Chapter 6
Designing Antibacterial Surfaces for Biomedical Implants

Vy T.H. Pham, Chris M. Bhadra, Vi Khanh Truong, Russell J. Crawford, and Elena P. Ivanova

Abstract The infection of biomaterials, particularly medical implants, represents a significant challenge during surgical implantation processes and the subsequent recovery period for the recipient of the implant.

Infections arising from such surgical procedures not only adversely affect the well-being of the patient; they also place a significant burden on the healthcare systems of many countries around the world. A great deal of effort has been made in attempts to minimise or prevent pathogenic bacteria from contaminating these biomaterials. These efforts have included the development of techniques for rendering the surfaces anti-fouling through chemical modification or functionalization of the surface. Recent focus, however, has been placed on the production of antibacterial surfaces. Developments in the area of nanofabrication have allowed the chemical and physical characteristics of the surface of implant materials to be modified such that the molecular to micro-scale topological features can now be accurately controlled.

This chapter will provide an overview of the current approaches and techniques being used or are being developed in the design of antibacterial metallic implant surfaces. Such surfaces can be subjected to a number of chemical and physical modification techniques to achieve this aim, with the resulting surfaces being found to not only exhibit antibacterial behaviour, but also biocompatibility.

Keywords Implant surfaces • Antibacterial • Bactericidal • Titanium • Biocide-releasing surfaces • Nanoparticles • Surface topography

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6.1 Introduction

Bacterial infection of biomaterials has long been a significant problem, both during implant surgery and after the implantation process (Lavernia et al. 2006; Bozic and Ries 2005; Montanaro et al. 2011; Arciola et al. 2012; Campoccia et al. 2013a, b; Busscher et al. 2012; Whitehouse et al. 2002). Infection that results from receiving a contaminated biomedical implant substantially affects the quality of life for the patient and represents a large burden to society by increasing the number of clinical procedures having to be undertaken in the healthcare system, thus increasing the costs of health care. For example, it has been estimated that antibiotic treatment and revisionary surgery for replacement of an infected total hip causes the primary procedure costs to triple to an average of $75,000 in the US (Lavernia et al. 2006; Bozic and Ries 2005). It has been found that infections that have arisen from orthopaedic surgery prolonged the hospital stay for patients by a median of 2 weeks each, approximately doubled the re-hospitalisation rates, and increased healthcare costs by more than 300 % (Whitehouse et al. 2002). Complications arise as a result of the the pathogenic bacteria developing resistance to the natural host defense mechanisms and antibiotics. This means that treatment of the infection whilst the implant is still resident in the patient is usually unsuccesful, leading to the necessity for surgical intervention (Olson et al. 2002; Davies 2003; Vasilev et al. 2009; Høiby et al. 2010). It appears that only a low dose of inoculum is required to result in the infection of an implant; in an animal model study, it was found that 100 colony-forming units (cfu) of *S. aureus* were sufficient to infect 95 % of the subcutaneous implants used in the study (Arciola et al. 2012; Campoccia et al. 2013b; Song et al. 2013). Most of the microorganisms that cause implant infections are present in the host flora, of which the most frequent are Staphylococci, Streptococci, Pseudomonas species and coliform bacteria (Campoccia et al. 2013a, b; Olson et al. 2002; Davies 2003).

In an effort to combat implant-associated bacterial infection, recent focus has been placed on the development of antibacterial surfaces (Campoccia et al. 2013a, b; McLean et al. 1993; Yoshinari et al. 2001; Wan et al. 2007a, b; Zhao et al. 2009; Vasilev et al. 2009; Rautray et al. 2010; Glinel et al. 2012; Hajipour et al. 2012; Hasan et al. 2013). With the current growth in the development of novel nanofabrication tools, modifying the chemical and physical characteristics of implant surfaces has been shown to control the molecular to micro-scale topological features of many substrate surfaces. This chapter will provide an insight into the current methods being used for the design of antibacterial surfaces on implant materials and a rationale for the applicability of the unique surfaces being produced.

6.2 Strategies Being Used for the Design of Antibacterial Implant Surfaces

6.2.1 Overview

Implantable biomaterials have long been used for restoring joint function, reduce pain or stabilise fractured bones (Pourbaix 1984; Cui and Luo 1999; Wan et al. 2007a, b; Zhao et al. 2009; Rautray et al. 2010; Minagar et al. 2012; Andani et al. 2014). Antibacterial implant materials need to be both antibacterial and biocompatible (Anselme et al. 2010; Zhao et al. 2009, 2014; Vasilev et al. 2009; Busscher et al. 2012; Campoccia et al. 2013a, b). In 1987, Anthony Gristina first introduced the concept of a "race for the surfaces" to describe the competition that exists between the integration of tissue cells onto the surface of an implant material and the adhesion of pathogenic bacteria (Gristina 1987; Gristina et al. 1988). Clearly, it is desirable for the host tissue to have the opportunity to establish itself over the surface of a biomedical implant in order that the implant surface is connected to the body, allowing further tissue generation to take place without the invasion of pathogenic bacteria. It is therefore not surprising that a variety of alternative approaches have been developed for the construction of biomaterials that exhibit antibacterial properties that can simultaneously support the host tissue integration and effectively deal with any bacteria coming into contact with the surface.

