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Review

Nanoparticles and the control of oral infections

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ABSTRACT

The potential of antimicrobial nanoparticles to control oral infections is reviewed. Such particles can be classified as having a size no greater than 100 nm and are produced using traditional or more novel techniques. Exploitation of the toxic properties of nanoparticles to bacteria, fungi and viruses, in particular metals and metal oxides, as well as their incorporation into polymeric materials have increased markedly over the past decade. The potential of nanoparticles to control the formation of biofilms within the oral cavity, as a function of their biocidal, anti-adhesive and delivery capabilities, is now receiving close attention. Latest insights into the application of nanoparticles within this field, including their use in photodynamic therapy, will be reviewed. Possible approaches to alter biocompatibility and desired function will also be covered.

1. Introduction

Nanotechnology represents the ability to image, manipulate and model functionalities on the nanometre scale. This discipline includes the study of nanoparticles, which can be classified as particles with a size no greater than 100 nm. Those particles with an antimicrobial function have received considerable attention within a range of diverse fields, including medicine and dentistry. These include spherical, cubic and needle-like nanoscaled particles (ca. 5–100 nm) and near-nanoscaled devices (up to micrometres) [1]. Properties of nanoparticles, e.g. their active surface area, chemical reactivity and biological activity, are often radically different from particles of a greater size [2]. For example, the antimicrobial effectiveness of metallic nanoparticles has been suggested to be due both to their size and high surface-to-volume ratio. In theory, these characteristics should allow them to interact closely 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 as well as coated onto surfaces to provide a variety of potential antimicrobial and anti-adhesive applications within the oral cavity [4,5].

The oral cavity provides habitats for a wide diversity of microorganisms including bacteria, yeasts and viruses, with 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 colonisation including surfaces on the teeth, mucosa and tongue, and the opportunity to survive as a biofilm [6,7]. 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. These are mainly localised and include dental caries, gingivitis, periodontitis, candidiasis, endodontic infections, orthodontic infections and peri-implantitis [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 micro-organisms normally found in the mouth, such as the main plaque-related diseases, namely dental caries and periodontal disease, or from 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 both on the natural and restored tooth surface.

Plaque-related diseases are probably the most common bacterial diseases occurring in man. Dental caries (dental decay) is a destructive condition of the dental hard tissues that, if unchecked, can progress to inflammation and death of vital pulp tissue, with 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. [6]. Periodontal diseases can involve both the soft and hard tissues and are the most common inflammatory destructive conditions that affect man. They are initiated by components of the plaque that develops on the hard root surface adjacent to the soft tissues of the supporting periodontium and may be confined to the

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gingiva (gingivitis) or extend to the deeper supporting structures with destruction of the periodontal ligament and the alveolar bone that supports the teeth (periodontitis). Such loss of attachment, with associated periodontal pocket formation, may ultimately lead to loosening and loss of the affected teeth. *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* are regarded as the major pathogens in advancing periodontitis [8]. Furthermore, it has been recently suggested that there is an association between the oral microbiota and systemic diseases such as cardiovascular disease and complications during pregnancy [9,10].

Prevention of dental caries and the periodontal diseases is traditionally targeted at the mechanical or non-specific control of dental plaque, as this is the precipitating factor. However, the individual response of the host and other confounding factors can influence disease initiation and progression. Antimicrobial approaches, including the use of antimicrobial agents, represent a valuable complement to mechanical plaque control. Such strategies should ideally prevent plaque biofilm formation without affecting the biological equilibrium within the oral cavity, which is inhabited by up to 1000 different species of bacteria at 10^8–10^9 bacteria per millilitre of saliva or per milligram of dental plaque [11]. Use of nanotechnology 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.

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 [12]. This infection is a major cause of dental implant failure whereby the induced inflammatory changes in the soft tissues surrounding the implant lead to 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) [13]. Current forms of treatment are often inadequate, with chronic infection often requiring implant removal and expensive resective and regenerative procedures in an attempt to restore and reshape the supporting tissue [13]. Nanoparticle-based implant coatings may well offer useful osteoconductive and antimicrobial functionalities to prevent dental implant failure.

