Chapter 14
The Use of Antimicrobial Nanoparticles to Control Oral Infections

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14.1 Introduction

Nanotechnology represents the ability to image, manipulate and model functionalities on the nanometer scale. Nanoparticles can be classified as particles of a size no greater than 100 nm, and their unique attributes to combat infection have received considerable attention. Nanomaterials are increasingly finding uses in products such as antimicrobial surface coatings and semiconductors. Such nanoparticulate materials include spherical, cubic and needle-like nanoscaled particles (approximately 5–100 nm) and near-nanoscaled devices (up to micrometers) (Cushing et al. 2004). The properties of nanoparticles, for example hardness, active surface area, chemical reactivity and biological activity, can be dramatically different from those of micrometer-sized particles (Allaker and Ren 2008), and indeed the biocidal effectiveness of metallic nanoparticles has been suggested to be due to both their size and their high surface-to-volume ratio. Such characteristics should allow them to closely interact with microbial membranes, the effect not being solely due to the release of metal ions (Morones et al. 2005). Metallic and other nanoparticles are now being combined with polymers or coated onto surfaces which may have a variety of potential antimicrobial applications within the oral cavity (Monteiro et al. 2009; Hannig et al. 2007).

The mouth supports the growth of a wide diversity of micro-organisms including bacteria, yeasts, viruses, and (on occasions) protozoa. Bacteria are the predominant components of this resident microflora, and the high species diversity found reflects the wide range of endogenously derived nutrients, the varied types of habitat for colonisation, and the opportunity to survive on surfaces provided by a biofilm. Within this context, a biofilm can be classed as an aggregate of microorganisms in which cells adhere to each other and to a surface (Marsh and Martin 2009).

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However, perhaps more commonly than elsewhere in the body, the relationship between this flora and the host in the oral cavity can be disrupted in a number of ways, resulting in the development of disease of the oral structures.

Potential habitats suitable for attachment within the oral cavity include either non-shedding, hard tooth surfaces or soft, constantly replaced epithelial surfaces, and conditions vary with respect to oxygen levels and anaerobiosis, availability of nutrients, exposure to salivary secretions or gingival crevicular fluid, masticatory forces and other variables such as oral hygiene procedures. As a result, the composition of the microbial flora of the mouth varies considerably from site to site and at different times. The composition of the oral microflora, in addition to being variable, is highly complex. Up to 1,000 different species of bacteria at 10^8–10^9 bacteria per mL saliva or mg dental plaque are known to be associated with the oral cavity, and it has been suggested that only 50% of the bacteria found at this site can be cultured (Marsh and Martin 2009).

In the majority of cases, infections of the oral cavity are bacterial, fungal or viral. Most bacterial infections within the oral cavity are polymicrobial in nature, and it is quite unusual to find any that are clearly due to a single species. The relative contribution of different bacterial components in such infections is thus difficult to determine. Oral infections may arise either from an endogenous source, i.e. one yielding microorganisms normally found in the mouth, such as plaque-related dental caries ('tooth decay') and periodontal disease ('gum disease'), or an exogenous source yielding micro-organisms not normally found as part of the oral microflora. Dental caries and periodontal disease involve the adherence of bacteria and development of biofilms on both the natural and restored tooth surface. The use of nano-sized antimicrobials offers the possibility to control the formation of these and other oral biofilms through the use of nanoparticles with biocidal, anti-adhesive and delivery capabilities.

14.2 Biofilms and Oral Infections

Biofilms of oral bacteria and yeasts can cause a number of localised diseases in the oral cavity, including dental caries, periodontal disease, candidosis ('oral thrush'), endodontic ('tooth root and pulp disease'), orthodontic ('dental braces') and dental implant ('titanium root') infections (Marsh and Martin 2009).

14.2.1 Formation and Properties

The survival of microorganisms within the oral cavity is dependent on their ability to adhere to surfaces and subsequently develop into a biofilm, a process influenced by the physical and chemical properties of the underlying surface (Hannig and Hannig 2009). On the tooth surface, the initial colonisers adhere to the acquired

pellicle, a salivary-/dietary-derived proteinaceous layer, which can then influence the subsequent sequence of colonisation by microorganisms (Marsh and Bradshaw 1995). The acquired pellicle also contains several antibacterial components such as secretory immunoglobulin A (IgA) and lysozyme, and provides both barrier and buffering functions (Hannig and Joiner 2006). Both de- and remineralisation processes of the teeth are also mediated by the pellicle. In terms of bacterial colonisation, many of the proteins that make up the pellicle act as receptors for the specific interaction with adhesins on the surface of pioneer bacterial species (Hannig and Joiner 2006). This layer is therefore of particular relevance as regards the interactions of both bacteria and nanoparticles with the tooth surface.

The strength of the forces involved in the initial attachment of bacteria is critical to their survival and the subsequent growth of the biofilm. The major growth of dental plaque mass then occurs by bacterial cell division within the biofilm rather than by co-aggregation at the surface of the developing biofilm (Kolenbrander et al. 2006). The initial communities of bacteria found within the supragingival plaque (above the gum margin) biofilm are of a relatively low diversity in comparison to those present in the mature communities of both supragingival and subgingival (below the gum margin) plaque. Initial colonisers include *Streptococcus oralis*, *S. sanguinis* and *S. mitis*. The coaggregating partners with these bacteria would then include predominantly Gram-negative species, for example *Eikenella corrodens*, *Veillonella atypica* and *Prevotella loescheii*. Coaggregation bridges between these early colonisers and *Fusobacterium nucleatum*, are common and the latter then co-aggregates with numerous late colonisers. Late colonisers include *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Treponema denticola* and *Porphyromonas gingivalis* (Kolenbrander et al. 2006). The interactions between oral bacteria are integral to the development and maturation of the biofilm. Such interactions occur at a number of levels and include physical contact, metabolic exchange, molecular communication and genetic material exchange.

Oral biofilms will accumulate on both the hard and soft tissues, and this community of microbial species is embedded in a matrix of bacterial components, salivary proteins/peptides and food debris (Marsh and Bradshaw 1995). Extracellular polymeric substances, produced by bacteria in a mature biofilm, contain large amounts of polysaccharides, proteins, nucleic acids and lipids. These maintain the structural integrity of the biofilm and provide an ideal matrix for bacterial cell growth and survival (Sutherland 2001). The biofilm mode of growth is thus clearly distinguished from planktonic growth by a number of features, which includes the resistance to antimicrobial agents at concentrations that approach 1,000 times greater than that required to kill planktonic microorganisms (Jenkinson and Lamont 2005; Lewis 2001). This is of major significance in the development of nano-antimicrobials and the extrapolation of in vitro findings.

14.2.2 Oral Biofilms and Disease

14.2.2.1 Dental Caries and Periodontal Disease

Dental caries is a destructive condition of the dental hard tissues that can progress to inflammation and death of vital pulp tissue, and if untreated it may lead to the eventual spread of infection to the periapical area of the tooth and beyond. The disease process involves acidogenic plaque bacteria, including *Streptococcus mutans*, *S. sobrinus* and *Lactobacillus* spp. (Hardie 1992), whereas periodontal diseases can involve both the soft and hard tissues and are initiated by components of the plaque biofilm that develop on the hard root surface adjacent to the soft tissues of the supporting periodontium. Periodontal disease may be confined to the gingiva (gingivitis) or extend to the deeper supporting structures with destruction of the periodontal ligament and the alveolar bone that supports the teeth (periodontitis). This loss of attachment, with associated periodontal pocket formation, may ultimately lead to loosening and loss of the affected teeth. *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are now regarded as the major pathogens in advancing periodontitis (Ximenez-Fyvie et al. 2000).

The prevention of dental caries and periodontal diseases is traditionally targeted at mechanical or non-specific control of the plaque biofilm because this is the precipitating factor. The use of antimicrobial agents represents a valuable complement to mechanical plaque control (Baehni and Takeuchi 2003). Such strategies should ideally control plaque biofilm formation without significantly affecting the biological equilibrium within the oral cavity. However, actual periods of exposure to antimicrobial agents during tooth brushing and mouth rinsing can be very short, and may amount to about 30 s, rather than the recommended 2 min (van der Ouderaa 1991).

