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CHAPTER

# Nanoparticles and the Control of Oral Biofilms

10

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## CHAPTER OUTLINE



**Nanobiomaterials in Clinical Dentistry.**

## 10.1 Introduction

Nanoparticles can be classified as particles of a size no greater than 100 nm, and their unique attributes to combat infections have received considerable attention within a range of diverse fields, including medicine and dentistry. Nanomaterials are increasingly finding uses in products such as antimicrobial surface coatings and semiconductors. These include spherical, cubic, and needle-like nanoscaled particles (approximately 5−100 nm) and near-nanoscaled devices (up to micrometers) [1]. Properties of nanoparticles, for example, their active surface area, chemical reactivity, and biological activity, can be dramatically different from those of micrometer-sized particles [2], 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. These characteristics should allow them to closely interact with microbial membranes, and thus elicit an antimicrobial effect that is not solely due to the release of metal ions [3]. Metallic and other nanoparticles are now being combined with polymers and other base materials and coated onto surfaces which may have a variety of potential antimicrobial applications within the oral cavity [4,5].

The oral cavity supports the growth of a wide diversity of microorganisms including bacteria, yeasts, and viruses—members of all groups being associated with oral infections. Bacteria are the predominant components of this resident microflora, and the diversity of species found in the oral cavity reflects the wide range of endogenously derived nutrients, the varied types of habitat for colonization including surfaces on the teeth, mucosa, and tongue, and the opportunity to survive as a biofilm. An oral biofilm can be classed as an aggregate of microorganisms in which cells adhere to each other and to a surface [6]. However, the relationship between this microflora and the host 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 the nonshedding 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 (GCF), masticatory forces, and other variables such as oral hygiene procedures. The composition of the microbial flora of the mouth thus varies considerably from site to site and at different time points. Up to 1000 different species of bacteria at $10^8$−$10^9$ bacteria per milliliter saliva or per milligram dental plaque are known to be associated with the oral cavity, and it has been suggested that only 50% of the bacteria found at these sites can be cultured [6].

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 and periodontal disease, or an exogenous source yielding microorganisms 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 the

restored tooth surface. The use of nanotechnology offers the possibility to control the formation of these and other oral biofilms through the use of nanoparticles with biocidal, antiadhesive, and delivery capabilities.

## 10.2 Biofilms and oral infections

Biofilms of oral bacteria and yeasts can cause a number of localized diseases in the oral cavity, including dental caries, gingivitis, periodontitis, candidiasis, endodontic infections, orthodontic infections, and peri-implantitis [6].

### 10.2.1 Formation and properties of oral biofilms

Within the oral cavity, the survival of microorganisms 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 [7]. On the tooth surface, the initial colonizers adhere to the acquired pellicle, a salivary/dietary-derived proteinaceous layer, which can then influence the subsequent sequence of colonization by microorganisms [8]. The acquired pellicle also contains several salivary components such as secretory immunoglobulin A (sIgA) and lysozyme, and these provide both barrier and buffering functions [9]. Both de- and remineralization processes of the teeth are also mediated by the pellicle. In terms of bacterial colonization, 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 [9]. The pellicle layer is therefore of particular relevance for 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 coaggregation at the surface of the developing biofilm [10]. The initial communities of bacteria found within the supragingival plaque biofilm are of a relatively low diversity in comparison to those present in the mature communities of both supra- and subgingival plaque. Initial colonizers include *Streptococcus oralis*, *Streptococcus sanguinis,* and *Streptococcus mitis*. The coaggregating partners with these bacteria would then include predominantly gram-negative species, e.g., *Veillonella atypica, Eikenella corrodens,* and *Prevotella loescheii*. Coaggregation bridges between these early colonizers and *Fusobacterium nucleatum* are common and the latter then coaggregates with numerous late colonizers. Late colonizers include *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Treponema denticola,* and *Porphyromonas gingivalis* [10]. The interactions between oral bacteria are integral to biofilm development and maturation and include physical contact, metabolic exchange, molecular communication, and genetic material exchange.

Biofilms will accumulate on both the hard and soft oral tissues, and this community of microbial species is embedded in a matrix of bacterial components, salivary proteins/peptides, and food debris [8]. 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 [11]. The biofilm mode of growth is clearly distinguished from planktonic growth by a number of features,

which includes the resistance to antimicrobial agents at concentrations that approach 1000 times greater than that required to kill planktonic microorganisms [12,13]. This is of particular significance in the development of nanoantimicrobials and the extrapolation of in vitro findings.

## 10.2.2 Oral biofilms and disease

### 10.2.2.1 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*, *Streptococcus sobrinus,* and *Lactobacillus* spp. [14], whereas the 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. *P. gingivalis*, *Tannerella forsythia,* and *T. denticola* are now regarded as the major pathogens in advancing periodontitis [15].

Prevention of dental caries and periodontal diseases is traditionally targeted at mechanical or nonspecific control of the plaque biofilm because this is the precipitating factor. The use of antimicrobial agents represents a valuable complement to mechanical plaque control [16]. 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 [17].

