intra- an extra-cellular polymers can also change.

In addition to the nature of the injury (e.g. skill of the surgeon) and the health status of the host (e.g. suppressed proliferative capacity of cells), the nature of the implant may significantly affect the hemostasis, inflammation, repair, and remodeling processes, and thus directly influence the tissue healing outcomes. For example, copper-based coatings have been shown to effectively reduce infections on the

surfaces of titanium implants. Implantation of copper-modified titanium implants has been associated with increased total and tissue macrophages and MHC-class-II-positive cells in a murine model (Andreas et al. 2013). As the tissue reactions persisted beyond the Cu release, it was concluded that surface-bound Cu may have also contributed to the increased local inflammatory response. In Cu/Ti coatings, an increasing Cu fraction led to an increase in the degree of hemolysis, limited break down of platelets and reduction in platelet adhesion (Liu et al. 2012a). At Cu concentrations above 10 wt.%, significant inhibition of endothelial cell adhesion and proliferation was observed. Given the important role of chemical factors released by plasma and cells in mediating the inflammatory response, implant-released chemicals may interfere with correct tissue regeneration (Yu et al. 2013). Furthermore, the lysosomal proteases and reactive oxygen species abundant at the site of inflammation may promote degradation of the implant material, thus further exacerbating the release of the active compounds.

The importance of the provisional matrix formation on tissue integration should not be underestimated (Anitua et al. 2014; Abrahamsson et al. 2004). The matrix is composed of many components; fibronectin, collagen and thrombospondin bind to fibrin and the platelet granule components of the initial clot. A major constituent of the matrix, fibrin induces neovascularization, promotes cell attachment (Thomson et al. 2013), and provides a scaffold for cell migration and tissue formation. The matrix also releases a wide array of mitogens, chemoattractants, cytokines, and growth factors that regulates cellular proliferation. The formation of the provisional matrix may be considerably altered by the surface properties of the materials (Tejero et al. 2014; Lang et al. 2011), and thus needs to be considered when developing anti-infective coatings.

Importantly, the inflammatory response to the surface properties of the biomaterial needs to be considered in all of its complexity. Specifically, it is important to look beyond the cellular and tissue morphology of the peri-implant region to consider the densities, activities and functions of other cells which may be impacted by endogenous and autacoid mediators (Anderson 2001; Anderson and Miller 1984). Moreover, while the histopathological consequences of acute inflammation are relatively uniform, the same cannot be said for chronic inflammation, where multiple factors can alter the histological appearance of the process. Enduring stimulations, e.g. those by a controlled-released antimicrobial agent, chemical or morphological surface features of the implant, may contribute to the establishment of chronic inflammation. The foreign body response is also influenced by the surface topography, configuration and the surface-to-volume ratio of the implant, with smoother implants having been associated with low levels of macrophages and foreign body giant cells in the peri-implant milieu and a tendency to develop a fibrous capsule. Rough, porous and fabric-like surfaces, on the other hand, have more macrophages and foreign body giant cells in the proximity to the implant, and are less likely to become encapsulated.

7.1.5 Evaluating Biocompatibility

Application of any type of physical or chemical modification to the surface of the implant will directly affect the dynamics of the above described host-implant relationships (Triantafillopoulos and Papaioannou 2014). It is therefore essential to determine whether or not the modified implant is still sufficiently biocompatible for the intended application. This can be done by assessing a series of biological responses, and then comparing the magnitude and duration of the adverse changes in homeostatic mechanisms of the host against those for the unmodified implant.

Consideration needs to be given to the nature and duration of the implant-tissue contact. For example, for medical devices that come into direct contact with blood, good hemocompatibility is paramount. Silver is an element with well-known antibacterial properties that has many promising applications, however, contradictory reports exist about the ability of silver nanoparticles to induce platelet aggregation and procoagulant activation, potentially leading to increased risk of cardiovascular events (Jun et al. 2011; Smock et al. 2014; Shrivastava et al. 2009; AshaRani et al. 2009). The effect of the implant on the mechanisms of thrombosis, coagulation, platelet activation, and production of blood and its components, i.e. blood cells, hemoglobin, blood proteins, will depend on the surface physico-chemical properties of the implant, as well as its geometry, contact conditions, and flow dynamics.

The contact duration is regarded as limited for contact time of less than 24 h, prolonged when implant-tissue contact is maintained for over 24 h to 1 month and permanent thereafter. The location of the implant and the types of tissues and bodily fluids that will be in direct contact with the implant are also considered.

For anti-infective modifications that involve biodegradation and controlled release of particles and/or chemical substances into the peri-implant milieu, the transport, accumulation, degradation and metabolism of these release by-products by other tissues may be of significance. Even very small amounts of metal ions, antimicrobials, biomolecules, nano- and micro-particles can give rise to allergic or sensitization reactions, with the severity of the reaction dependent on the properties of the leachable agent and the sensitivity of the host.

In cases where these biomaterials are implanted into susceptible individuals or are present in higher amounts, leachable agents can induce systemic toxicity, directly affecting the immune, hematologic, central nervous and cardiovascular systems, amongst others. In addition to leachable agents, any structural components of bacteria, yeasts, and molds, organic and inorganic dusts, nanoparticles, and diesel exhaust particles that may remain on the surface of the implant post-sterilization may provoke an inflammatory (innate) response and be fever-producing (pyrogenic) (Mazzotti et al. 2007).