14.2.2.2 Peri-implantitis

Implant systems are increasingly being used to replace missing teeth, and most integrate with bone without complications. Small amounts of plaque consisting mainly of streptococci and *Actinomyces* spp. will accumulate on successful implants. However, in peri-implantitis, anaerobic Gram-negative organisms predominate (Allaker and Hardie 1998). This infection is a key cause of dental implant failure whereby the induced inflammatory changes in the soft tissues surrounding oral implants lead to a progressive destruction of the supporting bone (classified as peri-implantitis and seen in up to 43% of implant-treated subjects) or soft tissues (classified as peri-implantitis mucositis and seen in up to 50% of implant-treated subjects) (Zitzmann and Berglundh 2008). Current forms of treatment are often inadequate and may result in chronic infection requiring implant removal and costly resective and regenerative procedures in an attempt to restore and reshape the implant support (Zitzmann and Berglundh 2008). The incorporation of nanoparticles into

implant coatings may well offer useful osteoconductive and antimicrobial functionalities to prevent dental implant failure.

14.2.2.3 Candidosis

The development of candidosis, including denture stomatitis (chronic atrophic candidosis), which can affect up to 65% of edentulous individuals (Chandra et al. 2001), involves the formation of a biofilm. Despite the use of antifungal drugs to treat denture stomatitis, infection can often become re-established. Chandra et al. (2001), using a poly (methyl methacrylate) (PMMA) biofilm model, demonstrated, that *C. albicans* biofilms are potentially highly resistant to the currently used antifungal agents, with resistance developing with time and showing a correlation with biofilm maturation.

14.2.3 Control of Oral Biofilms

Issues surrounding the uptake and penetration of antimicrobial agents into biofilms are key considerations in the administration of therapeutics (Stewart 2003). This is of particular importance within the oral cavity when these agents have to reach fewer accessible stagnation sites or through plaque to the enamel. Thus, there remains an interest in the development of plaque control measures that require a minimum of public compliance and professional health care intervention (Wilson 1996). Within this context, antimicrobial nanoparticles may be of particular value if retained at approximal teeth surfaces and below the gum margin. The anti-caries potential of fluoride and other more conventional antimicrobial/antiplaque agents, which are mostly deployed in mouthwashes and toothpastes, have been well characterised (Baehni and Takeuchi 2003). However, the potential of nanoparticles as constituents of topical agents to control oral biofilms through either their biocidal or anti-adhesive capabilities is now emerging as an area worthy of serious consideration. The studies by Robinson and co-workers using the 'Leeds in situ model', a device that allows dental plaque to develop in situ on a removable human enamel surface, have helped in the assessment of novel antimicrobial agents and take into account the very complex microbial composition and architecture of plaque biofilms (Watson et al. 2005). The use of such intact biofilms on natural tooth surfaces would be of particular value to a study of the penetration of nanoparticles and released ions in situ. This model has indicated that plaque contains voids and channels, sometimes extending completely through the biomass to the underlying enamel (Wood et al. 2000). The presence of channels may have considerable influence on the transfer of nanoparticles through biofilms. The main considerations are the physico-chemical characteristics of the particular nanoparticles used, including the surface charge and degree of hydrophobicity, the surface area-to-mass ratio of the plaque biofilm and the ability of the particles to adsorb to/be taken up at the

biofilm surface. Within this context, nanoparticles are potentially useful because it is possible to alter their surface charge, hydrophobicity, and other physical and chemical characteristics (Nel et al. 2009).

14.3 Nanometals and the Control of Oral Infections

14.3.1 Nanometals as Antimicrobial Agents

Metals have been used for centuries as antimicrobial agents. Silver, copper, gold, titanium and zinc have attracted particular attention, each having different properties and spectra of activity. Many oral products, including toothpastes, now incorporate powdered (micron-sized) zinc citrate or acetate to control the formation of dental plaque (Giersten 2004). Powdered titanium dioxide is also commonly used as a whitener in toothpastes.

With respect to nanoparticles, the antimicrobial properties of silver (Sondi and Salopek-Sondi 2004) and copper (Cioffi et al. 2005a) have received the most attention. Both of these have been coated onto or incorporated into various materials (Li et al. 2006), including PMMA (Boldyryeva et al. 2005) and hydrogels (Lee and Tsao 2006). An inverse relationship between nanoparticle size and antimicrobial activity has been clearly demonstrated, where nanoparticles in the size range of 1–10 nm have been shown to have the greatest biocidal activity against bacteria (Morones et al. 2005; Verran et al. 2007). Indeed, it has been shown that smaller silver nanoparticles are more toxic than larger particles, more so when oxidised (Lok et al. 2007). At the nanoscale, Ag+ ions are known to be released (leached) from the surface (Benn and Westerhoff 2008). Sotiriou and Pratsinis (2010) propose that the antimicrobial activity of small (<10 nm) nanosilver particles is dominated by Ag+ ions, while for larger particles (>15 nm), the contributions of Ag+ ions and particles to the antibacterial activity are comparable, the Ag+ ion release being proportional to the exposed nanosilver surface area.

As a result of their small size, particular nanoparticles may be able to offer other advantages to the biomedical field through improved biocompatibility (Kim et al. 2007). Also, it appears that bacteria are far less likely to acquire resistance to metal nanoparticles than they are to other conventional and narrow-target antibiotics (Pal et al. 2007). This is thought to occur because metals may act on a broad range of microbial targets, and many mutations would have to occur in order for microorganisms to resist their antimicrobial activity. Shape may also affect the activity of nanoparticles. Indeed, it has been demonstrated that the shape of silver nanoparticles can influence antimicrobial activity, as has been shown in the case of *Escherichia coli* (Pal et al. 2007). Truncated triangular silver nanoplates with a {111} lattice plane as the basal plane showed the greatest biocidal activity compared with spherical and rod-shaped nanoparticles. The differences appear to be explained by the proportion of active facets present in nanoparticles of different shapes.

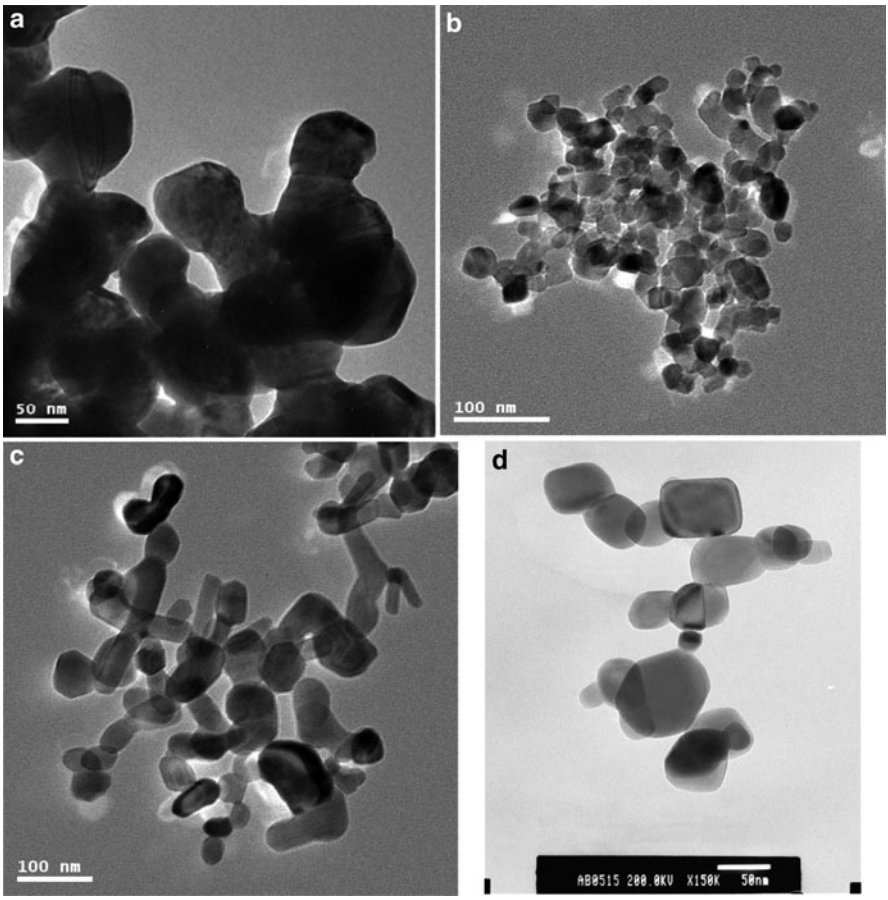

Fig. 14.1 Transmission electron microscopy images of agglomerated silver (**a**), titanium dioxide (**b**), zinc oxide (**c**) and copper oxide (**d**) nanoparticles