### 10.2.2.2 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 *Streptococcus* and *Actinomyces* spp. will accumulate on successful implants. However, in peri-implantitis, anaerobic gram-negative organisms predominate [18]. 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-implant mucositis and seen in up to 50% of implant-treated subjects) [19]. 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 supporting tissue [19]. The incorporation of nanoparticles into implant coatings may well offer useful osteoconductive and antimicrobial functionalities to prevent dental implant failure.

### 10.2.2.3 Candidiasis

The development of candidiasis, including denture stomatitis (chronic atrophic candidiasis), which can affect up to 65% of edentulous individuals [20] involves the formation of a biofilm. Despite the use of antifungal drugs to treat denture stomatitis, infection can often recur. Chandra et al. [20],

using a poly(methyl methacrylate) (PMMA) biofilm model, demonstrated that *Candida albicans* biofilms are potentially highly resistant to the currently used antifungal agents, with resistance developing with time and showing a correlation with biofilm maturation.

### 10.2.3 Control of oral biofilms

Agents classified as antiplaque generally function by removing or disrupting biofilms or by preventing the formation of a new biofilm. However, they do not necessarily kill the microorganisms within the biofilm. Whereas, agents classified as antimicrobial act by inhibiting the growth (bacteriostatic) or killing (bactericidal) microorganisms, as defined by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), respectively. The uptake and penetration of antimicrobial agents into biofilms are key considerations in the administration of therapeutics [21]. This is of particular importance within the oral cavity when these agents have to reach less accessible stagnation sites or through plaque to the enamel. The development of plaque control measures that require a minimum of patient compliance and professional health-care intervention are therefore of particular interest [22]. Within this context, antimicrobial nanoparticles may be of particular value if retained at approximal teeth surfaces and below the gum margin. The anticaries potential of fluoride and other conventional antimicrobial/antiplaque agents, which are mostly deployed in mouthwashes and toothpastes, have been well characterized [16]. The potential of nanoparticles as constituents of topical agents to control oral biofilms through either their biocidal or antiadhesive capabilities has now emerged as an area that should be given serious consideration. The studies by Robinson et al. 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 extremely complex microbial composition and architecture of plaque biofilms [23]. 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. This model has indicated that plaque contains voids and channels, sometimes extending completely through the biomass to the underlying enamel [24] and may have considerable influence on the transfer of nanoparticles through biofilms. The main considerations are the physical and 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 [25].

## 10.3 Antimicrobial nanoparticles and oral biofilm control

### 10.3.1 Nanoparticulate metals 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 [26]. Powdered titanium dioxide is also commonly used as a whitener in toothpastes.

With respect to nanoparticulate metals, the antimicrobial properties of silver [27] and copper [28] have received the most attention. Both of these have been coated onto or incorporated into various base materials [29], including PMMA [30] and hydrogels [31]. An inverse relationship between the size of nanoparticles and antimicrobial activity has been clearly demonstrated, where particles in the size range of 1−10 nm have been shown to possess the greatest biocidal activity against bacteria [3,32]. Indeed, it has been shown that smaller silver nanoparticles are more toxic than larger particles, more so when oxidized [33]. At the nanoscale, $Ag^+$ ions are known to be released (leached) from the surface [34]. Sotiriou et al. [35] proposed 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.

Particular nanoparticles, as a result of their small size, may be able to offer other advantages to the biomedical field through improved biocompatibility [36]. Also, it appears that bacteria are far less likely to acquire resistance to metal nanoparticles than they are to other conventional and narrow-spectrum antibiotics [37]. 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 the microorganisms to resist their antimicrobial activity. Shape may also affect the activity of nanoparticles. It has been demonstrated that the shape of silver nanoparticles can influence antimicrobial activity, as has been shown in the case of *Escherichia coli* [37]. 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.

Exploitation of the toxic properties of nanoparticulate metals and metal oxides, such as titanium dioxide ($TiO_2$; Figure 10.1B) and zinc oxide (ZnO; Figure 10.1C), in particular those that produce reactive oxygen species (ROS) under UV light, are finding increased use in antimicrobial formulations, with silver metal nanoparticles (5−40 nm) having been reported to inactivate most microorganisms, including HIV-1 [38]. The high reactivity of nano-titanium dioxide and nano-silicon dioxide ($SiO_2$) is exploited extensively for their bactericidal properties in filters and coatings on substrates such as polymers, ceramics, glasses, and alumina [39]. Significant activity using metal and metal oxide nanoparticles and their compound clusters against fungal and bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* has recently been demonstrated. These have also shown the capability to inactivate viruses, including SARS (severe acute respiratory syndrome), H1N1 swine flu, 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 upon 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 in regards to 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 [40]. MBCs were found to be in the range of 0.1−5 mg/mL. In comparison, traditional antibiotics are effective at concentrations 1000-fold lower. NiO, Ni, $Al_2O_3$, $TiO_2$ (in the absence of UV light), $Si_3N_4$, WC (tungsten carbide), and $ZrO_2$ were found to lack antimicrobial activity at the concentrations tested. The oral pathogens *P. gingivalis*, *F. nucleatum*, *Prev. intermedia,* and *A. actinomycetemcomitans* were also found to be susceptible to Ag and CuO nanoparticles under anaerobic conditions with MBC values in the range 0.025−2.5 mg/mL [41].