Given that many modern antimicrobial and tissue regeneration strategies are based on the controlled release of biologically active molecules, including growth factors, cytokines, antimicrobial factors and proteins, stem cell stimulating factors, complement proteins, and chemotactic factors, it is important to consider the immunotoxic and immunogenic potential of these reactive agents (Maeda et al. 2007;

Bielecki et al. 2007; Chandorkar et al. 2015). Once released inside the host, these molecules, their fragments or their degradation by-products can be recognized by the adaptive immunity, triggering the humoral and cellular response. As such, exposure to these substances can lead to immunosuppression, immunostimulation, or autoimmunity, manifested as histopathological changes, host tissue autoimmune damage, and compromised ability of the host to protect itself from foreign agents. For example, an inorganic compound, protein or lipid component in the drug delivery system may perform as an adjuvant, stimulating autoimmune response; similarly, a foreign biomolecule may induce antibodies that will react with host molecules, leading undesirable tissue damage via complement pathway (Anderson 2001).

Systemic toxicity is typically evaluated as a function of exposure time, with relevant adverse reactions being observed within 24 h after first contact (acute), after 14–28 days of repeat exposure (sub-acute), and up to 90 days of exposure (sub-chronic). Chronic toxicity arises from long term exposure, and is characterized by persistent or progressively deteriorating dysfunction of cells, organs or multiple organ systems. Unlike other types of toxicity, it is difficult to evaluate and predict the chronic toxicity potential of the modified implant, due to the obvious difficulty in maintaining the experimental conditions, such as nutrition, health, and lifestyle parity between test groups.

Implant-related physical, chemical and biological agents may lead to genotoxicity. For example, particles and ions leached from the surface of metallic bone implants have been shown to increase DNA damage, gene mutations, and chromosome aberrations in the adjacent bone marrow and chromosome translocations and aneuploidy in the peripheral blood (Gajski et al. 2014; Karahalil et al. 2014). Such mutations, mistimed event activation, and direct DNA damage may lead to the development of cancer, although the risk of implant-leached products inducing systemic cancers is regarded as very low (Christian et al. 2014; Moalli et al. 2014). The carcinogenicity of the compound is patient-specific in that it depends on the ability of the individual to activate or detoxify genotoxic substances, and to repair deletions, breaks and/or rearrangements within DNA. Interestingly, biomaterial-associated tumor development in animals has been demonstrated to be associated with the physical configuration, not the chemical composition of the implant (Moalli et al. 2014). Implants with a smooth surface and large surface area, such as thin sheets and discs, are more carcinogenic than biomaterials with irregular surface morphologies, such as meshes and porous solids.

Implant-derived particles and reactive chemical species may affect reproductive toxicity. For instance, the reproductive potential of male mice injected with CoCr nanoparticles (to simulate leaching of particles and ions from CoCr alloy implants) was lower than that of the control group, with reduced epididymal sperm motility, viability and concentration, and higher abnormal sperm rate (Wang et al. 2013). Popular anti-microbial agents, silver and titanium dioxide nanoparticles were also found to be cytotoxic and cytostatic for primary testicular cells, causing apoptosis, necrosis and decreased proliferation (Asare et al. 2012). Oxidative stress is the likely driver for the testicular damage and pathological changes. Indeed, the

production of reactive oxygen species and DNA damage are regarded as the underlying mechanism of nanoparticle cytotoxicity (Taylor et al. 2012). Compared to bulk materials, nanoparticles have heightened chemical and biological reactivity due to much higher surface-to-volume ratio, which means that even inert biocompatible materials, e.g. gold (Zhao et al. 2013b), can become toxic. Specific toxicity is highly dependent on the size, shape, polarization and surface functionalization of the particle.

Nanoparticles may induce teratogenicity, halting the development or leading to congenital malformation of the fetus, and negatively affecting post-natal development of the offspring. For example, graphene oxide is a promising antimicrobial material (Liu et al. 2011; Podila et al. 2013), which, when modified with silver nanoparticles, becomes even more strongly antibacterial towards pathogens such as *E. coli* (Ma et al. 2011). When maternal mice were exposed to graphene oxide during lactation, the filial mice developed significantly slower, gaining less body weight and length compared to control group (Fu et al. 2015). The intestinal villus of the filial mice was found to be notably shorter in the offspring mice in the treatment group, indicating a possible mechanism of toxicity.

A critical determinant of toxicity is the dose to which the host is exposed. Biodegradable and drug-release antimicrobial coatings are designed to release tightly controlled quantities of biochemically active substances into the peri-implant milieu. The release rate balances microbial efficacy against host toxicity. Premature biodegradation *in vivo*, i.e. excessive elimination of the therapeutic compound, may tip that balance. In addition to toxicity, distorted degradation kinetics may result in the premature exhaustion of the anti-bacterial agent. The suboptimal levels of the released agent may not be sufficient to effectively kill bacteria, potentially acting as a positively driver for the development of antibiotic resistivity.

In addition to the active ingredient, toxicity can arise from other seemingly benign degradation products, including very low amounts of impurities, additives and chemicals used in the synthesis and processing of a biomaterial. The chemical and morphological properties of the released agents will be affected by the biodegradation mechanism and on the health status of the host (Baran et al. 2014). Medically compromising systemic conditions, e.g. diabetes, and inflammatory diseases may increase the rate of biomaterial degradation (Alani and Bishop 2014). Higher blood glucose levels and lower pH levels, for instance, has been shown to the rate of electrochemical corrosion of titanium dental implants (Tamam and Turkyilmaz 2014). It is challenging to predict all the potential interactions among the degradation products, and also between these products and other biochemical agents used for the treatment of the patient. One of the issues is associated with the difficulty in identifying appropriate biomarkers for detection of premature degradation onset and progression (Sumner et al. 2014; Grainger et al. 2013).