2. Control of oral biofilms

Agents classified as 'antiplaque' generally function by removing or disrupting biofilms or prevent the formation of a new biofilm. However, such agents do not necessarily kill the micro-organisms within the biofilm, whereas agents classified as antimicrobial act by inhibiting the growth of or by killing micro-organisms, as defined by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), respectively. Uptake and penetration of antimicrobial agents into biofilms are key considerations in the administration of therapeutics [14]. Biofilm mode of growth is clearly distinguished from planktonic growth by a number of features, including resistance to antimicrobial agents at concentrations that approach 1000 times greater than those required to kill planktonic micro-organisms [15,16]. This is of significance in the development of nano-antimicrobials and the extrapolation of in vitro findings. Uptake and penetration are of particular importance within the oral cavity when these agents have to reach less accessible stagnation sites or pass through plaque to the enamel. Development of plaque control measures that require a minimum of patient compliance and professional healthcare intervention are therefore of particular interest [17]. 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, has been extensively tested [18]. The potential of nanoparticles as constituents of topical agents to control oral biofilms through either their biocidal or anti-adhesive capabilities has now emerged as an area worthy of serious consideration. Studies 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 [19]. 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 [20], which 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 and penetrate at the biofilm surface. Nanoparticles are potentially useful within this context because it is possible to alter their surface charge, hydrophobicity, and other physical and chemical characteristics [21].

3. Antimicrobial nanoparticles and control of oral biofilms

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. Some of the most fundamental breakthroughs in medicinal history can be attributed to the antimicrobial properties of metals. Use of mercury as a medicinal agent can be traced back to the 10th century in Europe and the 2nd century BC in China. Skin diseases and syphilis were treated with inorganic mercury compounds, and more recently organomercurial compounds have been used as antiseptics and disinfectants [22]. Copper and zinc salts have also been investigated with respect to their use as antiseptics and as antifungal agents in the treatment of tinea pedis (athlete's foot). Many oral products, including toothpastes, now incorporate powdered (micron-sized) zinc citrate or acetate to control the formation of dental plaque [23].

With respect to nanoparticulate metals, the antimicrobial properties of silver [24] and copper [25] have received the most attention. Both of these have been coated onto or incorporated into various test materials [26], including poly(methyl methacrylate) (PMMA) [27] and hydrogels [28]. 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 have the greatest killing activity against bacteria compared with larger particles [3,29]. Indeed, it has been shown that smaller silver nanoparticles are more toxic than larger particles, and even more so when oxidised [30]. At the nanoscale, Ag^+ ions are known to be released from the surface of base materials incorporating nanoparticles [31]. Sotiriou and Pratsinis proposed that the antimicrobial activity of small (<10 nm) nanosilver particles is dominated by Ag^+ ions, whilst for larger particles (>15 nm) the contributions of Ag^+ ions and particles to the antibacterial activity are comparable, with the Ag^+ ion release being proportional to the exposed nanosilver surface area [32].

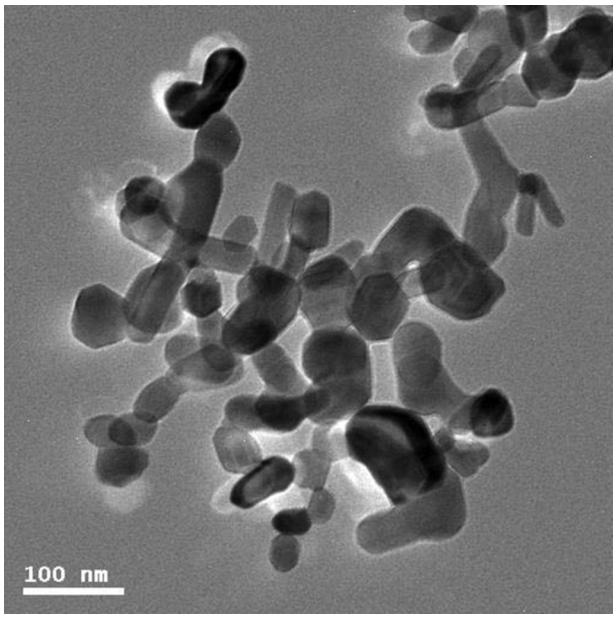

Fig. 1. Transmission electron microscopy image of zinc oxide nanoparticles.