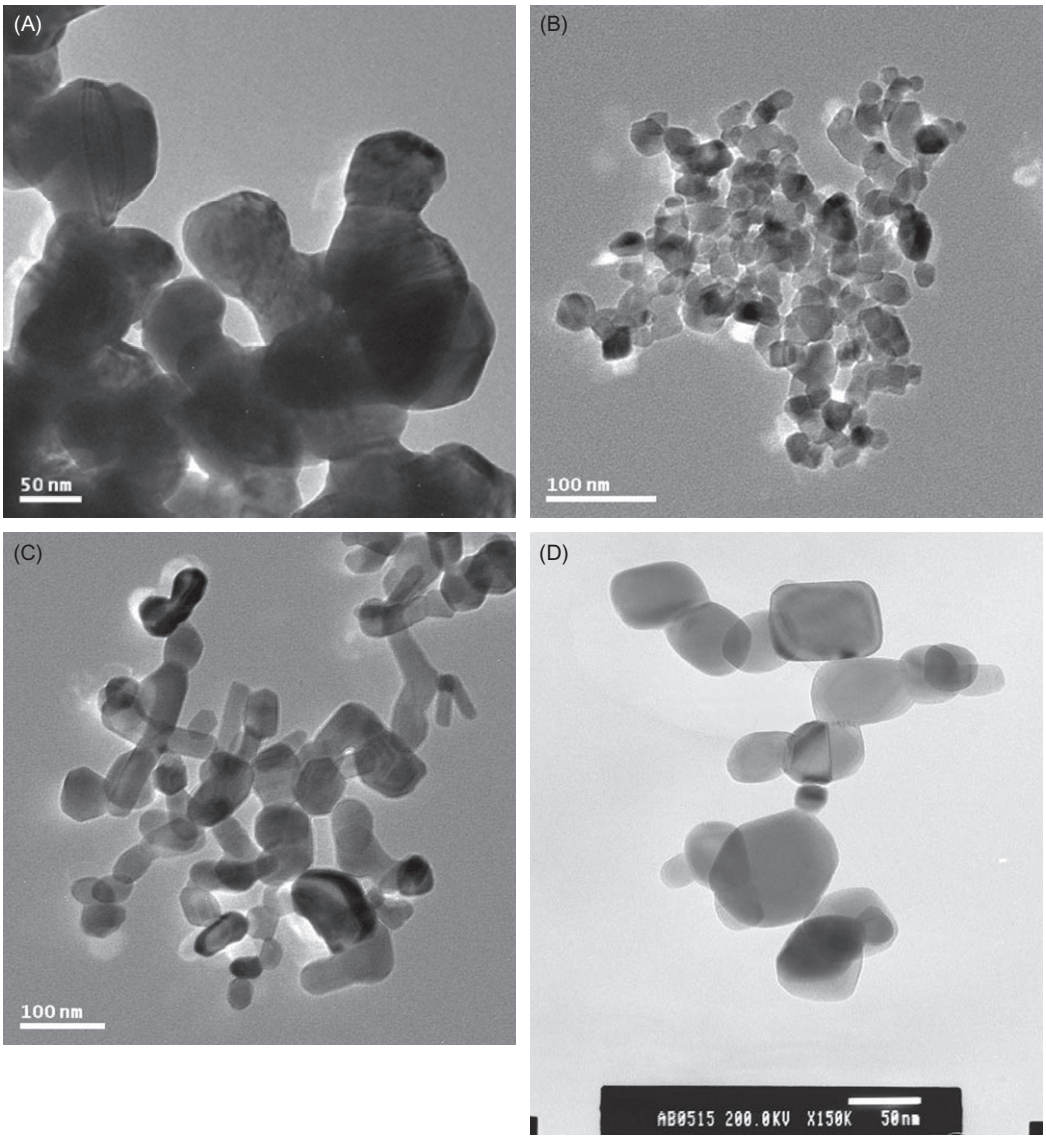


**FIGURE 10.1**

TEM images of agglomerated silver (A), titanium dioxide (B), zinc oxide (C), and copper oxide (D) nanoparticles.

#### 10.3.1.1 Silver (Ag)

The antimicrobial actions of elemental silver, $Ag^+$ ions, and silver compounds have been extensively investigated [4]. 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 [42]. 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 in tissue conditioners, acrylic resins, and mouth rinses within the dental field [43–46]. Silver nanoparticles (Figure 10.1A), either alone or together with other antimicrobial agents, have shown particularly encouraging results [27,47,48]. 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 synthesize polymer-nanocomposites, surfaces that comprised this material 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 [49].