Here, the different approaches currently being used to reduce the vulnerability of medical devices to bacterial attachment will be discussed. The current designs for antibacterial biomaterials can be classified into two major groups, classified according to their mode of action. The first is antifouling surfaces, which have the ability to repel or prevent bacteria from adhering to their surface. The second is bactericidal surfaces, which have the ability to damage or kill any pathogenic bacteria coming into contact with the surface (Fig. 6.1).

6.2.2 Antifouling Surfaces

Bacterial adhesion to an implant surface occurs when bacteria are able to make contact with a surface that provides them with a favourable environment for changing from their planktonic state to that of being sessile; this is most often accompanied by the production of a bacterial biofilm (Olson et al. 2002; Anselme et al. 2010; Arciola et al. 2012; Foster et al. 2014). It is clear that an infection arising from the presence of pathogenic bacteria would not occur if the bacteria involved were

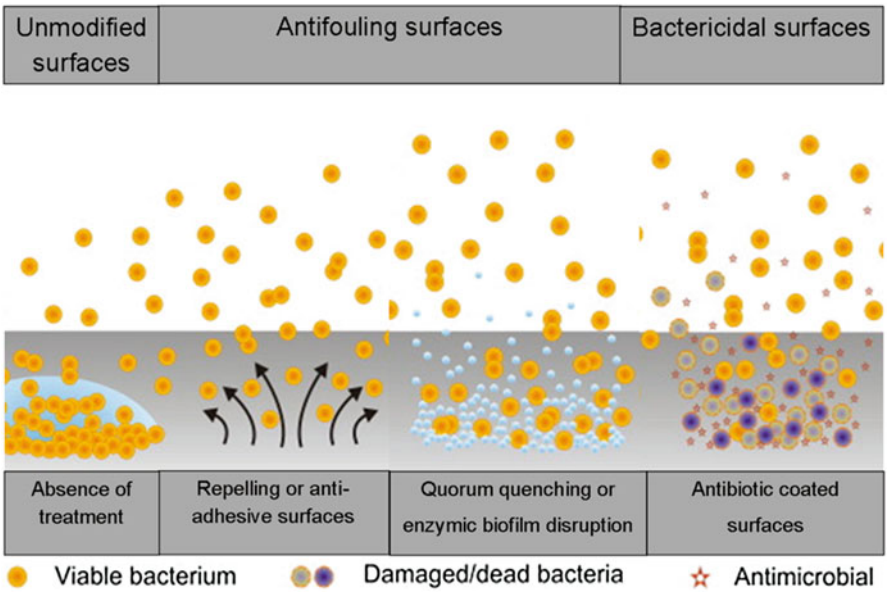

Fig. 6.1 Schematic representation of the different strategies currently being used in the design of antibacterial surfaces (Adapted from Campoccia et al. 2013a)

unable to initially colonise the medical device. The complex mechanisms associated with bacterial attachment have long been studied in order to gain an understanding into the methods by which metallic antibacterial surfaces can be designed such that this event can be prevented. A wide range of chemico-physical properties and functional groups on both the substrate and pathogen have been modified in order to modulate the attachment of these bacteria (Fusetani 2004, 2011; Hasan et al. 2013; Bazaka et al. 2011a, 2012; Webb et al. 2011; Crawford et al. 2012). More recently, surface architectures that contain specific surface porosity, roughness and geometry have been used to produce metallic biomaterial surfaces that are resistant to microbial colonisation (Meng et al. 2014; Anselme et al. 2010; Webb et al. 2011; Crawford et al. 2012; Bazaka et al. 2012).

Metallic biomaterial devices are often exposed to body fluids and a rich protein environment at the site of surgical implantation (Arciola et al. 2003; Campoccia et al. 2013a, b). It is known that a variety of host proteins promote bacterial attachment and the formation of biofilms. These are called microbial surface components recognizing adhesive matrix molecules, or MSCRAMMs, and these include collagen, fibrinogen, fibronectin, laminin, vitronectin, clumping factor A and B, bone-sialoprotein, elastin, IgG and other possible components (Lv et al. 2013; Montanaro et al. 2011; Patti et al. 1994; Arciola et al. 2012; Foster et al. 2014; Foster and Höök 1998; Hauck et al. 2006; Lambris et al. 2008). The biomaterial surfaces are therefore required to support the adsorption of host adhesins onto their surface to ensure

the successful subsequent integration of tissue, whilst at the same time being able to repel the bacteria or be anti-adhesive towards them.