Particular nanoparticles, as a result of their small size, may be able to offer other advantages to the biomedical field through improved biocompatibility [33]. Also, bacteria are far less likely to acquire resistance to metal nanoparticles than they are to other conventional and narrow-spectrum antibiotics [34]. 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 micro-organisms to resist their antimicrobial activity. Shape may also affect the activity of nanoparticles, as demonstrated with the shape of silver nanoparticles and antimicrobial activity against *Escherichia coli* [34]. Truncated triangular silver nanoplates with a {1 1 1} 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, in particular those that produce reactive oxygen species when exposed to ultraviolet (UV) light, such as titanium dioxide (TiO_2) and zinc oxide (ZnO) (Fig. 1), are finding increased use in antimicrobial applications, with silver metal nanoparticles (5–40 nm) having been reported to inactivate most micro-organisms, including human immunodeficiency virus type 1 (HIV-1) [35]. The high reactivity of nano-based TiO_2 and silicon dioxide (SiO_2) is exploited extensively for their bactericidal properties in filters and coatings on polymers, ceramics, glasses and alumina [36]. Significant activity using metal and metal oxide nanoparticles and their compound clusters against bacterial pathogens such as meticillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* has also been demonstrated. Nanoparticle preparations, including those based upon nickel, zirconium, copper, titanium, zinc, aluminium, silicon, silver and tungsten, have been compared with respect 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 shown [37]. 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*, *Fusobacterium nucleatum*, *P. intermedia* and *A. actinomycetemcomitans* were also found to be susceptible to Ag and CuO nanoparticles under anaerobic conditions, with MBCs in the range 0.025–2.5 mg/mL [38].

3.1.1. Silver (Ag)

The antimicrobial actions of elemental silver, Ag^+ ions and silver compounds have been extensively investigated [4]. In comparison with other metals, silver is relatively less toxic to human cells, albeit at very low concentrations. Silver has been considered for a range of biomedical applications, including use within the dental field as an antibacterial component in dental resin composites [39]. Silver also exhibits a strong affinity for zeolite, a porous crystalline material of hydrated aluminosilicate that 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 [40–43]. Silver nanoparticles, either alone or together with other antimicrobial agents, have shown particularly encouraging results [24,44,45]. Use of silver salt nanoparticles instead of elemental silver or complex silver compounds to prevent biofilm formation on surfaces both for biomedical and more general use has been investigated. Using silver bromide (AgBr) precipitation to synthesise polymer–nanocomposites, surfaces comprised of this material were shown to resist biofilm formation. Through controlling the size of the embedded AgBr, it was also shown to be possible to modify the release of biocidal Ag^+ ions [46].

The mechanism of antimicrobial activity of silver is not completely understood but is likely to involve multiple targets, in contrast to the more defined targets of antibiotics. Studies have shown that the positive charge on the Ag^+ ion is critical for antimicrobial activity, which allows the electrostatic attraction between the negative charge of the bacterial cell membrane and positively charged nanoparticles [33]. With regard to molecular mechanisms of the inhibitory action of Ag^+ ions on micro-organisms, it has been shown that DNA loses its ability to replicate [47], and the expression of ribosomal subunit proteins and other cellular proteins and enzymes necessary for ATP production becomes inactive [48]. It has also been hypothesised that Ag^+ ions affect membrane-bound respiratory enzymes [49]. Sondi and Salopek-Sondi demonstrated structural changes and damage to bacterial membranes resulting in cell death [24]. These particular studies suggest that sulphur-containing proteins in the membrane or inside the cells as well as phosphorus-containing elements such as DNA are likely to be the preferential binding sites for silver nanoparticles. The relative 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. 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 [33].