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 [36]. In regards to molecular mechanisms of the inhibitory action of $Ag^+$ ions on microorganisms, it has been shown that DNA loses its ability to replicate [50], and the expression of ribosomal subunit proteins and other cellular proteins and enzymes necessary for ATP production become inactive [51]. It has also been hypothesized that $Ag^+$ ions affect membrane-bound respiratory enzymes [52]. 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 [27] demonstrated structural changes and damage to bacterial membranes resulting in cell death. These particular studies suggest that sulfur-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 [36].

#### 10.3.1.2 Copper (Cu)

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 activity against microorganisms. As with silver, it is thought that copper acts by combining with the −SH groups of key microbial enzymes. Yoon et al. [53] demonstrated superior antimicrobial activity with copper nanoparticles against *E. coli* and spore forming *Bacillus subtilis* when compared to silver

nanoparticles. However, other studies demonstrate silver to have superior activity to copper against a wide range of different species and strains [40].

The antimicrobial properties of both silver and copper nanoparticles were also investigated by Ruparelia et al. [54] using strains of *E. coli*, *B. subtilis,* and *S. aureus*. The bactericidal effect of the nanoparticles was compared using disc diffusion tests and MIC and MBC determinations. 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 [55]. Released copper ions within the cell may then disrupt nucleic acid and key enzymes [56]. In theory, a combination of silver and copper nanoparticles may give rise to a more complete bactericidal effect, especially against a mixed population of bacteria. Indeed, the studies of Ren et al. [40] 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 nanocopper 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) of nanosilver.

#### 10.3.1.3 Gold (Au)

Gold shows a weak antimicrobial effect in comparison to silver and copper. 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 color in the visible range and contrast strongly for imaging by electron microscopy [57]. 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 (PEG) coated to allow dispersion) on *E. coli* at various concentrations, demonstrated no significant activity [58]. Studies with PEG-coated gold nanoparticles also showed no activity against *E. coli*. However, the growth of the gram-negative *Proteus* species and *P. aeruginosa* was inhibited at a concentration of 1.0 mg/mL (R.P. Allaker, unpublished observations).

### 10.3.2 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 [59]. However, certain metal oxides are now coming under close scrutiny because of their potential toxic effects [60]. Oxides under consideration as antimicrobial agents include those of copper, zinc oxide, titanium dioxide (titania), and tungsten trioxide ($WO_3$).

#### 10.3.2.1 Copper oxide (CuO and $Cu_2O$)

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 [61,62]. Copper oxide is relatively cheap, easily mixed with polarized 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 [59].

Copper oxide (CuO) nanoparticles have been characterized, both physically and chemically, and investigated with respect to potential antimicrobial applications [40]. It was found that nanoscaled 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 (Figure 10.1D). CuO nanoparticles in suspension showed activity against a range of bacterial pathogens, including MRSA and *E. coli*, with MBCs ranging from 0.1 to 5.0 mg/mL. As with silver, studies of CuO nanoparticles incorporated into polymers suggest that the release of ions may be required for optimum killing [40]. 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 [63].

$Cu_2O$ (copper (I) oxide; cuprous oxide) is a red powder and can also be produced as nanoparticles. Similar activity to CuO (copper(II) oxide; cupric oxide) has been shown against a range of species and strains [40].

#### *10.3.2.2 Zinc oxide (ZnO)*

As in the case of other nanoparticulate metals and metal oxides, the antimicrobial mechanisms of zinc are not completely understood. Nano-zinc oxide has received increasing attention, not only because it is stable under harsh processing conditions but also because it is generally regarded as safe and biocompatible [59]. 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 [64,65,66]. The proposed mechanisms of antibacterial activity include induction of ROS [67,68] and damage to the cell membrane with subsequent interaction of the nanoparticle with the intracellular contents [64].

Liu et al. [69] investigated the antimicrobial properties of ZnO nanoparticles against *E. coli* strain O157:H7 (verocytotoxin-producing). This strain was significantly inhibited as shown using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses to assess the morphological changes of bacterial cells. Leakage of intracellular contents and a degree of membrane disorganization were 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), a higher concentration of zinc oxide (particle size: approximately 15−20 nm; surface area: 47 $m^2$/g) is required to have growth inhibitory (0.5−2.5 mg/mL) and killing effects ($>$2.5 mg/mL) against a range of pathogens including *E. coli* and MRSA (K. Memarzadeh and R.P. Allaker, unpublished observations). While with those organisms implicated in oral infections, including *A. actinomycetemcomitans*, *P. gingivalis*, *Prev. intermedia* and *F. nucleatum*, greater sensitivity was demonstrated under anaerobic conditions, with growth inhibitory and killing concentrations of 0.25−2.5 and 0.25−2.5 mg/mL, respectively [41].