Chemical methods can be used to construct microbe-repellent surfaces by attaching antifouling molecules to the surfaces of implant materials (Neoh et al. 2012; Campoccia et al. 2013a, b). Common chemical modification approaches include rendering the surfaces superhydrophobic/superhydrophilic or highly hydrated or non-charged, each of these being unfavourable for bacterial adhesion under certain circumstances (Fig. 6.1) (Campoccia et al. 2013a, b). One of the most common coatings to render the surface hydrophilic is poly(ethylene glycol) (PEG). The inhibition mechanism of such surfaces is based on the dynamic motion and steric repulsion of hydrated polymer chains, which prevents bacterial attachment (Harris et al. 2004; Maddikeri et al. 2008). In addition, polycationic polymers exhibit antifouling effects have been used by directly coating or grafting them onto biomedical devices (Chua et al. 2008; Subbiahdoss et al. 2010; Shi et al. 2008; Hu et al. 2010; Siedenbiedel and Tiller 2012). Heparin coatings have also been shown to exhibit a high anti-adhesive effect for bacteria by increasing the hydrophilicity of the surfaces. The heparin forms a highly hydrated layer between the pathogens and the substrate (Ruggieri et al. 1987; Arciola et al. 1993). It was reported that heparin can inhibit the extent of *S. epidermidis* binding to fibronectin, thus preventing the subsequent colonisation of the surface (Arciola et al. 2003; Bustanji et al. 2003). Another approach, where quorum-sensing inhibitors (e.g. furanones and their derivatives) are incorporated onto biomedical device surfaces, was used to disrupt the processes responsible for the formation of a biofilm (Fig. 6.1) (Fusetani 2004, 2011). This approach, however, has significant drawbacks in terms of the long-term stability of the coating and the possible cytotoxicity of these additives in biomedical applications. Current approaches use surface topography as the factor by which the degree of bacterial adhesion and subsequent biofilm formation can be controlled or prevented. Techniques such as this represent a more robust method for creating surfaces that repel or control the extent of microbe attachment (Webb et al. 2011, 2014; Hasan et al. 2013; Grinthal and Aizenberg 2014; Bazaka et al. 2012; Crawford et al. 2012). For example, superhydrophobic surfaces have been shown to exhibit antifouling characteristics and can be obtained by physically modifying the micro- and nanostructures of biomaterial surfaces by mimicking natural surface structures such as that of the lotus leaf (Truong et al. 2012; Fadeeva et al. 2011; Crick et al. 2011). By tailoring the precise and specific surface topographical parameters, these surfaces have shown promising results in their ability to limit the initial adhesion of pathogenic bacteria (Webb et al. 2011, 2014; Hasan et al. 2013).

6.2.3 Bactericidal Surfaces

Another common approach in the prevention of biofilms on biomedical devices is the utilization of bioactive antibacterial agents that act by contact killing the bacteria. These techniques involve coating the substrate with various immobilized

antimicrobial substances such as antibacterial peptides (Brouwer et al. 2011; McCloskey et al. 2014; Salwiczek et al. 2014), quaternary amines (Mei et al. 2012; Schaer et al. 2012), nitric oxide (Fox et al. 2010; Nablo et al. 2005) or antibacterial metals (silver, zinc, cobalt, aluminium and copper) (Kawashita et al. 2000; McLean et al. 1993; Heidenau et al. 2005; Lemire et al. 2013; Stafford et al. 2013; Prantl et al. 2010; Wan et al. 2007a). These substances are not released from the substrate, and as such they directly interact with any pathogenic bacteria coming in contact with the surface (Williams and Worley 2000). Bioactive antibacterial coatings have been used extensively in applications that require the surface to be self-sterilizing over extended periods (Williams and Worley 2000; Campoccia et al. 2013a).

Silver and its derivatives are some of the earliest bactericidal agents that have been largely applied in a wide range of applications (Zhao et al. 2009; Kawashita et al. 2000; McLean et al. 1993; Nomiya et al. 1997; Dueland et al. 1982; Richards 1981; Bayston et al. 2010). Other metals that have also been reported to exhibit bactericidal effects, mostly in their composite form, include zinc, cobalt, aluminium and copper (Heidenau et al. 2005; Lemire et al. 2013; Samanovic et al. 2012; Stafford et al. 2013; Hoene et al. 2013; Prantl et al. 2010; Shirai et al. 2009; Wan et al. 2007a, b). The use of antimicrobial metals is, however, often associated with certain degree of cytotoxicity. This can have an impact on the host cell response, leading to the loss of cell viability and the failure of tissue integration (Heidenau et al. 2005; Hoene et al. 2013; Paasche et al. 2011; Vasilev et al. 2009). This occurs mainly as a result of corrosion of the metal in the physiological environment, which causes the release of metal ions at relatively high concentrations, leading to local toxicity and occasionally metal accumulation in the target organs (Campoccia et al. 2013b; Lemire et al. 2013; Vasilev et al. 2009). The mechanisms responsible for the antibacterial activity of metals and metal ions is not fully understood. Gordon et al. suggested that silver interacts with thiol groups, causing the inactivation of critical enzymes in the respiratory chain and the induction of hydroxyl radicals (Gordon et al. 2010).

Another emerging strategy for the manufacture of antimicrobial surfaces is the incorporation of biocide-releasing surfaces such as those containing nanoparticles. The extent of the bactericidal effect of these surfaces depends on the size, shape, concentration and chemical composition of the nanoparticles (Cui et al. 2012b; Zhang et al. 2013; Hajipour et al. 2012). While the exact mechanisms of the antimicrobial activity are also not fully understood, most nanoparticles are seen to generate reactive oxygen species and damage the cell membranes (Cui et al. 2012b; Zhang et al. 2013; Hajipour et al. 2012). For example, gold nanoparticles exhibit bactericidal effects against *E. coli* by inhibiting ATP synthase activity, followed by the inhibition of the ribosome sub-unit in tRNA binding (Cui et al. 2012b). There is still a lack of knowledge on the toxicology of nanoparticles, with most of the available data being inconsistent and largely non-reproducible (Campoccia et al. 2013a; Yildirimer et al. 2011). The negative impact of nanoparticles in biomedical applications includes the induction of apoptosis, introduction of toxic effects to the genome and the possible translocation of nanoparticles to distant tissues and

organs, with an associated risk of systemic effects (Yildirimer et al. 2011; Campoccia et al. 2013a).