7.2 Modern Strategies to Minimize Implant-Associated Infections

There exists a wide spectrum of substances and technological methodologies that can be used to fabricate biomaterials with anti-infective features (Jadalannagari et al. 2014), including those based on bio-inspired and biomimetic surface topographies (discussed in Chap. 2), electroactive and mechano-responsive surfaces (Chaps. 4 and 8), and natural antimicrobial agents (Chap. 2). Broadly, anti-infective approaches can be divided into two categories: antifouling, i.e. those that repel microbes; and antimicrobial, those that prevent cell proliferation (bacteriostatic) and/or kill bacteria (bactericidal). In the following sections, we will discuss several examples of the modern strategies to minimize implant-associated infections, focusing on the challenges in merging the need to withstand bacterial load with that to support function restoration and tissue healing.

7.2.1 Anti-adhesive Surfaces

Device related infections take place when bacteria coat the surface or infiltrate the lumen of an implant. The peri-implant milieu is abundant in nutrients, providing suitable growth conditions for bacterial growth and replication. On the surfaces of implants, bacteria can be present in both planktonic and biofilm forms, the former affording mobility whereas the latter providing means of protection, selective transport of biomolecules, environmental regulation and communication for resident cells. The presence of biofilm markedly increases the likelihood of bacteria evading host defense mechanisms, antimicrobial agents and extended periods of unfavorable environmental conditions. The release of extracellular polymeric substances, e.g. polysaccharides, amyloid fibrils, lipids and nucleic acid (Claessen et al. 2014), is critical to establishment, development and functioning of any biofilm. For example, adhesins enable cell attachment to living tissue or an abiotic substrate and act as receptors for adhesins on other bacteria. A detailed overview of the process of bacterial attachment and biofilm formation can be found in Chap. 1.

Since adherence is a critical first step in pathogenesis, many strategies have been developed to control bacterial and protein adhesion via steric repulsion, low surface energy and electrostatic repulsion. This can be achieved by modifying the surface to be hydrophilic, negatively charged, and/or with low surface free energy (Yu et al. 2011). In the case of hydrophilic polymers, water is attracted to the surface, encapsulating it in a thin repellent layer that is associated with the polymer via hydrogen bonds. This layer creates steric barrier for the biomolecules and cells, preventing their attachment.

The appropriate chemistry is typically obtained by surface functionalization, e.g. plasma treatment (Bazaka et al. 2011, 2012; Jacobs et al. 2012), or application of coatings and polymer brushes (Knetsch and Koole 2011; Hook et al. 2012; Chauhan

et al. 2014). Hydrophilicity of the material may also be altered by controlling surface morphology (Zheng et al. 2010). For example, metallic surfaces can be made superhydrophobic using chemical etching or laser ablation (Fadeeva et al. 2011). Amongst polymer brushes, amphiphilic copolymers (Liu et al. 2012b; Zhou et al. 2014), zwitterionic polymers (Cheng et al. 2008) and patterned polymers are common. Polymer chemistries based on polyethylene glycol (PEG) or its derivatives are common (Ye and Zhou 2015; Yang et al. 2014), as they are non-immunogenic and effectively resist protein fouling and thrombosis (Chen et al. 2011). The efficacy of PEG modification is strongly dependent on the molecular weight, branching and surface chemistry of the coating (Beloin et al. 2014). In addition to PEG, hydrophilic polymers based on hyaluronic acid (Liu et al. 2014) such as poly-N-vinylpyrrolidone (Liu et al. 2013a), poly(dimethylaminoethyl methacrylate) (Lih et al.; Tu et al. 2013), have also been used.

There are many possible configurations in which polymer brushes can be assembled, and enumerate chemistries that can be used to fine tune their properties for a desired application or to enhance the antifouling property of the surface. Broadly, polymer can be attached in the form of a homopolymer, as mixed polymer or block co-polymer brushes, assembled from functional particles or as layer-by-layer films, incorporating nano- an micro-sized particles, in a form of cross-linked polymer matrixes, and so on (Lih et al.; Wang et al. 2014). For example, the antifouling efficacy of fluorinated amphiphilic copolymers composed of 2-perfluorooctylethyl methacrylate and 2-hydroxyethyl methacrylate was greater than that of their constituent homopolymers, suggesting a synergistic mechanism of fouling inhibition (Zhao et al. 2013c). The stability of the polymer layer is dependent on the method of immobilization, with physically absorbed (self-assembled) brushes being less chemically stable than covalently linked molecules, e.g. grafted-to or grafted-from polymer chains.