#### 10.3.2.3 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 [70]. $TiO_2$ is widely used in a number of applications, as a powder and increasingly in a nanoparticulate form, and is generally considered to be nontoxic 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 interleukin 1α [71]. The anatase form of nano-$TiO_2$ and UV light excitation are required to ensure maximum antimicrobial activity. $TiO_2$ 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 [72]. The study of Tsuang et al. [73] demonstrated $TiO_2$-mediated photocatalytic and bactericidal activities against obligate aerobes (*P. 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: approximately 18 nm; surface area: 87 $m^2$/g) 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, respectively (K. Memarzadeh and R.P. Allaker, unpublished observations). While with those organisms implicated in oral infections, including *A. actinomycetemcomitans*, *P. gingivalis*, *Prev. intermedia,* and *F. nucleatum*, growth inhibitory and killing concentrations under anaerobic conditions are in the same order at 0.25−2.5 and >2.5 mg/mL, respectively [41].

### 10.3.3 Oral applications of nanoparticulate metals and metal oxides

Silver nanoparticles are being investigated to reduce bacterial and fungal adhesion to oral biomaterials and devices, e.g., incorporation into denture materials (Figure 10.2) [4] and orthodontic adhesives [74]. 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. [74] clearly 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. 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 [75]. 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. The structural characteristics of composites would need to be carefully assessed with a 10% ZnO loading.

With reference to dental implants, numerous companies 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 a HA material available in nanophase and a nanocrystalline silver-based antimicrobial coating that should reduce the potential for bacterial

colonization. The antibacterial properties of an amorphous carbon film [76] incorporating silver nanoparticles in a 40–60 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.

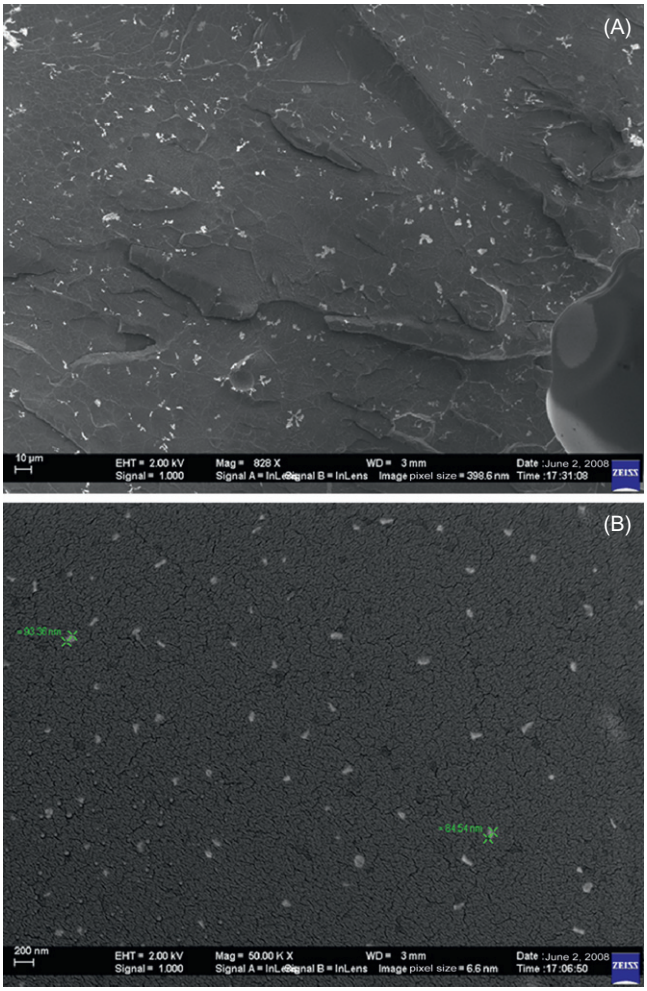


**FIGURE 10.2**

Scanning electron micrograph of a fractured PMMA/Ag nanocomposite containing approximately 0.04% w/w silver. Distribution of silver particles in the PMMA acrylic resin is 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 from Ref. [4].*

### 10.3.4 Quaternary ammonium compounds

Quaternary ammonium poly(ethylene imine) (QA-PEI) nanoparticles as an antimicrobial to incorporate into restorative composite resins have been developed [77] (Figure 10.3). This may have distinct advantages over the currently used composite resins employed to restore hard tissues, which