The major problem, however, is that biofilms display an increased tolerance towards antimicrobial agents, which substantially restricts our ability to treat biofilm-related infections in clinical settings. While the increased resilience of biofilms towards antibiotics is multifactorial, this resistance can be attributed to the presence of persistent bacteria, those that can enter into a specific phenotype state that allows them to survive in the presence of 1000 times the minimum inhibitory concentration of bactericidal antibiotics (Olson et al. 2002; Davies 2003). Persistent cells have recently been the subject of increased investigation with a view to limiting their biofilm-associated antibiotic tolerance. The current strategy for preventing the formation of biofilms has been to develop ways by which the initial bacterial adhesion step can be inhibited, which will subsequently limit the growth of the biofilm (Hasan et al. 2013; Fusetani 2004).

Recently, the effects of surface topography on the attachment responses of bacterial and mammalian cells has been under investigation in an effort to obtain an insight into the competition that takes place when bacteria and host tissue compete for attachment on a substrate surface (Hasan et al. 2013). When trying to prevent biofilms from forming on medical implant surfaces, a common approach is to develop a surface structure that can both physically inhibit the growth of bacteria, but at the same time promote tissue integration. Nano-structured surfaces have shown numerous promising results. Interesting reports have demonstrated that surfaces containing nanopillar arrays that mimic the structure of dragonfly wings can exhibit a bactericidal effect to not only Gram positive and Gram negative bacteria, but also their spores (Hasan et al. 2013; Ivanova et al. 2013; Bazaka et al. 2012). Recent research has also shown that mammalian cells are biocompatible with these high aspect ratio structured surfaces that contain complex geometries, in which the cells appeared to be able to maintain their viability, adhesion to the surface and their subsequent cellular activities (Kim et al. 2007; Robinson et al. 2012; Elnathan et al. 2014; Jahed et al. 2014). For the reasons previously described, the ability to design surfaces that possess antimicrobial properties without the need for the surface to contain antibiotics or chemical additives represents a significant step forward in developing implant materials that are less likely to be the cause of post-operative infections.

6.3 Fabrication Techniques

Since the advent of micro- and nano-technology, a number of fabrication techniques have been developed that can be used to modify the surface properties of metallic implants on a molecular-, nano- and micro-scale. Antibacterial metallic surfaces can be fabricated via two principal methods: chemical and physical modification (Jeon et al. 2014; Lv and Feng 2006; Yoshinari et al. 2001; Vasilev et al. 2009;

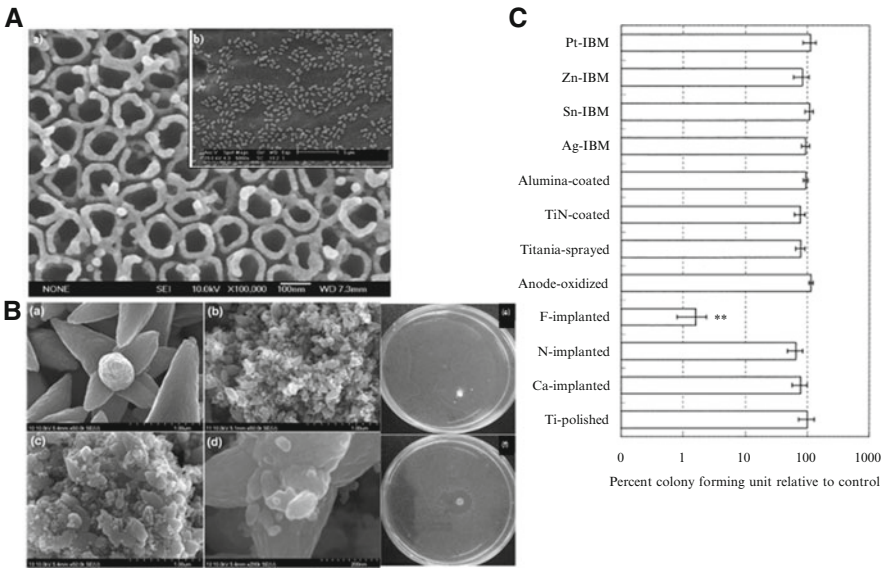

Fig. 6.2 Representative antibacterialmetallic surfaces fabricated via various chemical fabrication techniques. (**A**) (*a*) SEM images of the *top-view* of TiO_2 nanotube surfaces; the nanotubes are approximately 40–97 nm in diameter and 300 nm in length. (*b*) (inset) the changed morphologies of the surface after the adhesion patterns of *Streptococcus mutans*, cultured for 48 h (Adapted with permission from Cui et al. 2012a, b). (**B**) FE-SEM images of (*a*) pure ZnO, (*b*) TiO_2/ZnO, (*c*) Ag/TiO_2/ZnO particles, and (*d*) high magnification of (*c*). (*e*) and (*f*) represent the zones of inhibition tests for (*top*) TiO_2/ZnO and (*bottom*) Ag/TiO_2/ZnO composite surfaces towards *E. coli* (Adapted with permission from Pant et al. 2013). (**C**) Colony-forming units relative to control against *P. gingivalis* on 1 cm^2 plates for 48 h against different ion-implanted Ti surfaces (Adapted with permission from Yoshinari et al. 2001)

Hasan et al. 2013; Salwiczek et al. 2014). Techniques such as surface chemical functionalization, chemical vapour deposition, anodic oxidation, hydrothermal treatment and ion implantation involve chemical reactions occurring at the surface to modify the surface properties. Techniques such as physical vapour deposition, layer-by-layer coating, and sol-gel coating utilise physical adsorption on implant surfaces without altering the surface chemistry.