Improved anti-infective outcomes can be achieved by synergistically merging antifouling and antibacterial chemistries, e.g. by coating the implant surface with brush-like polycarbonates containing pendent adhesive dopamine, antifouling PEG, and antibacterial cations (Yang et al. 2014). Such a surface can ensure that bacterial cells able to overcome antifouling chemistry and attach to the implant surface are prevented from multiplying and forming a biofilm (Yang et al. 2014). In another example, antifouling poly(sulfobetaine methacrylate) and bactericidal polymer, N-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride polymers are integrated to achieved a similar result (Wang et al. 2015). Heparin and other biomolecules can be loaded into such a coating to enhance surface hemocompatibility and improve host cell attachment (Almodóvar et al. 2013; Zhu et al. 2013). Coating the surface of the implant with heparin and heparin-like molecules has been demonstrated to reduce bacterial attachment and biofilm formation. Presence of heparin or albumin results in a negatively charged surface that reduces bacterial adhesion (as cells bear a net negative charge on their surface under normal pH conditions) and the deposition of fibronectins (Francolini and Donelli 2010).
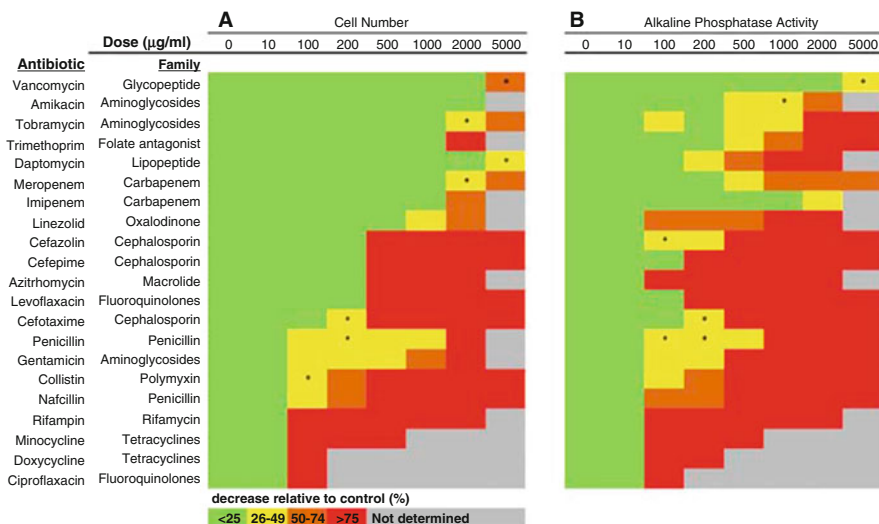
From a biocompatibility point of view, most anti-fouling surfaces are non-toxic, as the effect is typically achieved by surface chemistry rather than a release of

antimicrobial agents. At the same time, the antifouling action is generally non-specific, i.e. it may alter the attachment of host biomolecules and cells. While this may be desired for urinary or vascular catheter applications or wound dressings, it may impede the establishment of a tissue-implant interface where integration is desired. To circumvent this problem, antifouling polymer brushes can be functionalized or loaded with biomolecules that promote host cell adhesion, tissue formation and spreading. For example, improved tissue compatibility was obtained by functionalizing brushes of the block copolymer Pluronic F-127 with contact-killing antimicrobial peptides and host cell adhesion promoting arginine-glycine-aspartate (Muszanska et al. 2014) (Figs. 7.1 and 7.2).

Higher control over host cell adhesion can be obtained by specifically targeting bacterial adhesion, by using strategies that hinder biogenesis of fimbrial adhesins (Lo et al. 2013; Nait Chabane et al. 2014; Shamir et al. 2010). Production of functional extracellular amyloid fibers by uropathogenic *E. coli* and other Enterobacteriaceae can be effectively hindered *in vitro* and *in vivo* by using ring-fused 2-pyridones, such as FN075 and BibC6 (Cegelski et al. 2009). Pyridone-based pilicides inhibit the assembly of type 1 pili and thus interfere with pathogenesis and formation of curli-dependent and type 1-dependent biofilms. Another target is the FimH, an adhesion located at the tip of type 1 fimbriae and responsible for binding to mannosylated glycoproteins on human cells (Totsika et al. 2013). Here, small molecule mannosides can be used to bind FimH thus making it unavailable for binding to eukaryotic receptors (Guiton et al. 2012). Glycoclusters based on a cyclic oligo-(1→6)-β-D-glucosamine core can be employed to inhibit bacterial lectin LecA of *P. aeruginosa* (Gening et al. 2013), interfering with the colonization ability
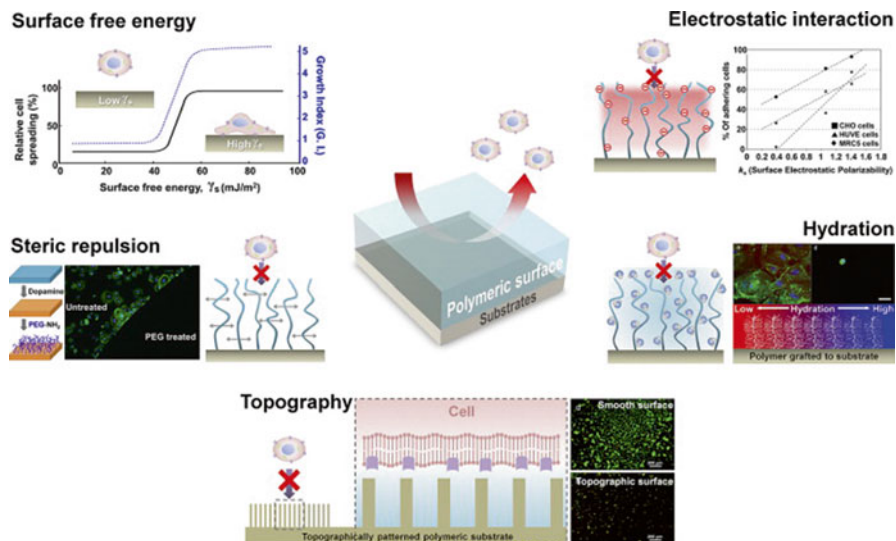
Fig. 7.1 Interactions between host cells and anti-adhesive surfaces (Reproduced with a permission (Lih et al.))