3.1.2. Copper (Cu)

In comparison with silver, comparatively few studies have reported the antimicrobial properties of copper. 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 micro-organisms. As with silver, it is thought that copper acts by combining with the –SH groups of key microbial enzymes. Yoon et al. demonstrated superior antimicrobial activity with copper nanoparticles against *E. coli* and spore-forming *Bacillus subtilis* compared with silver nanoparticles [50]. Yet other studies demonstrate silver to have superior activity to copper against a wide range of different species and strains [37].

3.1.3. Gold (Au)

Gold shows a weak antimicrobial effect in comparison with 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 colour in the visible range and contrast strongly for imaging by electron microscopy [51]. Despite all the current and potential applications for gold nanoparticles, there remains little information as to how these particles affect micro-organisms. Growth inhibition studies to measure the effect of gold nanoparticles, coated with polyethylene glycol (PEG) to allow dispersion, on *E. coli* at various concentrations demonstrated no significant activity [52]. This is supported by other studies with PEG-coated gold nanoparticles that also showed no activity against *E. coli*. However, growth of the Gram-negative *Proteus* spp. and *P. aeruginosa* was inhibited at a concentration of 1.0 mg/mL (R. Allaker, unpublished observations).

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 [53]. On the other hand, certain metal oxides are now coming under close scrutiny because of their potential toxic effects [54]. Oxides under consideration as antimicrobial agents include those of copper, zinc, titanium and tungsten. 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 [55–57].

3.2.1. Copper oxide (CuO and Cu_2O)

Copper oxide (CuO) is a semi-conducting compound with a monoclinic structure. CuO is the simplest member of the family of copper compounds and exhibits a range of useful physical properties, such as high-temperature superconductivity, electron correlation effects and spin dynamics [58,59]. It is relatively cheap, easily mixed with polarised liquids (i.e. water) and polymers, and relatively stable in terms of chemical and physical properties.

CuO nanoparticles have been physically and chemically characterised and investigated with respect to possible antimicrobial applications [37]. Nanoscaled CuO, as generated by thermal plasma technology, was found to demonstrate particle sizes in the range of 20–95 nm with a mean surface area of 15.7 m^2/g (Fig. 2). CuO nanoparticles in suspension show activity against a range of bacterial pathogens, including MRSA and *E. coli*, with MICs ranging from 0.1 mg/mL to 5.0 mg/mL. As with silver, studies of CuO nanoparticles incorporated into polymers suggest that release of ions may be required for optimum killing [37]. Incorporation of nano CuO into porous elastomeric polyurethane films has demonstrated potential for a number of applications [60].

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 of bacteria [37].

3.2.2. Zinc oxide (ZnO)

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 and biocompatible [53]. Zinc is also an important trace element in the human body. The proposed mechanisms of antibacterial activity with respect to nano zinc oxide include generation of reactive oxygen species [61,62] and damage to the cell membrane with subsequent interaction of the nanoparticle with the intracellular contents [55]

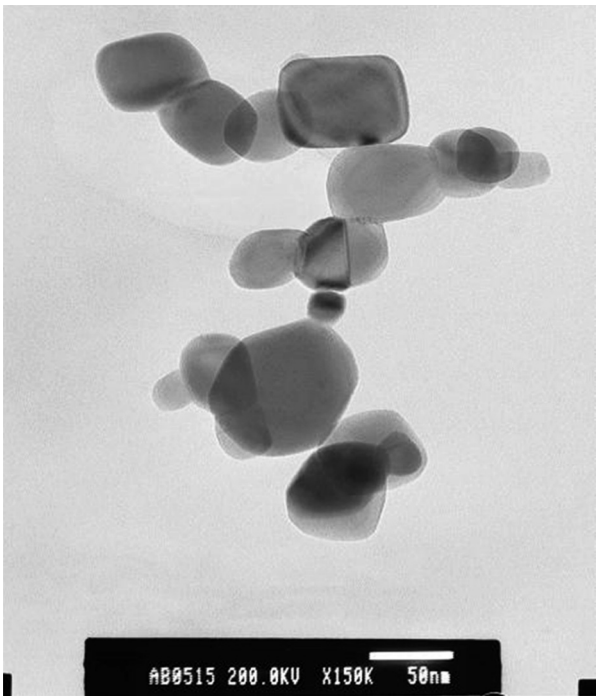

Fig. 2. Transmission electron microscopy image of copper oxide nanoparticles.