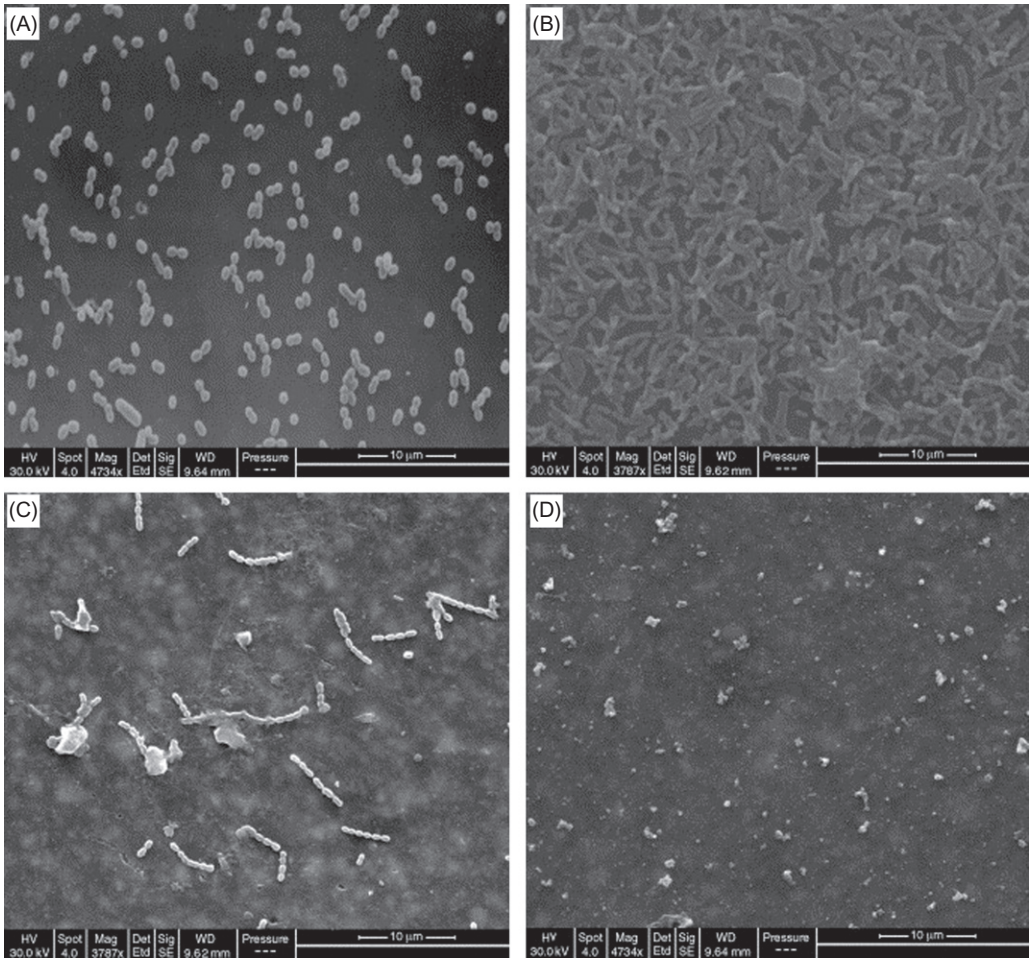


**FIGURE 10.3**

Scanning electron micrograph (4000 × magnification) of *S. mutans* in contact with composite resin (Z250, 3M ESPE Dental) with and without 1% w/w quaternary ammonium polyethylenimine (PEI) nanoparticles. (A) After 1 h incubation without nanoparticles. (B) After 24 h incubation without nanoparticles showing bacterial growth and typical biofilm formation. (C) After 1 h of incubation with nanoparticles. (D) After 24 h of incubation with nanoparticles. There is a decrease in the amount of *S. mutans* present illustrating the bactericidal properties of PEI nanoparticles.

*With permission from Ref. [77].*

are known to possess several disadvantages including development of biofilms on both teeth and the restorative material [4]. 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.

QA-PEI nanoparticles at a concentration of 1% w/w enabled complete in vitro growth inhibition of *S. mutans* to be achieved for at least 3 months [78]. 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 particles 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 optimize the release characteristics of QA-PEI and other potentially useful nanoparticulates from dental materials will be required.

## 10.4 Antiadhesive nanoparticles and oral biofilm control

### 10.4.1 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 [79]. Although the antimicrobial irrigants (without chitosan) are used 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 [80]. The in vitro study of Kishen et al. [81] 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 dentin. In theory, such surface treatment could prevent bacterial recolonization and biofilm formation in vivo.

### 10.4.2 Silica and silicon nanoparticles

Particles of a nano and micro size based upon the element silicon, designed to rapidly deliver antimicrobial and antiadhesive capabilities to the desired site within the oral cavity, have received attention [82]. 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 have actively sought new directions in this area through the use of porous silicon and nanocrystalline silicon technology to carry and deliver antimicrobials, e.g., triclosan. These may well offer advantages to 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 [83]. 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 mineralization 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 [84]. Modified surfaces were shown to reduce attachment and growth of *C. albicans*, with the greatest effect observed with 7 and 14 nm particles. These effects could possibly be attributed to the surface topography or slow dissolution of the bound silica. Such treatment has the advantages of being nontoxic, simple to apply and adaptable to three-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 [85]. 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 *P. aeruginosa*, *E. coli*, *S. aureus*, *Staphylococcus epidermidis,* and *C. 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 antibiofilm efficacy [25].