Exploitation of the toxic properties of nanoparticulate metals and metal oxides, in particular those that produce reactive oxygen species under UV light, such as titanium dioxide (TiO_2; Fig. 14.1b) and zinc oxide (ZnO; Fig. 14.1c), are finding increasing use in antimicrobial formulations, with silver metal nanoparticles (5–40 nm) having been reported to inactivate most microorganisms, including HIV-1 (Elechiguerra et al. 2005). The high reactivity of nano-titanium dioxide and nano-silicon dioxide (SiO_2) is exploited extensively for their bacteriocidal properties in filters and coatings on substrates such as polymers, ceramics, glasses and alumina (Han et al. 2005). Significant activity using nanoparticles and their compound clusters (as produced by thermal plasma technology) against fungal and bacterial pathogens such as meticillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* has recently been demonstrated. These have also shown the capability to inactivate viruses, including SARS, H1N1 Influenza and H5N1 Bird Flu. For

example, new broad-spectrum materials (5–60 nm) can reduce virus levels by 80–100% through direct or indirect contact. Nanoparticle preparations, including those based on nickel (Ni, NiO), zirconium (ZrO_2), copper (Cu, CuO, and Cu_2O), titanium (TiO_2), zinc (ZnO), aluminum (Al_2O_3), silicon(IV) nitride (Si_3N_4), silver (Ag), and tungsten carbide (WC) have been compared as regards their antimicrobial potential. Significant activity with Ag, ZnO, TiO_2 (in the presence of UV light), SiO_2, Cu, Cu_2O, and CuO against bacterial pathogens, including MRSA and *Pseudomonas aeruginosa,* has been demonstrated. Minimum bacteriocidal concentrations (MBC) were found to be in the range of 0.1–5 mg mL^{-1}. In comparison, traditional antibiotics are effective at concentrations 1,000-fold lower. NiO, Ni, Al_2O_3, TiO_2 (in the absence of UV light), Si_3N_4, WC, and ZrO_2 were found to lack antimicrobial activity at the concentrations tested. The oral pathogens *Streptococcus intermedius*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* were also found to be susceptible to Ag and CuO nanoparticles (Ren et al. 2009) with MBC values in the range 0.025–2.5 mg mL^{-1} (R.P. Allaker and M.A. Vargas-Reus, 2011).

14.3.1.1 Silver (Ag)

The antibacterial and antiviral actions of elemental silver, Ag+ ions, and silver compounds have been extensively investigated (Monteiro et al. 2009). In comparison to other metals, silver is relatively less toxic to human cells, albeit at very low concentrations. Ag+ ions have been considered for a range of biomedical applications, including their use within the dental field as an antibacterial component in dental resin composites (Herrera et al. 2001). Silver also exhibits a strong affinity for zeolite, a porous crystalline material of hydrated aluminosilicate which can bind up to 40% Ag+ ions within its structure. Silver zeolite has been incorporated into tissue conditioners, acrylic resins, and mouth rinses within the dental field (Casemiro et al. 2008; Kawahara et al. 2000; Matsuura et al. 1997; Morishita et al. 1998). Silver nanoparticles (Fig. 14.1a), either alone or together with other antimicrobial agents, have shown particularly encouraging results (Sondi and Salopek-Sondi 2004; Li et al. 2005; Rai et al. 2009). The use of silver salt nanoparticles instead of elemental silver or complex silver compounds to prevent biofilm formation on surfaces for both biomedical and more general use has been investigated. Using silver bromide precipitation to synthesise polymer–nanocomposites, surfaces were shown to resist biofilm formation. It was also shown to be possible, through controlling the size of the embedded AgBr, to modify the release of biocidal Ag+ ions (Sambhy et al. 2006).

In comparison to conventional antimicrobials, surprisingly little is known about how nanoparticles behave in relation to microorganisms, particularly at the cellular level. The mechanism of the antimicrobial activity of silver is not completely understood, but is likely to involve multiple targets in comparison to the more defined targets of antibiotics. Studies have shown that the positive charge on the Ag+ ion is

critical for antimicrobial activity, allowing the electrostatic attraction between the negative charge of the bacterial cell membrane and positively charged nanoparticles (Kim et al. 2007). In terms of the molecular mechanisms of inhibitory action of Ag+ ions on microorganisms, it has been shown that DNA loses its ability to replicate (Feng et al. 2000), and the expression of ribosomal subunit proteins and other cellular proteins and enzymes necessary for ATP production becomes inactivated (Yamanaka et al. 2005). It has also been hypothesised that Ag+ ions affect membrane-bound respiratory enzymes (Bragg and Rainnie 1974). However, the precise mechanism(s) of biocidal activity of silver nanoparticles against bacteria remains to be fully elucidated. The work of Sondi and Salopek-Sondi (2004) demonstrated structural changes and damage to bacterial membranes resulting in cell death. These particular studies suggest that sulphur-containing proteins in the membrane or inside the cells and phosphorus-containing elements, such as DNA, are likely to be the preferential binding sites for silver nanoparticles. The contribution of Ag+ ion release from nanoparticles to the overall antimicrobial activity remains unclear. It is suggested that a bacterial cell in contact with silver nanoparticles will take up Ag+ ions, which possibly in turn will inhibit respiratory enzymes and so help to generate free radicals, and subsequent free radical-induced damage to the cell membrane. In order to determine the relationship between free radical formation and antimicrobial activity, the use of antioxidants does suggest that free radicals may be derived from the surface of silver nanoparticles (Kim et al. 2007).

14.3.1.2 Copper (Cu)

Alongside silver, copper is a traditionally well-known antimicrobial material. In comparison to silver, relatively few studies have reported the antimicrobial properties of copper. It is suggested that copper may well have a similar mode of action to that of silver. However, it remains unclear as to the precise mechanism by which copper nanoparticles exert their antimicrobial activity. As with silver, it is thought that copper partly elicits its antimicrobial activity by combining with the –SH groups of key enzymes. Yoon et al. (2007) demonstrated superior antimicrobial activity with copper nanoparticles against *E. coli* and *Bacillus subtilis* when compared to silver nanoparticles. However, in the author's laboratory, silver consistently demonstrated superior activity to copper with a wide range of different species and strains (Ren et al. 2009).

The antimicrobial properties of both silver and copper nanoparticles were also investigated by Ruparelia et al. (2008) using strains of *E. coli*, *B. subtilis,* and *S. aureus*. The bacteriocidal effect of the nanoparticles was compared using disc diffusion tests, and minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) determinations in batch cultures. Bacterial sensitivity was found to differ according to the species tested and the test system employed. For all strains of *S. aureus* and *E. coli*, the action of silver nanoparticles was found to be superior. Strain-specific variation for *S. aureus* was negligible, while some strain-specific variation was observed for *E. coli*. A higher sensitivity, as shown

with *B. subtilis*, may be attributed to more amine and carboxyl groups (in comparison to other species) on the cell surface, these groups having a greater affinity for copper (Beveridge and Murray 1980). Released copper ions within the cell may then disrupt nucleic acid and key enzymes (Stohs and Bagchi 1995). In theory, a combination of silver and copper nanoparticles may give rise to a more complete bacteriocidal effect, especially against a mixed population of bacteria. Indeed, the studies of Ren et al. (2009) demonstrated that populations of Gram-positive and Gram-negative bacteria could be reduced by 68% and 65%, respectively, in the presence of 1.0 mg mL^{-1} nano-copper oxide within 2 h. This was significantly increased to 88% and 100%, respectively, with the addition of a relatively low concentration (0.05 mg mL^{-1}) of nano-silver.

14.3.1.3 Gold (Au)

In comparison to silver and copper, gold generally shows a weak antimicrobial effect. However, gold nanoparticles are employed in multiple applications involving biological systems. The binding properties of gold are exceptional, and this makes it particularly suitable for attaching ligands to enhance biomolecular interactions. Gold nanoparticles also exhibit an intense colour in the visible range and contrast strongly for imaging by electron microscopy (Lin et al. 2002). Despite all the current and potential applications for gold nanoparticles, there remains little information as to how these particles affect microorganisms. Growth inhibition studies, to measure the effect of gold nanoparticles (polyethylene glycol-coated to allow dispersion) on *E. coli* at various concentrations, demonstrated no significant activity (Williams et al. 2006). Studies in the author's laboratory with PEG-coated gold nanoparticles also showed no activity against *E. coli*. However, the growth of the Gram-negative species *Proteus* and *Pseudomonas aeruginosa* was inhibited at a concentration of 1.0 mg mL^{-1}.