A Cell Number **B** Alkaline Phosphatase Activity

Dose (µg/ml): 0 10 100 200 500 1000 2000 5000

Antibiotic	Family
Vancomycin	Glycopeptide
Amikacin	Aminoglycosides
Tobramycin	Aminoglycosides
Trimethoprim	Folate antagonist
Daptomycin	Lipopeptide
Meropenem	Carbapenem
Imipenem	Carbapenem
Linezolid	Oxalodinone
Cefazolin	Cephalosporin
Cefepime	Cephalosporin
Azitrhomycin	Macrolide
Levoflaxacin	Fluoroquinolones
Cefotaxime	Cephalosporin
Penicillin	Penicillin
Gentamicin	Aminoglycosides
Collistin	Polymyxin
Nafcillin	Penicillin
Rifampin	Rifamycin
Minocycline	Tetracyclines
Doxycycline	Tetracyclines
Ciproflaxacin	Fluoroquinolones

decrease relative to control (%)

<25 26-49 50-74 >75 Not determined

Fig. 7.2 Effect of various concentrations of antibiotics on osteogenic cell viability and activity. The mean % decreases in osteoblast cell number (**a**) and ALP activity (**b**) are classified as <25 %, 26–50, 51–74, and >75 % of control after incubation with 0, 10, 100, 200, 500, 1000, 2000, and 5000 µg/mL of each antibiotic for 10 and 14 days (n=5–6 per dose after data are pooled). Not determined: ALP activity and/or cell number was untestable for some of the antibiotics due to precipitation or incompatibility with the test assays used. Decreases in osteoblast cell number and ALP activity >25 % were significant, $p < 0.05$, with exceptions indicated by (*) where the value at that dose was not different from control (Reproduced with permission (Rathbone et al. 2011))

of the pathogen. Furthermore, the biofilm-forming ability of *P. aeruginosa* can be compromised by targeting several specific functions of the pathogen. For example, maltose derivatives with bulky hydro-carbon groups inhibit the swarming motility and cell adhesion of *P. aeruginosa*, demonstrating biofilm inhibition and dispersion (Shetye et al. 2014).

Another potential target is small messenger molecules that enable pathogen cell-to-cell communication within a colony. Being a co-operative process, biofilm formation is inherently dependent on information being effectively transported from cell to cell. Quorum sensing is responsible for synchronization of gene expression, co-ordinated switch between planktonic and sessile states, biofilm formation, maturation and disassembly (Solano et al. 2014; Singh et al. 2000). By targeting the major signaling pathways of cells, bacterial communication and thus biofilm formation can be compromised. Sulfathiazole and azathioprine has been shown to inhibit diguanylate cyclases (Sambanthamoorthy et al. 2012, 2013; Antoniani et al. 2010, 2013). This enzyme is responsible for production of c-di-GMP, a small messenger molecule responsible for switching from planktonic to biofilm modality.

Nitric oxide is another molecule that can interfere with biofilm formation and induce dispersal of *P. aeruginosa* (Barraud et al. 2009). Nitric oxide increases cell motility by decreasing the levels of the secondary messenger cyclic di-GMP and

decreasing production of adhesins, making these cells more susceptible to antibiotic agents (Li et al. 2013). Nitric-oxide producing compounds can be easily immobilized on the surfaces of implants, releasing NO under specific *in vivo* conditions (Li et al. 2013; Duong et al. 2014). A controlled release of low levels of NO may also enhance the biocompatibility of the surfaces by reducing platelet activation and adhesion, and inhibiting thrombus formation. NO has a complex role in tissue inflammation (Laroux et al. 2001), where under normal physiological conditions, NO has an anti-inflammatory effect. At the same time, overproduction of NO can promote inflammation, with large amounts of NO being released by cytokine-activated macrophages, contributing to pathogenesis of inflammatory disorders, vasoconstriction, and tissue damage (Sharma et al. 2007).

7.2.2 Antibacterial Surfaces

Most strategies aimed at reducing biofilm-associated infections combine an anti-fouling property with antimicrobial activity (Francolini et al. 2015; Salwiczek et al. 2014), either through elution of antimicrobial agent or a surface killing. Antimicrobial agents range from conventional (systemic) antibiotics (Gao et al. 2011), e.g. nafcillin, levofloxacin, daptomycin, gentamycin, vancomycin (Bastari et al. 2014; Beenken et al. 2014; Ordikhani et al. 2014; Wu et al. 2014; Cashman et al. 2013), to metallic ions such as silver (Wong and Liu 2010; Chernousova and Epple 2013; Perez et al. 2014; Jo et al. 2014) and copper (Kalaivani et al. 2014; Ye et al. 2014; Chen et al. 2014), nitrofurazone (Kottur et al. 2015; Johnson et al. 2012), chlorhexidine (Jamal et al. 2014), quaternary ammonium compounds (Asri et al. 2014; Shalev et al. 2012; Cheng et al. 2012; Bakhshi et al. 2013), antibacterial peptides (Muszanska et al. 2014; Cleophas et al. 2014; Rapsch et al. 2013; Etayash et al. 2013) and anionic nanoporous hydrogels (Li et al. 2011; Hook et al. 2012). These agents are highly diverse in their modes of action. For example, an antimicrobial polycationic hydrogel based on dimethyldecylammonium chitosan-graft-PEG methacrylate and PEG diacrylate attracts sections of the anionic membrane of the pathogen into the internal nanopores of the hydrogel (Li et al. 2011). This process leads to bacterial membrane disruption and subsequent death. Synthetic macromolecular antimicrobials are being researched for their potential to physically destroy cell membranes of the pathogens thus preventing them from developing drug-resistance. Examples of this type of antibacterials include biodegradable cationic polycarbonates containing propyl and hexyl side chains quaternized with various nitrogen-containing heterocycles, such as imidazoles and pyridines (Ng et al. 2014). Although not based on a particular chemistry, surface capable of contact killing using morphological features are also being investigated (Ivanova et al. 2012, 2013).