6.3.1 Chemical Modification

This section highlights the techniques that employ processes such as wet chemistry and high energy sources to alter the surface characteristics of metal surfaces. Here, we present a general overview of the chemical modification techniques that can be used to produce antibacterial metallic surfaces in Figs. 6.2 and 6.3.

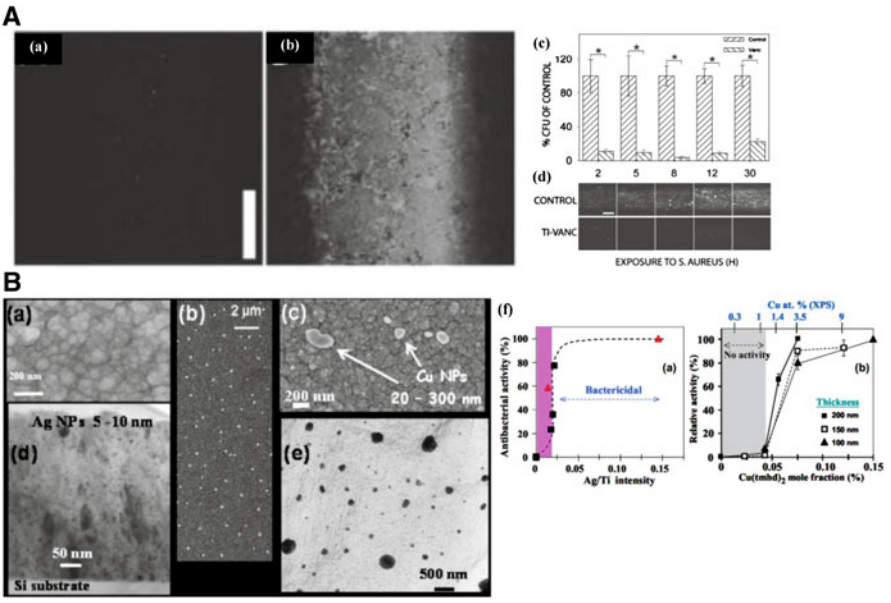

Fig. 6.3 (**A**) Confocal microscopic images of (*a*) a control Ti rod and (*b*) Ti rods covalently linked with vancomycin (vanc); (*c*) The antibacterial efficiency of the control and Ti-vanc rods towards *S. aureus* incubated at a time period from 0 to 30 h. The Ti-Vanc rods showed fewer adherent colonies at all times than control rods. (*d*) Represents the fluorescent stains for the viable cells on the control Ti surface (Adapted with permission from Antoci et al. 2007). (**B**) SEM images of (*a*) Ag-TiO_2 and (*b*, *c*) Cu-TiO_2 films. (*d*) TEM cross section micrograph of Ag-TiO_2 and (*e*) planar view of Cu-TiO_2 coatings show the metal nanoparticle distribution in the TiO_2 matrix through chemical vapour deposition (CVD). (*f*) The influence of Ag (*left*) and Cu (*right*) content of M-TiO_2 nanocomposite coatings on the antibacterial behaviour against *S. aureus*. The *coloured zone* corresponds to inactive surfaces according to the JIZ test (Adapted with permission from Maury et al. 2014)

6.3.1.1 Chemical Functionalisation

Chemical functionalization of various metallic surfaces has been one of the most commonly used techniques for preparing antibacterial surfaces (Zobrist et al. 2011; Ogaki et al. 2010; Gerberich and Bhatia 2013). This fabrication technique is suitable for designing antifouling and bactericidal surfaces that can kill bacteria on contact. The functionalisation of inert metallic surfaces is, however, a challenge. To overcome this problem, there are two strategies used for functionalising surfaces such that they contain antifouling or bactericidal agents (Yuan et al. 2011; Gadenne et al. 2013; Antoci et al. 2007; Godoy-Gallardo et al. 2014; Holmberg et al. 2013; Chen et al. 2013): These are: (1) developing adhesion coatings, such as self-assembled monolayers (SAM); or (2) functionalising metallic surfaces with active groups, such as thiol (-SH), hydroxyl (-OH), amine (-NH_2) or carboxylic groups (-COOH).

The first strategy is to coat the surface with self-assembled monolayers (SAM) to act as an adhesion layer for the immobilisation of antibacterial agents (Yuan et al.

2011; Gadenne et al. 2013). For example, self-assembled monolayers of aminoundecyltrimethoxysilane were used as an adhesion layer on Ti substrates to covalently bond polysaccharides extracted from *Ulva rotundata* and *Ulva compressa* seaweed (Gadenne et al. 2013). Adhesion of *Pseudomonas aeruginosa* cells was shown to be reduced by 90 % on these surfaces compared to the control surface. Conjugation of poly(ethylene glycol) (PEG) brushes on stainless steel was used to couple with hydrolytic enzymes such as lysozyme (Yuan et al. 2011), creating a surface that exhibited a high degree of antifouling and bactericidal behaviour towards *E. coli* and *S. aureus* cells. The dual coupling of the PEG derivative along with lysozyme served a dual function; displaying antifouling properties towards bovine serum albumin protein and antibacterial behaviour towards the specific bacterial strains (Yuan et al. 2011).