14.3.2 Nanoparticulate Metal Oxides as Antimicrobial Agents

Nanoparticulate metal oxides have been of particular interest as antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies that have a high number of edges, corners and other potentially reactive sites (Stoimenov et al. 2002). However, certain metal oxides are now coming under close scrutiny because of their potential toxic effects (Karlsson et al. 2008). Oxides under consideration as antimicrobial agents include those of copper, zinc oxide, titanium dioxide (titania) and tungsten oxide (WO_3).

14.3.2.1 Copper Oxide (CuO)

Copper oxide (CuO) is a semi-conducting compound with a monoclinic structure. CuO has attracted particular attention because it is the simplest member of the

family of copper compounds and exhibits a range of potentially useful physical properties, such as high temperature superconductivity, electron correlation effects and spin dynamics (Cava, 1990; Tranquada et al. 1995). Limited information on the possible antimicrobial activity of nano CuO is available. Copper oxide is relatively cheap, easily mixed with polarised liquids (i.e. water) and polymers, and relatively stable in terms of both chemical and physical properties. Highly ionic nanoparticulate metal oxides, such as CuO, may be particularly valuable antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies (Stoimenov et al. 2002).

Copper oxide nanoparticles have been characterised, both physically and chemically, and investigated with respect to potential antimicrobial applications (Ren et al. 2009). It was found that nano-scaled CuO, as generated by thermal plasma technology, demonstrated particle sizes in the range 20–95 nm with a mean surface area of 15.7 $m^2\ g^{-1}$ (Fig. 14.1d). CuO nanoparticles in suspension showed activity against a range of bacterial pathogens, including MRSA and *E. coli*, with minimum bacteriocidal concentrations ranging from 0.1 to 5.0 mg mL^{-1}. As with silver, studies of CuO nanoparticles incorporated into polymers suggest that release of ions may be required for optimum killing (Ren et al. 2009). Incorporation of nano CuO into porous elastomeric polyurethane films has demonstrated potential for a number of applications. Studies have shown this approach to be effective against MRSA within 4 h of contact (Z. Ahmad and R.P. Allaker, unpublished observations).

14.3.2.2 Zinc Oxide (ZnO)

The antimicrobial mechanisms of zinc are not completely understood. In recent years, nano-zinc oxide has received increasing attention, partly because it is stable under harsh processing conditions but also because it is generally regarded as safe to man (Stoimenov et al. 2002). Studies have shown that some nanoparticulate metal oxides, such as ZnO, have a degree of selective toxicity to bacteria with a minimal effect on human cells (Brayner et al. 2006; Reddy et al. 2007; Zhang et al. 2007). The proposed mechanisms of antibacterial activity include induction of reactive oxygen species (Sawai, 2003; Jones et al. 2008) and damage to the cell membrane with subsequent interaction of the nanoparticle with the intracellular contents (Brayner et al. 2006).

In a study by Liu et al. (2009) the antimicrobial properties of ZnO nanoparticles were investigated against *E. coli* O157:H7. This strain was significantly inhibited as shown using SEM and TEM analyses to assess the morphological changes of bacterial cells. Leakage of intracellular contents and a degree of membrane disorganisation was observed. Using Raman spectroscopy, the intensities of lipid and protein bands were shown to increase after exposure to ZnO nanoparticles, whereas no significant change to nucleic acid was indicated. In comparison to silver nanoparticles (0.1 mg mL^{-1}), a higher concentration of zinc oxide (particle size: approx. 15–20 nm; surface area: 47 $m^2\ g^{-1}$) is required to have growth-inhibitory (0.5–2.5 mg mL^{-1}) and killing effects (>2.5 mg mL^{-1}) against a range of pathogens

including *E. coli* and MRSA. While with those organisms implicated in oral infections, including *A. actinomycetemcomitans*, *P. gingivalis*, *Prev. intermedia* and *F. nucleatum*, greater sensitivity was demonstrated, with growth inhibitory and killing concentrations of 0.25–2.5 and 0.25–2.5 mg mL^{-1}, respectively (R.P. Allaker, M.A. Vargas-Reus and K. Memarzadeh, 2011).

14.3.2.3 Titanium Dioxide (TiO_2)

Titanium dioxide (TiO_2) is the commonest titanium compound, and its ability to act as a photocatalytic antimicrobial compound is well established (Blake et al. 1999). TiO_2 is widely used in a number of applications, as a powder and increasingly in a nanoparticulate form, and is generally considered to be non-toxic at the concentrations normally employed. However, there are recent concerns that nano-titanium oxide may present a hazard to health through inflammation as generated by release of IL-1α (Yazdi et al. 2010). The anatase form of nano TiO_2 and UV light excitation are required to ensure maximum antimicrobial activity. Such TiO_2 photocatalysis is able to promote the peroxidation of the polyunsaturated phospholipid component of the microbial lipid membrane, induce loss of respiratory activity, and elicit cell death (Maness et al. 1999). In comparison to silver and copper nanoparticles, there have been relatively few studies on nano-titanium/-titanium dioxide. The study of Tsuang et al. (2008) demonstrated TiO_2-mediated photocatalytic and bacteriocidal activities against obligate aerobes (*Pseudomonas aeruginosa*), facultative anaerobes (*S. aureus, E. coli* and *Enterococcus hirae*) and obligate anaerobes (*Bacteroides fragilis*). Concentrations of titanium oxide (predominantly anatase phase; in the absence of UV light; particle size: approx. 18 nm; surface area: 87 m^2 g^{-1}) required to have a growth inhibitory and killing effect against a range of pathogens including *E. coli* and MRSA. have been shown to be 1.0–2.5 and >2.5 mg mL^{-1} respectively, while with those organisms implicated in oral infections, including *A. actinomycetemcomitans*, *P. gingivalis*, *Prev. intermedia* and *F. nucleatum*, growth inhibitory and killing concentrations are in the same order at 0.25–2.5 and >2.5 mg mL^{-1}, respectively (R.P. Allaker, M.A. Vargas-Reus and K. Memarzadeh, 2011).

14.3.3 Oral Applications of Nanoparticulate Metals and Metal Oxides

In order to reduce bacterial and fungal adhesion to oral materials and devices, silver nanoparticles are being investigated for a range of possible applications, for example, incorporation into denture materials (Fig. 14.2) (Monteiro et al. 2009) and orthodontic adhesives (Ahn et al. 2009). The optimum amount of silver nanoparticles used within such polymer materials will be of critical importance to avoid an adverse effect upon their physical properties. The study of Ahn et al. (2009) clearly

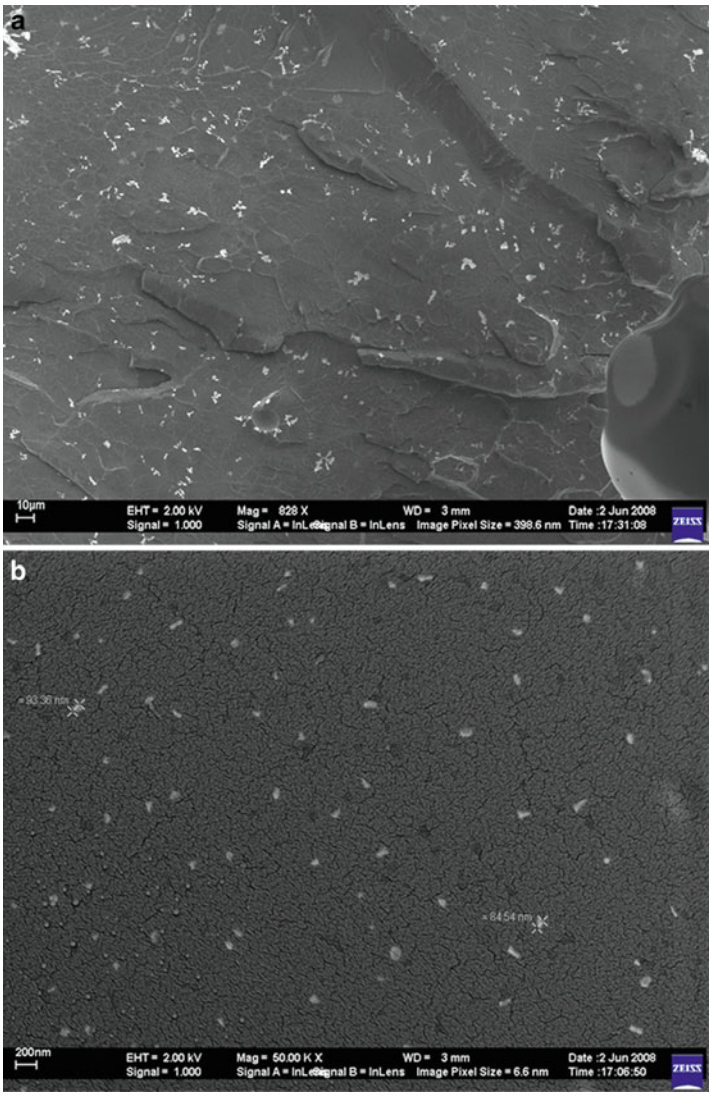

Fig. 14.2 Scanning electron micrograph of a fractured polymethyl methacrylate PMMA/Ag nanocomposite containing approximately 0.04% w/w silver. Distribution of silver particles in the PMMA acrylic resin shown. (**a**) *White areas* are agglomerated silver nanoparticles distributed in the PMMA (×828 magnification). (**b**) Silver nanoparticles (*white dots*) with approximate mean size 88 nm distributed in the PMMA matrix. (×50,000 magnification) (With permission; Monteiro et al. 2009)