7.2.2.1 Systemic Antibiotics

Conventional antibiotics have the advantage of having been thoroughly investigated, with well-defined host toxicity profiles and histories of clinical use that might detail potential long-term side effects. Since the doses of antibiotic released from the surface of implant are typically notably smaller than those used systemically, the toxicity of drug-eluting coatings is considered to be low. Indeed, one of the key advantages of drug-release coatings is the ability to locally deliver relatively high doses of antibiotic without inducing systemic toxicity. Nonetheless, the effects of antibiotics on cell viability and tissue regeneration should be considered. Osteoblasts treated with 21 different antibiotics over 0–5000 μg/mL concentrations for up to 14 days showed significantly lower cell number and osteogenic activity when exposed to rifampin, minocycline, doxycycline, nafcillin, penicillin, ciprofloxacin, colistin methanesulfonate, and gentamicin (Rathbone et al. 2011). On the other hand, osteoblast deoxyribonucleic acid content and alkaline phosphatase activity were least affected by amikacin, tobramycin, and vancomycin (Rathbone et al. 2011). Nevertheless, the majority of the antibiotics tested, a ≥50 % decrease in osteoblast cell number and/or osteogenic activity was observed. The decrease in the metabolic activity and thus the osteogenic potential of surviving cells may undermine bone repair.

7.2.2.2 Metals Salts

Metallic salts, such as those of silver, copper, zinc and mercury have been used to prevent and treat infections in wounds and burns for thousands of years. Long before the invention of polymer sutures, silver threads were also used to close wounds (Muffly et al. 2011), while silver-based solutions and creams (e.g. silver nitrate, silver sulfadiazine) were used as washes and ointments. Silver-based compounds precipitate cellular proteins and interfere with respiration of both aerobic and anaerobic bacteria, however the exact mechanism is yet to be fully elucidated (Chernousova and Epple 2013). Silver can also be used as part of combination therapy, where its ability to damage bacterial cells is used to potentiate antibiotic activity of conventional antibiotics (Morones-Ramirez et al. 2013) and thus target persister cells.

Current uses of silver primarily focus on a nano-sized particulate form of the metal (Rai et al. 2009; Wong and Liu 2010; Taheri et al. 2014). The nanoparticle form is characterized by high surface-to-volume ratio, which makes the particulate form of silver significantly more active with regard to its physicochemical and biological properties. It is believed that large surface area of nanoparticles allows them to establish good contact with the surfaces of target cell, whereas small size may facilitate particle penetration inside the pathogen. Larger surface area also enables more efficient release of silver ions, which can then interact with sulfur- and phosphorous-containing biomolecules in the bacterial membrane and DNA, respectively. Enhanced chemical reactivity of silver nanoparticles leads to the generation

of a large number of reactive oxygen species, which inflict further damage to the cells.

From a biocompatibility and cytotoxicity point of view, the major issue with the use of metallic ions is that their biological interactions are non-specific. Indeed, silver nanoparticles can easily breach host tissue and cell barriers, and interact with host biomolecules, e.g. enzymes, altering cell signalling and metabolic pathways (Gérard et al. 2010; Taylor 1985; Mouriño et al. 2012). Composition, dimensions, configuration and functionalization will affect the transport of chemical reactivity, and as such, the antibacterial efficacy and the potential toxicity of the nanoparticles (Li et al. 2012; Rai et al. 2009). For example, when human bone marrow mesenchymal stem cells and human hepatoma carcinoma cells were incubated in the presence of gold nanoparticles of different size, survival was 80 % in the case of 15 and 30 nm cells and less than 60 % for 5 nm particles (Fan et al. 2009). The increased levels of cell necrosis and altered osteogenic and adipogenic capabilities were attributed to the increase in the level of reactive oxygen species.

Anti-proliferative activity of silver nanoparticles was demonstrated in human lung fibroblasts (IMR-90) and glioblastoma cells, with treated cells exhibiting chromosome instability and mitotic arrest (AshaRani et al. 2009). Exposure to silver nanoparticles induced apoptosis in NIH3T3 fibroblast cells, inducing the release of cytochrome c into the cytosol and translocation of Bax to mitochondria through ROS and JNK (Hsin et al. 2008). Cellular apoptosis was also induced in RAW264.7 macrophage cells cultured in the presence of ~70 nm silver nanoparticles (Park et al. 2010). These particles were found to lower the intracellular glutathione level, increase NO secretion and TNF-α in protein and gene levels, and increase the gene expression of matrix metalloproteinases. It was demonstrated that intracellular oxidative stress due to the presence of agglomerated nanoparticles rather than ion damage was responsible for apoptosis in human hepatoma HepG2 cells (Kim et al. 2009). DNA damage may have also played a role, as hepatoma cells cultured with low dose silver nanoparticles showed an upregulation of DNA repair-associated genes (Kawata et al. 2009).

Oxidative stress was determined to be the cause of HeLa cell death, where oxidative stress-related genes, ho-1 and mt-2A, were expressed (Miura and Shinohara 2009). Coated silver nanoparticles also induced oxidative stress-type damage onto N27 rat dopaminergic neurons, where the size of the particle and type of coating determined the dominant pathway (Chorley et al. 2014). Poly(vinyl pyrrolidone)-coated particles were found to be more bioactive than citrate-coated particles, increasing intra-neuronal nitrite levels and inducing mitochondrial dysfunction (75 nm particles) and NRF2 oxidative stress pathway (10 nm particles) (Veronesi et al. 2014).