A second approach is to functionalise surface with reactive groups, then covalently bind antibiotics or antimicrobial peptides (AMP) to the surface (Antoci et al. 2007; Godoy-Gallardo et al. 2014; Holmberg et al. 2013; Chen et al. 2013). A number of studies have been reported for the successful tethering of antibiotics to metallic implant surfaces. For example, non-reactive Ti surfaces were chemically modified to carry amine groups that reacted with vancomycin (Antoci et al. 2007). This method was shown to be able to preserve the antibacterial properties of the modified Ti surfaces, while preventing the release of antibiotics into the surrounding body fluid. In other studies, AMP were conjugated onto metallic surfaces. For example, Ti surfaces were chemically modified to contain hydroxyl groups, onto which hydrophilic poly (ethylene glycol) was conjugated as a spacer between the AMP and the Ti surface (Gabriel et al. 2006).

6.3.1.2 Chemical Vapour Deposition

Chemical vapour deposition (CVD) is yet another chemical process that has been used to fabricate an antibacterial layer over substrate surfaces on a large scale (Wilkinson et al. 2013; Bazaka et al. 2010, 2011a, b, 2012; Dastjerdi and Montazer 2010; Maury et al. 2014; Varghese et al. 2013). In a deposition process, the substrate is exposed to antibacterial monomer precursors, which react with the substratum under plasma condition to synthesise antibacterial surfaces. These processes have been used to fabricate both inorganic and organic antibacterial coatings.

Inorganic coatings on metallic implant surfaces such as silver, TiO_2 and other photocatalytic metal oxides was performed using CVD (Dastjerdi and Montazer 2010; Maury et al. 2014; Varghese et al. 2013). Composite TiO_2 films, coupled with other metallic ions such as Ag^+ or Cu^{2+}, have also been synthesized using CVD (Maury et al. 2014). TiO_2 nano-composite films coupled with Ag have been shown to exhibit strong bactericidal efficiency against *S. aureus*, where a minimum film thickness of 100 nm has been found to be essential for rendering the film antibacterial in nature (Maury et al. 2014). Flame-assisted chemical vapour deposition has also been recently employed to fabricate silver-silica coatings on glass surfaces, the antibacterial efficiencies of which have been tested using *E. coli*, *S. aureus* and *P.*

aeruginosa (Varghese et al. 2013). A plasma deposition method has also been used to deposit Cu ions on titaniumalloy surfaces. This combination of Cu ions embedded onto Ti_6Al_4V surfaces has proven to be an effective method for preparing an antibacterial surface, since the amount of copper ions released from the surface resulted in killing ~99 % of the bacterial cells (Hempel et al. 2014).

For organic coatings to be prepared using CVD, monomers of antibacterial agents need to be used as precursors for the formation of antibacterial polymer films on a substrate (Bazaka et al. 2010, 2011a, b; Pegalajar-Jurado et al. 2014). For example, substrata have been encapsulated with polyterpenol thin films prepared from antibacterial terpinen-4-ol monomers using radio frequency plasma enhanced chemical vapour deposition (Bazaka et al. 2010, 2011b). These polyterpenol films were found to be effective against *P. aeruginosa* and *S. aureus* cells due to the preservation of original terpinen-4-ol molecules in the resulting film structure (Bazaka et al. 2010, 2011b). In another study, plasma polymerised thin films produced from antibacterial 1,8-cineole monomers were shown to inhibit the proliferation of *E. coli* and *S. aureus* (Pegalajar-Jurado et al. 2014).

6.3.1.3 Anodic Oxidation

Electrochemical anodic oxidation techniques have been used to fabricate highly ordered nanoporous nanotubes on metal surfaces (Cui et al. 2012a; Li et al. 2013; Yue et al. 2014; Minagar et al. 2012; Visai et al. 2011). In particular, TiO_2 nanotubes have been recognized as promising biomaterials, with proven biocompatibility, thermal stability and corrosion resistance. Enhancement of the antibacterial properties of TiO_2 nanotubes can be achieved through the use of UV illumination or the incorporation of antibiotic loadings (Cipriano et al. 2014; Cui et al. 2012a; Çalışkan et al. 2014; Chennell et al. 2013). For example, TiO_2 nanotube layers have been designed to become photocatalytic, which can disrupt the viability of *Streptococcus mutans* (Cui et al. 2012a), whereas the incorporation of gentamycin inside the nanotube patterns was found to improve their antibacterial properties (Çalışkan et al. 2014).

6.3.1.4 Hydrothermal Treatment

Hydrothermal synthesis is an environmental friendly process for synthesising antibacterial surfaces because the synthesis reaction is completely contained in aqueous solutions inside a closed system, making use of acidic or basic solutions as the reaction medium (Huo et al. 2013; Pant et al. 2013; Hebeish et al. 2013; Li et al. 2014; Wong et al. 2011). In such processes, the operating temperature is held above the boiling point of water to autogenously produce a saturated vapour pressure. As with the anodic oxidation technique, this technique is only suitable for the preparation of antibacterial inorganic coatings, such as silver, TiO_2, and other photocalytic materials (Huo et al. 2013; Pant et al. 2013; Hebeish et al. 2013; Li et al. 2014).