demonstrated that experimental composite adhesives (ECAs) had rougher surfaces than conventional adhesives due to the addition of silver nanoparticles, although bacterial adhesion to ECAs was shown to be less than that to conventional adhesives

and was not influenced by saliva coating. However, no significant difference between ECAs and conventional adhesives was shown as regards bond shear strength.

Biofilm growth is known to contribute to secondary caries and the failure of resin-based dental composites. Within this context, zinc oxide nanoparticles have undergone in vitro testing using biofilm culture test systems (Aydin Sevnic and Hanley 2010). ZnO nanoparticles blended into a variety of composites were shown to significantly inhibit *S. sobrinus* biofilm growth at concentrations not less than 10% w/w over a 3-day test period. However, at this concentration, the impact on the structural characteristics of composites would need to be carefully assessed.

As regards dental implants, numerous companies currently market novel synthetic hydroxyapatite (HA) materials, as the optimal osteoconductive implant coating available, and some companies have developed nanoscaled varieties. Some have employed coatings and application methods different from the conventional coating techniques, including an HA material available in nanophase and a nanocrystalline silver-based antimicrobial coating that in theory should reduce the potential for bacterial colonisation. The antibacterial properties of an amorphous carbon film (Almaguer-Flores et al. 2010) incorporating silver nanoparticles in a 40- to 60-nm size range and deposited onto a standard titanium material have been evaluated. A significant reduction in mixed biofilm counts compared to the standard titanium material was observed after 7 days using the coating with silver nanoparticles.

14.3.4 Other Nano-Based Antimicrobials

14.3.4.1 Quaternary Ammonium Nanoparticles

Quaternary ammonium poly (ethylene imine) (QA-PEI) nanoparticles as an antimicrobial to incorporate into restorative composite resins have been developed by Yudovin-Faber et al. (2008). These may have distinct advantages over the currently used composite resins employed to restore hard tissues, which are known to have several disadvantages including development of biofilms on both teeth and the restorative material (Monteiro et al. 2009). The traditional methods for preparing antibacterial composite materials have been to impregnate them with low-molecular-weight agents, such as Ag+ ions or iodine, that are then released slowly. Apart from the possible adverse effects on the mechanical properties of the composite, difficulties in controlling the release of such agents may be a potential drawback.

The use of QA-PEI nanoparticles at a concentration of 1% w/w enabled complete in vitro growth inhibition of *Streptococcus mutans* to be achieved for a duration of at least 3 months (Yudovin-Farber et al. 2008). The proposed mechanism of action of QA-PEI is suggested to be as a result of transfusion across, and damage to, the bacterial cell wall. The hydrophobic nature and positive charge of these nanoparticles are also thought to further enhance the antimicrobial activity. Surface chemical analysis of the restorative composite embedded with QA-PEI

demonstrated a surface modification of higher hydrophobicity and the presence of quaternary amines when compared to the unmodified material. Further studies to optimise the release characteristics of QA-PEI and other potentially useful nanoparticulates from dental materials will be required.

14.4 Anti-adhesive Nanoparticles and Oral Biofilm Control

14.4.1 Chitosan Nano- and Microparticles

Chitosan is a biopolymer derived by the deacetylation of chitin, a natural polymer occurring in the exoskeleton of crustaceans. Chitosan is positively charged and soluble in acidic to neutral solution, enabling it to bind to mucosal surfaces. Both chitosan nano- and microparticles have been investigated as a potential platform for local delivery of drugs (Wu et al. 2005). Although the currently used antimicrobial irrigants (without chitosan incorporation), employed to disinfect root canals in the treatment of endodontic infections, are capable of killing *Enterococcus faecalis*, the bacterium frequently associated with this condition, endodontic restorations often fail (Lin et al. 1992). The in vitro study of Kishen et al. (2008) demonstrated that root canal surfaces treated with cationic antibacterial nanoparticulates such as zinc oxide alone and a combination of zinc oxide and chitosan nanoparticulates are able to significantly reduce *E. faecalis* adherence to dentine. In theory such surface treatment could prevent bacterial recolonisation and biofilm formation in vivo.

14.4.2 Silica and Silicon Nanoparticles

Particles of a nano- and micro-size based upon the element silicon to rapidly deliver antimicrobial and anti-adhesive capabilities to the desired site within the oral cavity have received much attention (Stephen, 1993). Companies have used silica (silicon dioxide 'SiO_2' and often classed as 'microfine', but with a particle size within the definition of nanoparticles) in toothpastes for many years, and some are now actively seeking new directions in this area through the use of porous silicon and nanocrystalline silicon technology to carry and deliver antimicrobials, for example triclosan. These would offer advantages over some of the slower and more prolonged delivery systems under investigation.

The use of silica nanoparticles to polish the tooth surface may help protect against damage by cariogenic bacteria, presumably because the bacteria can more easily be removed. This has been investigated on human teeth ex vivo (Gaikwaad and Sokolov, 2008). Atomic force microscopy demonstrated lower nanometer-scale roughness obtained when silica nanoparticles were used to polish the surface of teeth as compared with conventional polishing pastes. It was also shown that

adherent *S. mutans* could be more easily removed. However, concerns remain as to the longevity of the effect, and whether the polished surface will inhibit mineralisation and plaque formation in vivo.

Spherical silica nanoparticles (up to 21 nm) deposited onto polystyrene surfaces by polycationic binding have been investigated with respect to the development of *C. albicans* biofilms and invasive filament formation (Cousins et al. 2007). Modified surfaces were shown to reduce attachment and growth of *C. albicans*, with the greatest effect observed with 7- and 14-nm particles. Such effects could possibly be attributed to the surface topography or slow dissolution of the bound silica. Such treatment has the advantages of being non-toxic, simple to apply and adaptable to 3-dimensional surfaces.

Other novel systems based upon silica have been investigated with respect to the control of oral biofilms. The use of nitric oxide (NO)-releasing silica nanoparticles to kill biofilm-based microbial cells has been described (Hetrick et al. 2009). The rapid diffusion of NO may well result in enhanced penetration into the biofilm matrix and therefore improved efficacy against biofilm-embedded bacteria. In vitro-grown biofilms of *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans* were exposed to NO-releasing silica nanoparticles. Over 99% of cells from each type of biofilm were killed via NO release. In comparison to small-molecule NO donors, the physicochemical properties, for example hydrophobicity, charge and size, of nanoparticles can be altered to increase anti-biofilm efficacy (Nel et al. 2009).

Bioactive glasses of the SiO_2–Na_2O–CaO–P_2O_5 system have been shown to possess antimicrobial activity through the release of ionic alkaline species over time and are under consideration as dentine disinfectants to offer an alternative to calcium hydroxide (Waltimo et al. 2007). Those in the form of amorphous nanoparticles with a size of 20–60 nm may show an advantage over micron-sized material as the decrease in glass particle size should increase, by more than tenfold, the active exchange surface of glass and surrounding liquid. In turn, this would substantially increase ionic release into suspension and enhance antimicrobial efficacy. Waltimo et al. (2007) monitored ionic dissolution profiles in simulated body fluid. Antimicrobial activity was assessed against *Enterococcus faecalis* as a pathogen often isolated from root canal infections. They found that a shift from a micron- to a nano-size increased the release of silica by a factor of 10 and elicited a pH elevation of at least 3 units. The killing efficacy was also significantly higher.