In the case of copper particles, the physical form of the particle is important, with dissolved Cu^{2+} ions contributing <50 % to cytotoxicity (Wang et al. 2012). A comparison between cell-particle interactions of nano-sized CuO and Ag showed that in the case of CuO, the particle was subject to rapid uptake by endocytosis, releasing copious amounts of copper ionic species within the cells (Cronholm et al. 2013). Even though silver particles were readily taken up by the cells, the intracellular

release of silver ions and hence the toxicity of the particles was low. The mechanism of toxicity also differed between different types of copper, where nanoparticles of copper and Cu-Zn alloy inflicted substantial damage onto cell membranes, whereas this behavior was not observed in the case of CuO nanoparticles and the micronsized Cu metal particles (Karlsson et al. 2013). Protein fouling in the presence of serum albumin inhibited cell toxicity of silver nanoparticles (Gnanadhas et al. 2013).

The systemic toxicity of medically-relevant metallic nanoparticles is not well studied (Alenius et al. 2014). Once eluted from the surface of the implant, the particles should theoretically be able to quickly and easily cross the blood barrier, being transported to other tissues in the body. There, these nanoparticles can potentially accumulate and degrade (Wong and Liu 2010). With regard to the cardiovascular system, sufficiently high doses of nanoparticles can alter the microcirculation, and promote thrombus formation and pro-inflammatory responses (Wong and Liu 2010), whilst in the central nervous system metallic nanoparticles may generate oxygen reactive species and damage the brain cells (Alenius et al. 2014). An iron- and oxygen-binding protein found in the muscle tissue, myoglobin can be affected by the presence of silver nanoparticles, where ~10 nm particles were shown to significantly augment its electron-transfer reactivity and catalytic ability toward hydrogen peroxide (Gan et al. 2004). They may also affect the reproductive ability of the host, as silver nanoparticles showed concentration dependent cytotoxicity towards mouse spermatogonial stem cells (Braydich-Stolle et al. 2005).

7.2.2.3 Chlorhexidine and Quaternary Ammonium Compounds

Chlorhexidine, a bisbiguanide, is an effective hospital disinfectant often used in surgery skin preparation and as an antimicrobial agent in medical devices (Opstrup et al. 2014; Silvestri and McEnery-Stonelake 2013) where they are employed to reduce extraluminal contamination, particularly in patients who require long-term vascular access (Khoo and Oziemski 2011). Recently, concerns have been raised about potentially significant allergic reactions to chlorhexidine-impregnated medical devices (Guleri et al. 2012). Severe allergic reactions, including anaphylactic shock have been reported for patients during the placement of a chlorhexidine impregnated central venous catheters (Khoo and Oziemski 2011; Faber et al. 2012; Bae et al. 2008), urological and rectal procedures (Jayathillake et al.; Bae et al. 2008). Approximately 10 % of patients with suspected perioperative allergic reactions were diagnosed with chlorhexidine (Opstrup et al. 2014). Wide exposure to chlorhexidine in hospital and non-medical environments may potentially sensitize some patients, leading to adverse reactions during surgery.

Quaternary ammonium compounds (QACs) are potent cationic antimicrobials active against a wide range of pathogens (Hegstad et al. 2010). QACs kill bacteria by interfering with cell membranes, primarily the cytoplasmic membrane, leading to membrane damage and loss of cellular content (Martín et al. 2014). QACs block potassium channels that are responsible for the passive movement of potassium ions

across the cell membrane and control the membrane potential in both eukaryotic and prokaryotic cells (Lenaeus et al. 2014). QACs may disrupt and denature structural proteins and enzymes. In anti-infective device applications, QACs can be loaded into coatings or surface immobilize for contact killing (Asri et al. 2014). As with other non-specific antimicrobial compounds, quaternary ammonium compounds display concentration-dependent eukaryotic cell toxicity with all types of exposure, namely inhalation, ingestion, and dermal application and irrigation of body cavities (Xue et al. 2004, 2012; Świercz et al. 2013; Dutot et al. 2008a, b). In susceptible individuals, contact with QACs may result in allergies that can range from mild localised irritation to anaphylactic reaction (Bello et al. 2009) and contact tissue damage (Kilic et al.). Mucosal contact with QACs may produce sensitization.

7.2.2.4 Antimicrobial Peptides

Produced by a large number of microorganisms, plants, invertebrates and animal species, antimicrobial peptides fulfil a broad range of roles in producing an innate immune response, specifically they hinder the growth and colonisation by infectious agents (Zasloff 2002; Bals et al. 1999). For example, resident epithelial cells (e.g. keratinocytes) produce a number of antimicrobial peptides and also signal the recruitment of circulating immune cells (e.g. neutrophils); once summoned, neutrophils also produce antimicrobial peptides, such as cathelicidin and β-defensins to fight the infection (Braff et al. 2005; Nizet et al. 2001). When adsorbed onto surfaces, nisin, an antimicrobial peptide, inhibits the attachment and biofilm formation capability of Gram-positive bacteria (Bower et al. 2002), whereas covalently-bound to the surface cathelin LL37 effectively kills *E. coli* on contact (Zaiou et al. 2003).

In Gram-positive and Gram-negative organisms, the antibacterial mechanism of the antimicrobial peptides is biophysical rather than biochemical in nature, destabilizing and disorganizing the structure of the membrane (Fitzgerald-Hughes et al. 2012). The cationic nature and amphipathic structure of antimicrobial peptides allows them to electrostatically attach to the negatively charged microbial surfaces (Powers and Hancock 2003; Forbes et al. 2013; Zasloff 2002). Subsequently, some peptides induce pore formation in the phospholipid bilayer, with death being a consequence of osmolysis (Augustyniak et al. 2012). A compromised cytoplasmic membrane also enables the translocated peptides to interact with intracellular biomolecules of the pathogen, inhibiting protein synthesis and conformation, interfering with DNA and metabolic activity, and suppressing cell multiplication (Brogden 2005). For example, the antibacterial action of nisin involves bacterial membrane permeation and disruption of the ability for the cell wall to undergo synthesis and regeneration (Hale and Hancock 2007).