(Fig. 3). Liu et al. investigated the antimicrobial properties of ZnO nanoparticles against verocytotoxigenic *E. coli* strain O157:H7 [63]. This strain was significantly inhibited as shown using scanning electron microscopy and transmission electron microscopy analyses to assess the morphological changes of bacterial cells. Leakage of intracellular contents and membrane disorganisation 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 with silver nanoparticles (0.1 mg/mL), a higher concentration of zinc oxide (particle size ca. 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 [64]. With those organisms implicated in oral infections, including *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *F. nucleatum*, greater sensitivity was demonstrated under anaerobic conditions, with growth inhibitory and killing concentrations of 0.25–2.5 mg/mL and 0.25–2.5 mg/mL, respectively [38].

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 agent is firmly established [65]. TiO_2 is widely used in a number of applications, as a powder and increasingly in a nanoparticulate form, and it is considered to be biocompatible 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 cytokine release [66]. The anatase form of nano TiO_2 and UV light excitation are required to ensure maximum antimicrobial activity, whereby 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 [67,68]. Concentrations of TiO_2 (predominantly anatase phase; in the absence of UV light; particle size ca. 18 nm; surface area 87 m^2/g) required to have growth inhibitory and killing effects against a range of pathogens including *E. coli* and MRSA have been shown to be 1.0–2.5 mg/mL and >2.5 mg/mL, respectively [64].

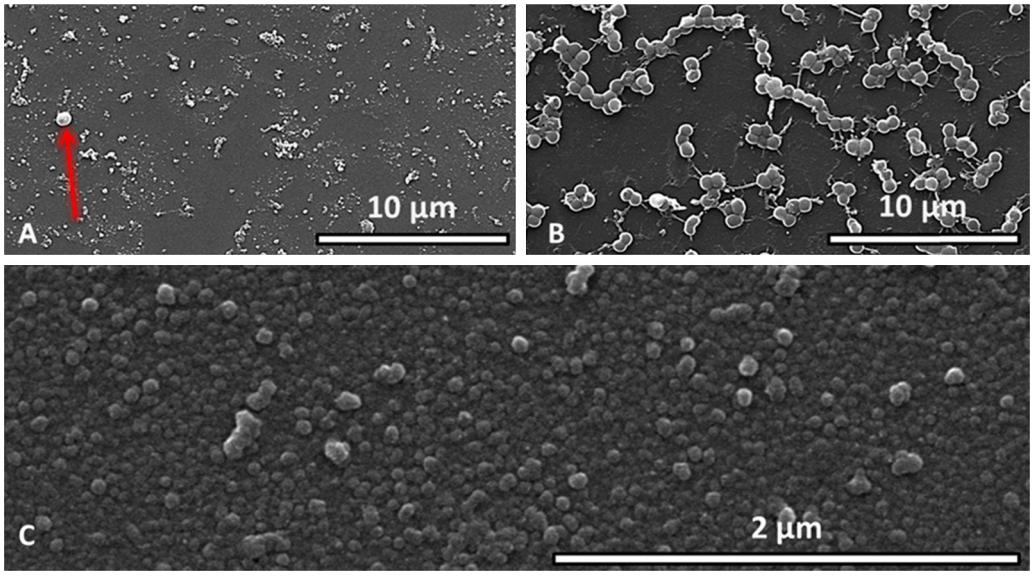

Fig. 3. Assessment of the bactericidal effect of nanoparticulate ZnO-coated glass substrates. (A) Arrow indicates an individual *Staphylococcus aureus* cell present on the coated surface. Debris present is likely to be remnants of dead bacteria. (B) A population of *S. aureus* present on an untreated surface. (C) High-resolution image to highlight the uniformity of the coated surface.