For example, a one-step hydrothermal synthesis has been used to fabricate ZnO and silver nanoparticles coupled with a passive TiO_2 layer to improve the antibacterial efficiency of photocatalytic TiO_2 layers (Pant et al. 2013). In addition, it has been reported that Ti nanowires doped with silver exhibited commendable antimicrobial efficiency towards various *Pseudomonas* species (Hebeish et al. 2013; Li et al. 2014).

6.3.1.5 Ion Implantation

Ion implantation has been used as a method for doping antibacterial inorganic ions onto the surface of biomedical implants under the immersion of plasma (Cui and Luo 1999; Rautray et al. 2010; Lu et al. 2012; Yoshinari et al. 2001; Zhao et al. 2009). Ti and Ti alloys modified with ion implantation (F^+) were shown to inhibit the growth of both *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitan* (Yoshinari et al. 2001; Rautray et al. 2010). In addition, it was shown that F+implanted surfaces did not inhibit the proliferation of fibroblast L929-cells (Yoshinari et al. 2001). Silver and copper ion-doped Ti surfaces prepared using ion implantation processes were shown to reduce the viability of *S. aureus* cells coming into contact with the surface (Wan et al. 2007a).

6.3.2 Physical Modification

Physical modification techniques coat metallic substrates through physical adsorption without altering the surface chemistry. Here, we present a general overview of the physical modification techniques that can be used for the preparation of antibacterial metallic surfaces (Fig. 6.4).

6.3.2.1 Physical Vapour Deposition (PVD)

Physical vapour deposition (PVD), due to its environmentally friendly characteristics, convenience and precision in deposition, has become one of the commonly used techniques in preparing uniform surface coatings (Zaborowska et al. 2014; Percival et al. 2005; Ip et al. 2006; Ivanova et al. 2011; Chang et al. 2013; Trivedi et al. 2014). A number of reports have shown that physical vapour deposition has also been used to deposit uniform coatings on titanium and its alloys. This technique fabricates an antibacterial surface by employing a twin-gun magnetron sputtering system to synthesize uniform coatings of silver and zirconium oxide (Zaborowska et al. 2014; Percival et al. 2005; Ip et al. 2006; Ivanova et al. 2011; Chang et al. 2013). This process is also commonly used to deposit silver coatings on titanium surfaces, since the silver coating provides a biocompatible surface and also helps to reduce the attachment and viability of bacteria (Zaborowska et al. 2014; Percival et al. 2005; Ip

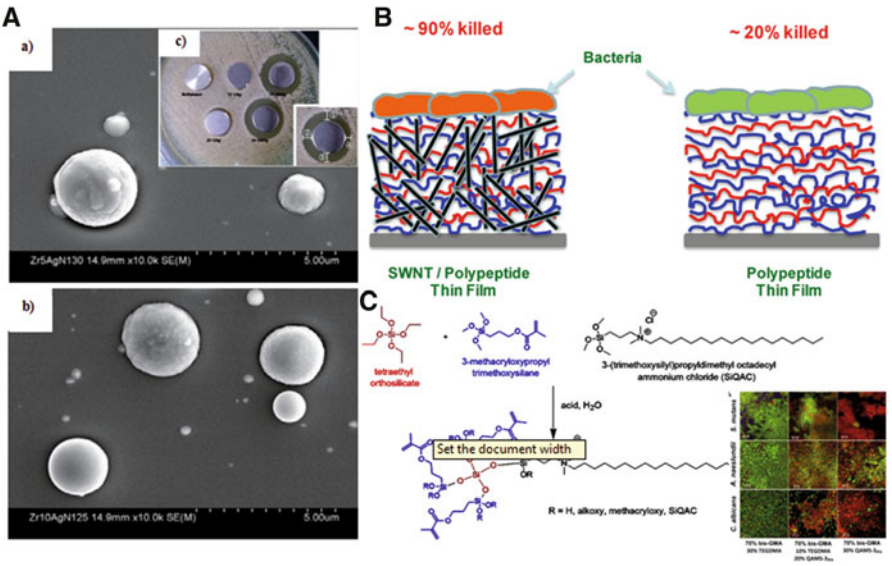

Fig. 6.4 Representative antibacterial metallic surface fabricated via various physical fabrication techniques. (**A**) FE-SEM images of the (*a*) TiAgN and (*b*) ZrAgN coatings fabricated through physical vapour deposition (PVD). *Scale bars* indicate 5 μm. *Inset* image (*c*) represents the clear zone of inhibitions of the coated samples against *S. mutans* after 18 h incubation (Adapted with kind permission from Kang and Lim 2014). (**B**) A schematic illustration of the antimicrobial property of the layer-by-layer assembly of single wall carbon nanotubes along with the polyelectrolytes poly(L-lysine) and poly(L-glutamic acid) (Adapted from Aslan et al. 2012). (**C**) Reaction scheme for producing quaternary ammonium silanes (QAMS) using tetra-alkoxysilane as attaching unit. Macromonomers with monofunctional (QAMS-1), bifunctional (QAMS-2) or trifunctional methacryloxy functionalities (QAMS-3) have been fabricated based on the molar ratio of the two trialkoxysilanes through the process of sol-gel coating. Inset image represents the antimicrobial activity of the polymerized resin through the confocal laser scattering microscopy images of 48 h microbial biofilms of *S. mutans* (*top*), *A. naeslundii* (*middle*) and *C. albicans* (*bottom*) respectively (Adapted with permission from Gong et al. 2012)

et al. 2006; Ivanova et al. 2011; Chang et al. 2013). Additionally, this system offers almost no cytotoxicity issues towards mammalian cells.