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 dentin disinfectants to offer an alternative to calcium hydroxide [86]. 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 10-fold, 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. [86] monitored ionic dissolution profiles in simulated body fluid. Antimicrobial activity was assessed against *E. faecalis* as a pathogen often isolated from root canal infections. They found that a shift from a micron- to a nanosize 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.

### 10.4.3 Hydroxyapatite and other calcium phosphate-based systems

The application of nanoscaled HA particles has been shown to impact on oral biofilm formation and provides a remineralization capability [87,88]. Biomimetic approaches, based upon HA nanocrystals which resemble the structure at the nanoscale of abraded dental enamel crystallites, should allow adsorbed particles to interact with bacterial adhesins, reduce bacterial adherence, and hence impact on biofilm formation [89].

A number of oral health-care products, including dentifrices and mouth rinses, have been developed containing nanosized apatite particles with and without protein-based additives [90,91]. 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™/MI Paste™) is a particular technology based upon ACP and stabilized by

CPP [92]. 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 remineralization of enamel subsurface lesions [91]. Analysis of plaque samples demonstrated CPP−ACP nanocomplexes to be localized in plaque on the surface of bacterial cells and essentially confirm the studies by Rose [93,94] who demonstrated tight binding to *S. 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 colonization as shown with CPP−ACP germanium treated surfaces [90].

## 10.5 Photodynamic therapy and the use of nanoparticles to control oral biofilms

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 photosensitizing agent and light sources to areas requiring treatment [95]. This approach is now being utilized 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 photoactivatable agent or sensitizer. The result of excitation is that the sensitizer moves from an electronic ground state to a triplet state which then interacts with microbial components to generate cytotoxic species [96]. One of the advantages of light-activated killing is that the resistance to action of singlet oxygen is unlikely to become widespread in comparison to that experienced with more traditional chemical antimicrobial agents. A sensitizer ideally should absorb light at red to near-infrared wavelengths because these wavelengths are able to penetrate more. The most commonly tested sensitizers on bacteria are tricyclic dyes (e.g., methylene blue and erythrosine), tetrapyrroles (e.g., porphyrins), and furocoumarins (e.g., 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) 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 [97]. 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 *S. mutans* cells demonstrated a 3-log reduction when treated with erythrosine and white light (500−650 nm) [98], while an approach using antibody- and erythrosine-labeled nanoparticles has shown the potential for targeting specific bacterial species in oral plaque biofilms (S. Wood et al., unpublished observations). These in vitro studies, employing constant-depth film fermenters with gold nanoparticles conjugated to erythrosine and antibody to either *S. 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 (i) direct toxicity of the sensitizer, (ii) indirect toxicity of the sensitizer in terms of

"by-stander" damage to adjacent host cells, (iii) penetration into the biofilm, (iv) light exposure time required to kill bacteria within in vivo biofilms, and (v) widespread relatively nonspecific bacterial killing [95]. The photosensitizer erythrosine has an advantage over other dyes because it is currently used in dentistry to visualize 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 GCF into the pocket, and most photosensitizers 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 GCF 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.

## 10.6 Biocompatibility of nanoparticles 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 body, non-nanoparticulate silver can accumulate within the body. However, the threat posed by these metals in a nanoparticulate form is far from clear [99]. In order to understand the mechanism of toxicity, a thorough knowledge of the toxicokinetic properties of nanoparticles is required. This includes information on the absorption, distribution, metabolism, and excretion of nanoparticles [100]. In theory, certain nanoparticles may be retained within the body for longer than the desirable time, 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, unpublished observations). However, a particle's surface chemistry, which in some cases can be modified, can govern whether it should be considered further for biomedical applications [25].

Toxicology and biodynamic studies suggest that silica, silicon, and chitosan nanoparticles are relatively safe if introduced via the oral route [99]. Testing of NO-releasing silica nanoparticles (at the highest concentration tested of 8 mg/mL) with fibroblasts demonstrated that cell proliferation was inhibited to a lesser degree than with chlorhexidine [85]. Likewise, 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 [78]. In comparison to other metals, silver is less toxic to human cells and is only ever used at very low concentrations in vivo [27]. For example, silver nanoparticles have been shown to inhibit *Candida* spp. at a concentration of 0.2 μg/mL, which is markedly less than the concentration (30 μg/mL) required to demonstrate a toxic effect against human fibroblasts [101].

The safe use of nanotechnology and the design of nanomaterials for biological applications, including the control of oral biofilms, involve a thorough understanding of the interface between

these materials and biological systems [25]. The interface comprises three interacting components: (i) the surface of the nanoparticle, (ii) the solid−liquid interface and the effects of the surrounding medium, and (iii) the contact zone with biological substrates. 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 [102]. For example, it has been shown that titanium dioxide nanoparticles [103] act to resist the formation of surface biofilms through increased hydrophilicity in comparison to an unmodified surface.