14.4.3 Hydroxyapatite and Other Calcium Phosphate-Based Systems

The application of nano-scaled hydroxyapatite particles has been shown to impact on oral biofilm formation and provides a re-mineralisation capability (Roveri et al. 2008; Cross et al. 2007). Biomimetic approaches, based upon hydroxyapatite nanocrystals which resemble the structure at the nano-scale of abraded dental

enamel crystallites, should allow adsorbed particles to interact with bacterial adhesins and reduce bacterial adherence, and hence impact on biofilm formation (Venegas et al. 2006).

A number of oral health care products, including dentrifices and mouth rinses, have been developed containing nano-sized apatite particles with and without protein-based additives (Rahiotis et al. 2008; Reynolds et al. 2003). It is suggested that the efficacy of these compounds can be attributed to the size-specific effects of the apatite nanoparticulates. Casein phosphopeptide (CPP) – amorphous calcium phosphate (ACP) nanocomplex (Recaldent™) – is a particular technology based upon ACP and stabilised by casein phosphopeptide (CPP) (Reynolds 2008). Use of this technology has demonstrated anticariogenic activity under both in vitro and in vivo conditions. The levels of calcium and phosphate ions in supragingival plaque have been shown to increase upon delivery of CPP-ACP in a mouth rinse form and promote remineralisation of enamel subsurface lesions (Reynolds et al. 2003). Analysis of plaque samples demonstrated CPP-ACP nanocomplexes to be localised in plaque on the surface of bacterial cells and essentially confirm the studies by Rose (Rose 2000a; Rose 2000b) who demonstrated tight binding to *Streptococcus mutans* and the intercellular plaque matrix to provide a calcium ion reservoir. As a result of interaction with calcium binding sites and the masking of bacterial receptors on salivary molecules, CPP-ACP is thought to reduce bacterial colonisation as shown with CPP-ACP germanium-treated surfaces. (Rahiotis et al. 2008).

14.5 Incorporation of Nanoparticles into Polymeric Materials for Possible Oral Use

14.5.1 Properties of Polymer Matrix Nanocomposites

Nanocomposites are usually solid combinations of a bulk matrix and a nano-dimensional phase(s), which differ in structural and chemical properties. The physical properties of the nanocomposite will thus differ markedly from those of the component materials. In mechanical terms, this is attributed to the high surface-to-volume ratio of the nanoconstituents. With polymer-nanocomposites, properties related to local chemistry, thermoset cure, polymer chain mobility, conformation, and ordering can all vary markedly and continuously from the interface with the nanophase into the bulk of the matrix.

Polymer–matrix nanocomposites (nanofilled polymer composites) are, in their simplest case, made by appropriately adding nanoparticulates to a polymer matrix to enhance its functionality (Manias 2007). This can be particularly effective in producing high-performance composites when optimum dispersion of the nano-filler is achieved, and the properties of such a filler can markedly enhance those of the matrix, for example by reinforcement of a polymer matrix with more rigid nanoparticles of ceramics or carbon nanotubes. The high aspect ratio and/or

the high surface area-to-volume ratio of nanoparticulates provide such superior properties.

Silver nanoparticles have been investigated with a view to improving both the physical and the antimicrobial properties of dental polymeric materials, for example, in denture materials (Fig. 14.2) (Monteiro et al. 2009) and orthodontic adhesives (Ahn et al. 2009). K.P. Lackovic et al. (2008) investigated the use of silver nanoparticles in an attempt to improve the physical and antimicrobial properties of orthodontic bracket-bonding cement. Incorporation of silver nanoparticles at a concentration of less than 1% w/v was found not to decrease the modulus of the cement tested. However, no significant effect on either the attachment or growth of the cariogenic bacterium *Streptococcus mutans* was observed. Thus, an optimum amount of silver nanoparticles used within polymer materials may well be of critical importance to avoid an adverse effect upon the physical properties. The study of Ahn et al. (2009) clearly demonstrated that experimental composite adhesives (ECAs) had rougher surfaces than conventional adhesives owing to the addition of silver nanoparticles. Bacterial adhesion to ECAs was shown to be less than that of conventional adhesives and was not influenced by saliva coating. No significant difference between ECAs and conventional adhesives was shown as regards bond shear strength.

14.5.2 Methods of Combining Nanometals with Polymers

Combining nanoparticulate metals with a polymer matrix such that they remain adequately dispersed is a process that has presented some problems. Three general methods to formulate polymer–matrix nanocomposites have been utilised:

1. In situ synthesis of nanoparticles in the polymer matrix by reduction of a metal salt in the matrix or evaporation of the metal at the heated surface of the matrix.
2. Polymerisation of the matrix around the nanoparticles.
3. Incorporation of pre-synthesised nanoparticles into a pre-synthesised polymer matrix with the aid of a blending solvent (Corbierre et al. 2005).

However, the first and second methods have a tendency to produce undesirable nanospecies and polydisperse (range of particle sizes) polymer matrices. An approach involving the impregnation of silicone (as used in a wide variety of devices) with nanoparticulate silver, using supercritical carbon dioxide, has been investigated (Furno et al. 2004). This may be particularly applicable as regards implantable devices, as their use is a major risk factor for hospital-acquired infection. The initiation of infection involving biomaterials requires an initial adhesion event to the device or more often to the patient-derived glycoprotein coating (conditioning film) which is deposited from the time of implantation (Green et al. 1999). Following adhesion, microbial proliferation leads to the development of a biofilm which is more resistant to the effect of antimicrobials. Although approaches to prevent this type of infection, including the use of coatings

with nanoparticulate silver, have been proposed, unsatisfactory clinical results and on occasion further complications, have occurred (Riley et al. 2002). Possible reasons for the lack of activity include the inactivation of the antimicrobial coating by plasma components and a lack of inherent durability of the coatings used. Impregnation of polymers may well be more beneficial than the use of coatings or addition into the mix. A significant level of surface- or near-surface-deposited silver nanoparticles should provide an initial "burst" effect. However, Furno et al. (2004) showed that the majority of the antimicrobial activity, against both biofilms and planktonic cells, was simply removed by washing the polymer discs impregnated with silver nanoparticles. Further sustained release with significant antimicrobial activity would need to be carefully evaluated. The protection of both inner and outer surfaces against bacterial colonisation by impregnation of an antimicrobial agent is advantageous (Wilcox et al. 1988) and has been demonstrated in the clinical setting (Darouiche et al. 1999). The continued release of Ag+ ions at antimicrobial concentrations even in the presence of a plasma protein conditioning film, and the ability to offer protection to both the inner and outer surfaces of a catheter, are two distinct potential advantages of polymer impregnation (Furno et al. 2004).

14.5.3 Polymeric Films Incorporating Nanometals

It is possible to enhance the properties of certain materials by encapsulation in a polymeric film, for example by encapsulating and modifying the surface properties of denture acrylic polymers with an inorganic silicone polymeric film (Thorne and Vittori 1997) to prevent diffusion of food contaminates, bacteria and the ingrowth and adherence of *Candida* spp. hyphae that may lead to failure. The use of hydrophobic polymer-based materials as occlusive thin films for the prophylaxis of dental caries, dental erosion and dentine hypersensitivity has more recently been explored in vitro (Nielsen et al. 2011).

Nanotechnology is now beginning to be able to provide the tools required to synthesise films or layers with embedded metal nanoparticles. For copper nanoparticles embedded in an inert, Teflon (polytetrafluoroethylene)-like matrix, Cioffi et al. (2005b) demonstrated significant antimicrobial activity in vitro as a result of ion release. It is suggested that in such an experimental situation a bacterial growth medium facilitates the release of metallic ions, possibly as a consequence of a reaction with the nutrient media constituents. A greater release of copper ions into a liquid medium could well be due to the presence of an oxide layer on the copper nanoparticles and reaction with chloride ions in the medium. The copper–fluoropolymer (Cu-CFx) nano-composite films in this study were deposited by dual ion-beam sputtering, a technique to deposit different polymeric and inorganic materials in a very controlled manner. Analysis of the layers revealed that the inorganic Cu(II) nanoparticles were evenly dispersed in the branched fluoropolymer matrix. Through the use of electrochemical atomic absorption spectroscopy, copper release kinetics in solutions was measured. A correlation was

shown between the chemical composition (copper loading) of the material surface, the concentration of copper ions released into the microbial culture broths, and the bioactivity of the Cu-CFx coating. The Cu-CFx layers, used as coatings, were shown to inhibit the yeast *Saccharomyces cerevisiae* and the bacterial species *E. coli*, *S. aureus* and *Listeria* spp. Such use of Cu-CFx coatings, which allow variable metal loading, demonstrate good stability upon storage and microbial inhibitory activity, is being explored in a variety of applied fields, including food chemistry and biomedicine.