Another slight variation of this technique, arc-ion plating (AIP), has also been used to fabricate TiAgN and ZrAgN alloys coatings (Kang and Lim 2014). These coatings serve as efficient antibacterial coatings by reducing the adhesion and viability of *S. mutans* (Kang and Lim 2014). AIP is a widely used technique in the biomedical industry due to its advantages, including its ability to produce a dense metal vapour, high ionization efficiency and high deposition rate (Joo et al. 2009). Plasma nitriding of stainless steel surfaces with Ag^+ ions has also resulted in the synthesis of highly efficient antimicrobial surfaces, which have been reported to eliminate almost all (~97 %) of the inoculating bacterial cells of *E. coli* and *S. epidermidis* in a 6 h time span by the method of contact killing (Dong et al. 2011).

6.3.2.2 Layer-by-Layer Coatings

Layer-by-layer self-assembly (LbL) is the technique that has been used to encapsulate antibiotics, antimicrobial peptides and nanomaterials onto metallic surfaces (De Villiers et al. 2011; Hammond 1999, 2004; Decher 1997; Linford et al. 1998). The technique has some essential steps, as follows: (1) A charged substrate is submerged in a solution of an oppositely-charged colloid, designed to assist the adsorbtion of the first monolayer, (2) this is followed by a washing cycle to remove unbound material and impede the contamination of the surface with the oppositely-charged colloid, (3) the coated substrate is re-submerged to finally adsorb the second layer and thus a series of multi-layered deposits is formed in this manner (De Villiers et al. 2011). There also have been instances where no washing step is required in the sample preparation, as in the case of strong electrolytes, where the polymer is strongly bound to the surface by electrostatic interactions (Linford et al. 1998).

Feature sizes lesser than 1 μm can be easily obtained, since the limitations of this technique are only restricted by the fairly large dimensions of the bound macromolecules in the solution (De Villiers et al. 2011). A recent use of this technique has been in the fabrication of antibacterial surfaces. Here, the antimicrobial efficiency of single-walled carbon nanotubes (SWNT) that were layer-by-layer assembled with poly (L-lysine) and poly (L-glutamic acid) were well studied for their resistance to the attachment of the bacterial strains of *E. coli* and S. *epidermidis* (Aslan et al. 2012, 2013). This assembly of multilayer films has the ability of reducing the proliferation rates of the bacterial cells by up to 90 % (Aslan et al. 2012, 2013). With LbL technology, multi-faceted surfaces with altered shapes can be conveniently coated with conformal ultra-thin films (Hammond 2004; Decher 1997). Layer by layer assembly has also been used to fabricate a cross-linked polymeric thin film using a polycation, N, N-dodecyl, methyl-polyethylenimine and a polyanion, poly (acrylic acid). Surfaces containing this combination have been reported to be highly effective against the commonly found infecting strains of *E. coli* and *S. aureus* (Wong et al. 2010). This film exhibited considerable antibacterial effectiveness by causing cell lysis when the cells came in contact with the surface of the polymeric film.

6.3.2.3 Sol-Gel Coating

Sol-gel coating processes involve the conversion of monomers into antibacterial colloidal solutions (sols) that acts as precursors for an integrated network (gel) of the coatings (Chun et al. 2007; Rivero et al. 2011; Talebian et al. 2014; Visai et al. 2011). Sol-gel TiO_2 coatings on stainless steel orthodontic wires have been shown to reduce the viability of *Streptococcus mutans* and *Porphyromonas gingivalis* (Chun et al. 2007). A novel antibacterial coating composed of an organic-inorganic hybrid matrix of tetraorthosilicate and a polyelectrolyte was successfully doped with Ag nanoparticles through sol-gel processes (Rivero et al. 2011). This specific coating has ability to eliminate the growth of *Lactobacillus plantarum*. Nickel oxide

nanoparticles that were immobilised in a sol-gel coating were also found to exhibit a high degree of antibacterial activity against *S. aureus* and *E. coli* cells (Talebian et al. 2014).

6.4 Future Perspectives

In general, this chapter provided an overview of the current approaches and techniques that have been developed in the design of antibacterialmetallic implant surfaces. Metallic implant surfaces can be modified to become either antifouling or bactericidal in nature. To achieve this, there are a number of chemical or physical modification techniques that have been developed for the fabrication of antibacterial metallic implant surfaces. In the last few decades, the surface chemical characteristics of a surface have been modified such that the resulting surface is not only antibacterial, but also biocompatible. Investigation of the cytotoxicity and biocompatibility of such modified materials requires significant resources. One of the currently emerging approaches for determining the cytotoxicity and biocompatibility of a surface is to develop surface micro- and nano-architectures that generate repulsive forces towards bacteria (Ivanova et al. 2012, 2013; Hasan et al. 2013). These techniques have proven to be a good starting point for the innovative design of metallic biomaterials, and represent an alternative approach to the methods that have traditionally been adopted. To date, only a limited number of studies have been performed to address these issues (Ivanova et al. 2012, 2013; Fadeeva et al. 2011). These recently developed methods for modifying the nanotopography of surfaces may prove to be very useful techniques for the fabrication of a new generation of antifouling or bactericidal biomaterials.

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