Given that antimicrobial peptides typically combine several mechanisms of action, they are less likely to prompt de novo resistance (Yeung et al. 2011). The killing efficacy, and potentially the antibacterial mechanism itself varies with peptide structure and the target organism (Augustyniak et al. 2012; Veiga et al. 2012;

Salick et al. 2007, 2009; Liu et al. 2013b; Zhou et al. 2011); however, there is only a limited understanding of all the mechanisms involved, which limits the optimization and clinical use of these peptides (Sahl et al. 2005). Further hindrance comes from the *in vivo* instability of these antimicrobial agents, whereby they are easily degraded by host enzymes, in addition to being affected by pH and osmotic conditions (Brogden and Brogden 2011). Various structural modifications have been proposed to improve peptide resistance to enzymatic proteolysis without compromising its killing efficacy (Zasloff 2002; Hamuro et al. 1999; Porter et al. 2000).

Being a foreign protein, host defense peptides can potentially be immunogenic. Host defense peptides are considered to be weakly immunogenic, owing to their small size and linear structure (Fitzgerald-Hughes et al. 2012). Nevertheless, antibodies were produced to human neutrophil defensins (Panyutich et al. 1991), bovine lactoferricin(R) (Shimazaki et al. 1996) and hCAP-18 (Sørensen et al. 1997). Furthermore, it is unclear whether the use of foreign peptides will lead to the development of peptide resistance in pathogenic bacteria, and thus compromise the natural ability of the host to fight infection (Fitzgerald-Hughes et al. 2012). The effect of synthetic human defense peptide-like molecules on the natural innate response has yet to be studied.

With regard to selectivity and cytotoxicity of antimicrobial peptides (Hancock and Sahl 2006), the comparative absence of negatively charged lipids on the surfaces of eukaryotic cells and their weak membrane potential gradient may afford some protection to host cells (Fitzgerald-Hughes et al. 2012). Some host defense peptides, e.g. magainin 2 and human cathelicidin LL-37, can translocate into the cytosol of mammalian cells, e.g. HeLa, TM12 and Chinese hamster ovary (CHO)-K1 cells (Imura et al. 2008; Takeshima et al. 2003). At higher concentrations of the magainin 2 peptide, cell toxicity was observed. The translocation across host cell is possible owing to a dual role of peptides such as LL-37, i.e. bacterial targeting and delivery of nucleic acids into the host cells (Zhang et al. 2010). Even though LL-37 is not haemolytic at antibacterial-relevant concentrations, *in vitro* cytotoxicity has been observed. Toxicity towards eukaryotic cells of orangutan, rhesus macaque and leaf eater monkey orthologues of LL-37 varied with the physical characteristics, with the leaf eater monkey peptide being most toxic (Tomasinsig et al. 2009). Cytotoxicity also depended on the nature and metabolic state of the target cells. At the same time, haemolytic activity was found to be similar among the tested peptides.

The preferential activity against Gram-positive and/or Gram-negative bacteria membranes over eukaryotic cells can be enhanced by controlling the ratio of D- to L-amino acids (Fernandez-Lopez et al. 2001; Oren and Shai 2000; Yin et al. 2012). Structural modifications, e.g. truncation or rearrangement, can be used to enhance membranolytic efficiency (Shimizu et al. 1998). If it is done while disregarding other relevant targets, however, the increased non-specific membranolytic activity can undermine surface bio-compatibility to the extent that this modification will have limited *in vivo* use. Target-specific antimicrobial peptides, where a *Pseudomonas*-specific targeting moiety is appended to a generally killing peptide novispirin G10, can both improve the speed and efficiency of bactericidal action (Eckert et al. 2006).

7.3 Conclusion

The host response to the act of implantation initiates a highly complex and multi-dimensional cascade of events, that start with an injury and proceeds to include blood-surface interactions, provisional matrix formation, acute and chronic inflammation, foreign body reaction, development of granulated tissue, healing and fibrous capsule formation. The exact events taking place will depend on a multitude of different parameters, including those pertinent to the host, those associated with the quality of the surgical procedure, and those that result from interactions between living host tissues and the surface and bulk characteristics of the biomaterial. Not surprisingly, predicting or even describing the biocompatibility of an implant is not trivial. The task becomes even more challenging when biodegradable and eluting surfaces are considered. It is these surfaces, however, that hold the greatest promise in mitigating biomaterial-associated infections. Indeed, where antifouling surfaces may succeed in some short term applications, long-term and permanent devices require a reliable means to ensure pathogen-free surfaces. Surface modification that synergistically combines multiple complementary lines of defense may provide the most effective and durable solution. Nevertheless, balancing the biocompatibility of an implant against the ability of their surfaces to be efficient over a range of bacterial loads is a difficult task. With the rise of alternative antimicrobial agents or configurations, more information is needed regarding their compatibility, toxicity and immunogenicity towards relevant host cells. In order to attain a more comprehensive picture, it is important to approximate the conditions under which implants will operate and the quantities of active agents that can potentially be leached out into the peri-implant milieu. A standardized experimental procedure with a focus on both the anti-infective efficacy and biocompatibility should provide for a better comparison between different antimicrobial strategies. It should also provide valuable information on which material and process optimization can be based to ensure appropriate cell integration; after all, for many applications, the ultimate aim is to achieve an adequate tissue integration and a functional implant.

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