Sacrificial-anode electrochemical synthesis of metal nanoparticles in the presence of tetraoctylammonium (TAO) salts have allowed Cioffi et al. (2005c) to prepare copper- and silver-containing coatings by combining the metal colloid with a polymer-dispersing matrix. The surfactants used were capable of providing physical and chemical stabilisation of the metal nanoparticles through the formation of a protective organic shell. These materials were then able to show a significant inhibitory effect on the growth of *E. coli* and *Saccharomyces cerevisiae*. The marked effects of such nanocomposites can be attributed to the synergistic effect of the antimicrobial metal and the TAO salts. This study has provided further support for the use of metal nanoparticles in disinfecting/antifouling paint and other coating formulations.

To determine whether the localisation of controlled loadings of silver nanoparticles within nanometer-thick polymeric films could kill bacteria yet support the growth of mammalian cells, poly(allylamine hydrochloride) and poly (acrylic acid) were prepared using layer-by-layer deposition and were then loaded with silver nanoparticles in the range 0.4–23.6 μg cm^{-2} (Agarwal et al. 2010). Suspensions of *Staphylococcus epidermidis* in contact with the film were reduced 6-log-fold with only 0.4 μg nanoparticles cm^{-2}. Polymeric films containing this concentration of silver nanoparticles were also shown to be non-toxic to mammalian fibroblast cells, allowing both growth and attachment.

14.6 Photodynamic Therapy and the Use of Nanoparticles to Control Oral Infections

Photodynamic therapy (PDT) is very well suited for the control of bacteria in oral plaque biofilms where there is relatively easy access for the application of the photosensitising agent and light sources to areas requiring treatment (Allaker and Douglas 2009). This approach is now being utilised within the clinical setting in some countries. The killing of microorganisms with light depends upon cytotoxic singlet oxygen and free radical generation by the excitation of a photo-activatable agent or sensitiser. The result of excitation is that the sensitiser moves from an electronic ground state to a triplet state that then interacts with microbial components to generate cytotoxic species (MacRobert et al. 1989). One of the advantages of light-activated killing is that resistance to the action of singlet oxygen is unlikely to become widespread in comparison to that experienced with more

traditional chemical antimicrobial agents. A sensitiser ideally should absorb light at red to near-infrared wavelengths because these wavelengths are able to penetrate more. The most commonly tested sensitisers on bacteria have been tricyclic dyes (for example methylene blue, erythrosine), tetrapyrroles (for example porphyrins) and furocoumarins (for example psoralen). The use of nanoparticles within this area is now under investigation. For example, a complex of biodegradable and biocompatible poly(lactic-co-glycolic acid) (PLGA) and colloidal gold nanoparticles, loaded with methylene blue and exposed to red light at 665 nm, have been tested against planktonic *E. faecalis* and in experimentally infected root canals (Pagonis et al. 2010). In theory, gold nanoparticle conjugates should have improved binding and cell wall penetration properties, and so should deliver a higher concentration of photoactive molecules. It remains to be fully established whether such conjugates will show an increased antibacterial activity when compared to more conventional treatments.

Most work on light-activated killing has been performed using suspensions of planktonic bacteria, with relatively few studies observing biofilm-grown microorganisms. In vitro biofilm-grown *Streptococcus mutans* cells demonstrated a 3-log reduction when treated with erythrosine and white light (500–650 nm) (Wood et al. 2006), while an approach using antibody- and erythrosine-labelled nanoparticles has shown the potential for targeting specific bacterial species in oral plaque biofilms (S.R. Wood, 2006). These in vitro studies, employing constant-depth film fermenters with gold nanoparticles conjugated to erythrosine and antibody to either *Streptococcus mutans* or *Lactobacillus casei*, have shown specific killing of target organisms in mixed-biofilm cultures.

Considerations in relation to the therapeutic use of light-activated killing of biofilms on host surfaces include: (1) direct toxicity of the sensitiser, (2) indirect toxicity of the sensitiser in terms of 'by-stander' damage to adjacent host cells, (3) penetration into the biofilm, (4) light exposure time required to kill bacteria within in vivo biofilms and (5) widespread relatively non-specific bacterial killing (Allaker and Douglas 2009). The photosensitiser erythrosine has an advantage over some other dyes because it is currently used in dentistry to visualise dental plaque in vivo, and so its lack of toxicity in the host is well established. For use in periodontitis, the dye needs to be applied subgingivally prior to fiber-optic laser light activation. However, when disease is present, the periodontal site has a marked flow of gingival crevicular fluid into the pocket, and most photosensitisers lose some activity in the presence of extraneous protein. Also, some have virtually no effect in the presence of saliva and other body fluids. This is because the agents complex with proteins and host cells in the gingival crevicular fluid and effectively compete for binding to bacteria. The use of nanoparticles as applied to PDT may help to overcome some of the issues associated with serum constituents.

14.7 Biocompatibility of Nano-Antimicrobials Within the Oral Cavity

Although the development and application of nanotechnology are of major importance in both industrial and consumer areas, knowledge regarding the possible toxicity of nanotechnology products to humans is limited. Whereas it is well known that copper in a non-nanoparticulate form is actively excreted from the normal body, non-nanoparticulate silver can accumulate within it. However the threat posed by these metals in a nanoparticulate form is far from clear (Seetharam and Sridhar 2006). In order to understand the mechanism of toxicity, a thorough knowledge of the toxico-kinetic properties of nanoparticles is required. This includes information on the absorption, distribution, metabolism and excretion (ADME) of nanoparticles (Hagens et al. 2007). In theory, certain nanoparticles may be retained within the body for longer than is desirable, and thus the safety profile becomes a matter of overriding significance. Nanomaterials are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot. Nanomaterials can enter the blood stream following inhalation or ingestion, and some can even penetrate the skin. In vitro studies with lung epithelial cells, enterocytes and skin keratinocytes indicate marked differences in susceptibility to metallic nanoparticles according to cell type tested (R.P. Allaker and M.A. Vargas-Reus, 2010). However, a particle's surface chemistry, which in some cases can be modified, can govern whether it should be considered further for biomedical applications (Nel et al. 2009).

14.7.1 Toxicity to Cells in the Oral Cavity

Toxicology and biodynamic studies suggest that silica, silicon, and chitosan nanoparticles are relatively safe if introduced via the oral route (Seetharam and Sridhar 2006). Testing of NO-releasing silica nanoparticles (at the highest concentration tested of 8 mg mL^{-1}) with fibroblasts demonstrated that cell proliferation was inhibited to a lesser degree than with chlorhexidine (Hetrick et al. 2009). Likewise, quaternary ammonium poly(ethylene imine) (QA-PEI) nanoparticles incorporated into composite resins to restore teeth at 1% w/w demonstrate no additional toxic effects on cultured cells or experimental animal tissue in comparison to unmodified composites (Yudovin-Farber et al. 2008). In comparison to other metals, silver is less toxic to human cells, and is only ever used at very low concentrations in vivo (Sondi and Salopek-Sondi 2004). For example, silver nanoparticles have been shown to inhibit *Candida* spp. at a concentration of 0.2 μg mL^{-1}, which is markedly less than the concentration (30 μg mL^{-1}) required to demonstrate a toxic effect against human fibroblasts (Panacek et al. 2009).

14.7.2 Alteration of Biocompatibility and Desired Function

The safe use of nanotechnology and the design of nanomaterials for biological applications, including the control of oral biofilms, involve a complete understanding of the interface between these materials and biological systems (Nel et al. 2009). The interface comprises three interacting components: (1) the surface of the nanoparticle, (2) the solid–liquid interface and the effects of the surrounding medium and (3) the contact zone with biological substrates (Fig. 14.3; Table 14.1). The nanoparticle characteristics of most importance as regards interaction with biological systems, whether mammalian or microbial, are chemical composition, surface function, shape and number of sides, porosity and surface crystallinity, heterogeneity, roughness, and hydrophobicity or hydrophilicity (Nel et al. 2006). For example, it has been shown that titanium dioxide nanoparticles (Luo et al. 2005) act to resist the formation of surface biofilms through increased hydrophilicity in comparison to an unmodified surface.