The characteristics of the surface layer, such as zeta 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 critically important. The contribution of surface charge to both mammalian and microbial interactions has been illustrated using surfactant-coated nanoparticles [104]. Antiadherent and antifungal effects were shown using buccal epithelial cells treated with nondrug-loaded poly(ethylcyanoacrylate) nanoparticles. Nanoparticles were prepared using emulsion polymerization and stabilized with cationic, anionic, or nonionic surfactants. Cationic surfactants, for example, cetrimide, which are known antimicrobial agents, were the most effective in reducing *C. 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. Nonionic surfactant-coated nanoparticles produced intermediate kill rates. These studies clearly demonstrate the importance of surface charge on the nanoparticle surface. It is suggested that the buccal epithelium could possibly be treated using polymeric-type nanoparticles in a mouthwash-type formulation; in theory, 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 [25]. 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 (to both eukaryotes and prokaryotes). Larger, more hydrophobic or poorly dispersed particles, which would be rapidly removed by the reticuloendothelial system, were shown to be less toxic. Karlsson et al. [60] have shown that metal oxide nanoparticles are more toxic than at first envisaged at concentrations down to 40 μg/mL 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, the comet assay to measure DNA damage and an oxidation-sensitive fluoroprobe to quantify the production of ROS [60]. 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, also showed significant cytotoxic and DNA-damaging effects. The potential mechanisms of toxicity for these and other selected nanoparticles are listed in Table 10.1.

In order to help prevent aggregation of nanoparticles, stabilizing (capping) agents that bind to the entire nanoparticle surface can be used; these include water-soluble polymers, oligo- and polysaccharides, sodium dodecyl sulfate, polyethylene glycol, and glycolipids. The specific impact of surface capping, size scale, and aspect ratio of ZnO particles upon antimicrobial activity and cytotoxicity have been investigated [105]. Polyethylene glycol-capped ZnO nanoparticles demonstrated

**Table 10.1** Nanoparticle Cytotoxicity to Mammalian Cells

| Nanoparticles | Cytotoxicity Mechanism |
|---|---|
| $TiO_2$ | ROS 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 |

*Adapted from Ref. [25].*

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 ROS is of overriding significance. Polyethylene glycol-capped nanoparticles were found to be highly toxic to human cells with a very low concentration (at 100 μM) threshold for cytotoxic action, whereas the concentration for antibacterial activity was 50 times greater (at 5 mM). It is hypothesized that the toxicity to eukaryotic cells is related to nanoparticle-enhanced apoptosis by upregulation of the Fas ligand on the cell membrane [105].

An understanding of the interface between biological systems and nanomaterials should enable design features to be used to control the exposure, bioavailability, and biocatalytic activities. A number of possible approaches are now being identified [25] including changing the 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.

## 10.7 Conclusions

The application of nanoscaled antimicrobials to control oral infections, as a function of their biocidal, antiadhesive, and delivering 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

**Table 10.2** Studies Presenting Data on Effects of Nanoparticles Against Oral Microorganisms

| Study | Study Design | Nanoparticles/ Materials Used | Parameters Studied | Results | Microbial Flora Studied |
|---|---|---|---|---|---|
| [41] | In vitro | Metals/metal oxides | Antimicrobial activity | Bactericidal in the range 0.025–2.5 mg/mL | *P. gingivalis, F. nucleatum, Prev. intermedia, A. actinomycetemcomitans* |
| [74] | In vitro | Composite adhesives with silver nanoparticles | Physical properties and antimicrobial activities | Antiadhesive properties and growth retardation | *S. mutans, S. sobrinus* |
| [75] | In vitro | Zinc oxide nanoparticles blended with resin-based dental composite | Antibiofilm activity | Inhibition of biofilm growth with concentration >10% w/w | *S. sobrinus* |
| [77] | In vitro | Composite resin with quaternary ammonium polyethylenimine nanoparticles | Antibiofilm activity | Inhibition of biofilm formation at 1 and 24 h | *S. mutans* |
| [81] | Ex vivo | Zinc oxide/chitosan nanoparticles | Antiadherence on treated root canal surfaces | Antiadherent | *E. faecalis* |
| [83] | Ex vivo | Silica nanoparticles | Antiadherence on polished teeth surfaces | Antiadherent | *S. mutans* |
| [84] | In vitro | Silica nanoparticles deposited onto polystyrene surfaces | Development of biofilm and invasive filament formation | Decreased attachment and growth | *C. albicans* |
| [85] | In vitro | Nitric oxide-releasing nanoparticles | Antibiofilm activity | >99% killing within biofilm | *C. albicans* |
| [86] | In vitro | Nanometric bioactive glass | Antimicrobial activity in simulated body fluid | Significant killing effects | *E. faecalis* |
| [92] | In vitro and in vivo | Casein phosphopeptide–amorphous calcium phosphate nanocomplex | Anticariogenic | Reduction of colonization | *S. mutans* |

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 help to determine whether or not it will have potential for oral applications. Approaches to alter biocompatibility and desired function are now being identified and these include changing the ability to aggregate, application of surface coatings, and altering oxidative state and charge density.

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