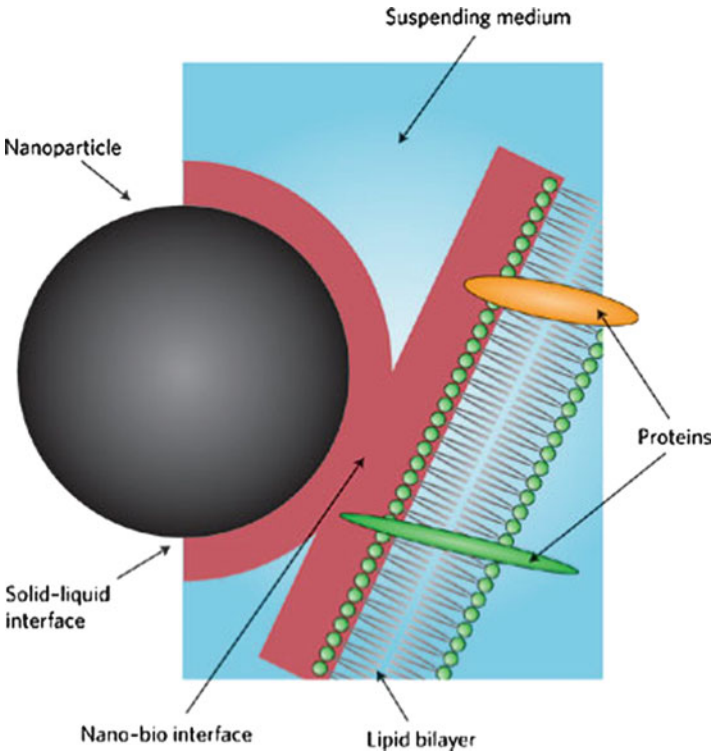

Fig. 14.3 Representation of the interface between nanoparticle and lipid bilayer. The properties of the material, modification of the surface properties of the material through interactions with the suspending medium, and the interactions of the solid–liquid interface with bio-molecules all impact on the interaction. See Table 14.1 for details (With permission; Nel et al. 2009)

Table 14.1 Biological, physical and chemical influences on the interface between nanomaterials and biological systems in man (Adapted with permission; Nel et al. 2009)

Nanoparticle
Size, shape and surface area
Surface charge, energy, roughness and porosity
Valence and conductance states
Functional groups
Ligands
Crystallinity and defects
Hydrophobicity and hydrophilicity
Suspending media
Water molecules
Acids and bases
Salt and multivalent ions
Natural organic matter (proteins, lipids)
Surfactants
Polymers
Polyelectrolytes
Solid–liquid interface
Surface hydration and dehydration
Surface reconstruction and release of free surface energy
Ion adsorption and charge neutralisation
Electrical double-layer formation, zeta potential, isoelectric point
Sorption of steric molecules and toxins
Electrostatic, steric and electrosteric interactions
Aggregation, dispersion and dissolution
Hydrophilic and hydrophobic interactions
Nano-bio interface
Membrane interactions: specific and non-specific forces
Receptor-ligand binding interactions
Membrane wrapping: resistive and promotive forces
Biomolecule interactions (lipids, proteins, DNA) leading to structural and functional effects
Free energy transfer to biomolecules
Conformational change in biomolecules
Oxidant injury to biomolecules
Mitochondrial and lysosomal damage, decrease in ATP

The characteristics of the surface layer, such as zeta potential (surface charge), nanoparticle aggregation, dispersion state, stability and hydration as influenced by the characteristics of the surrounding medium (including ionic strength, pH, temperature and presence of organic molecules or detergents) are of critical importance. The contribution of surface charge to both mammalian and microbial interactions has been clearly illustrated using surfactant-coated nanoparticles (McCarron et al. 2007). Both anti-adherent and antifungal effects were shown using buccal epithelial cells treated with non-drug-loaded poly (ethylcyanoacrylate) nanoparticles. Nanoparticles were prepared using emulsion polymerisation and stabilised with cationic, anionic or non-ionic surfactants. Cationic surfactants, for example cetrimide, which are known antimicrobial agents, were the most effective in reducing *Candida albicans*

blastospore adhesion, and showed a growth inhibitory and biocidal effect against the yeast. Production of nanoparticles with an anionic surfactant gave lower yields and wide particle size distributions. No evidence of killing against *C. albicans* was shown. Non-ionic surfactant-coated nanoparticles produced intermediate kill rates. These studies clearly demonstrate the importance of surface charge on the nanoparticle surface. It is envisaged that the buccal epithelium could possibly be treated using polymeric-type nanoparticles in a mouthwash-type formulation; effectively this would prime the potential target cells against adhesion and infection.

The in vivo screening of around 130 nanoparticles intended for therapeutic use has allowed detailed assessments as regards biocompatibility (Nel et al. 2009). It was shown that the main independent particle variables which determine compatibility are size, surface charge and dispersibility (particularly the effect of hydrophobicity). Cationic particles or particles with a high surface reactivity are more likely to be toxic (both to eukaryotes and prokaryotes). Larger, more hydrophobic or poorly dispersed particles, which are rapidly removed by the reticuloendothelial system, were shown to be less toxic. Karlsson et al. (2008) have recently shown that metal oxide nanoparticles are more toxic than at first envisaged at concentrations down to 40 μg mL^{-1} and show a high variation as regards different nanoparticle species to cause cytotoxicity, DNA damage and oxidative DNA lesions. Toxic effects on cultured cells were assessed using trypan blue staining together with the comet assay to measure DNA damage and an oxidation-sensitive fluoroprobe to quantify the production of reactive oxygen species (Karlsson et al. 2008). Copper oxide was found to be the most toxic and therefore may pose the greatest health risk. Nanoparticulate ZnO and TiO_2, both ingredients in sunscreens and cosmetics, showed cytotoxic and DNA-damaging effects. The potential mechanisms of toxicity for selected nanoparticles are listed in Table 14.2.

Table 14.2 Nanoparticle cytotoxicity to mammalian cells (Adapted with permission; Nel et al. 2009)

Nanoparticle	Cytotoxicity mechanism
TiO_2	ROS (reactive oxygen species) production
	Glutathione depletion and toxic oxidative stress
	Cell membrane disruption
ZnO	ROS production
	Dissolution and release of toxic cations
	Lysosomal damage
	Inflammation
Ag	Dissolution and Ag+ ion release inhibits respiratory enzymes and ATP production
	ROS production
	Disruption of membrane integrity and transport processes
Gold	Disruption of protein conformation
SiO_2	ROS production
	Protein unfolding
	Membrane disruption
Cu/CuO	DNA damage and oxidative stress

In order to help prevent aggregation of nanoparticles, stabilising (capping) agents that bind to the entire nanoparticle surface can be used; these include water-soluble polymers, oligo- and poly-saccharides, sodium dodecyl sulfate, polyethylene glycol and glycolipids. The specific roles of surface capping, size scale and aspect ratio of ZnO particles towards antimicrobial activity and cytotoxicity have been investigated (Nair et al. 2009). Polyethylene glycol-capped ZnO nanoparticles demonstrated an increase in antimicrobial efficacy with a reduction in particle size. Again, gram negative bacteria were more affected than gram positive, which suggests that a membrane damage mechanism of action rather than one involving the production of reactive oxygen species (ROS) is of overriding significance. Polyethylene glycol-capped nanoparticles were highly toxic to human cells with a very low concentration (100 μM) threshold for cytotoxic action, whereas the concentration for antibacterial activity was 50 times greater (5 mM). It is hypothesised that the toxicity to eukaryotic cells is related to nanoparticle-enhanced apoptosis by up regulation of the Fas ligand on the cell membrane.

An understanding of the interface between biological systems and nanomaterials should enable design features to be used to control the exposure, bioavailabilty and biocatalytic activities. A number of possible approaches are starting to be identified (Nel et al. 2009) including changing ability to aggregate, application of surface coatings, and altering charge density and oxidative state. However this may well compromise the intended selective toxicity of antimicrobial nanoparticles. It remains to be determined how potential mammalian toxicity issues will fully impact on the use of nanotechnology in the control of oral biofilms.

14.8 Concluding Remarks

The application of nano-antimicrobials to control oral infections, as a function of their biocidal, anti-adhesive and delivery capabilities, is of increasing interest. Their use as constituents of prosthetic device coatings, topically applied agents and within dental materials is currently being explored. Future developments are likely to concentrate on those nanoparticles with maximal antimicrobial activity and minimal host toxicity. Antimicrobial nanoparticulate metals have received particular attention as a result of their durability. Although certain nanoparticles may be toxic to oral and other tissues, the surface characteristics of a given particle will determine whether or not it will have potential for oral applications. Approaches to alter biocompatibility and desired function are now being identified and include changing the ability to aggregate, application of surface coatings, and altering oxidative state and charge density.

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