With those organisms implicated in oral infections, including *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *F. nucleatum*, growth inhibitory and killing concentrations under anaerobic conditions are in the same order at 0.25–2.5 mg/mL and >2.5 mg/mL, respectively [38].

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 [4] and orthodontic adhesives [69]. The optimum amount of silver nanoparticles used within such polymers will be of critical importance to avoid an adverse effect upon their physical properties. The study by Ahn et al. clearly demonstrated that experimental composite adhesives (ECAs) had rougher surfaces than conventional adhesives owing 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 [69].

Biofilm growth is known to contribute to secondary caries and the failure of resin-based dental composites. Within this context, ZnO nanoparticles have undergone testing using biofilm culture test systems [70]. ZnO nanoparticles blended into a variety of composites were shown to significantly inhibit *S. sobrinus* biofilm growth at concentrations in excess of 10% (w/w) over a 3-day test period. However, the structural characteristics of such 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. These include a HA material available in nanophase and a nanocrystalline silver-based antimicrobial coating that should reduce the potential for biofilm formation. The antibacterial properties of an amorphous carbon film [71] 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 with the standard titanium material was observed after 7 days using the coating with silver nanoparticles.

3.4. Quaternary ammonium compounds

Quaternary ammonium poly(ethylenimine) (QA-PEI) nanoparticles as an antimicrobial to incorporate into restorative composite resins have been developed [72]. This may be beneficial when compared with the currently used composite resins for hard tissue restoration, which are known to possess several disadvantages including development of biofilms both on teeth and on the restorative material [4]. 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 more than 3 months [73]. The proposed mechanism of action of nanoparticulate 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 antimicrobial activity. Surface chemical analysis of the restorative composite embedded with QA-PEI nanoparticles demonstrated a surface modification of higher hydrophobicity as well as the presence of quaternary amines when compared with the unmodified material. Further studies to optimise the release characteristics of QA-PEI and other potentially useful nanoparticulates from dental materials will be required.

4. Anti-adhesive nanoparticles and oral biofilm control

4.1. Silica and silicon-based nanoparticles

Particles of a nano and micro size based upon the element silicon have been designed to rapidly deliver antimicrobial and anti-adhesive capabilities to the desired site within the oral cavity [74]. Companies use silica (silicon dioxide 'SiO_2' and often classed as 'microfine', but with a particle size within the definition of nanoparticles) in toothpastes, 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.

Use of silica nanoparticles to polish the tooth surface may help protect against damage by cariogenic bacteria, presumably because these species can more easily be removed. This has been investigated on human teeth ex vivo [75]. Atomic force microscopy demonstrated lower nanometre-scale roughness obtained when silica nanoparticles were used to polish the surface of teeth 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 *Candida albicans* biofilms and invasive filament formation [76]. Modified surfaces were shown to reduce attachment and growth, with the greatest effect observed with 7 nm 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 non-toxic, 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 eradicate biofilm growth has been described [77]. Rapid diffusion of NO into the biofilm matrix probably provides 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. More than 99% of cells from each type of biofilm were killed as a result of NO release. Compared with small-molecule NO donors, the physicochemical properties, for example hydrophobicity, charge and size, of nanoparticles can be altered to increase antibiofilm efficacy [21].

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 [78]. Those in the form of amorphous nanoparticles, with a size range 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. monitored ionic dissolution profiles in simulated body fluid [78]. 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 [78].

4.2. Chitosan nanoparticles 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 nanoparticles and microparticles have been investigated as a potential platform for local delivery of drugs [79]. Although the antimicrobial irrigants (in the absence of chitosan) used to disinfect root canals in the treatment of endodontic infections are capable of killing *E. faecalis*, the bacterium frequently associated with this condition, endodontic restorations often fail. The in vitro study of Kishen et al. demonstrated that root canal surfaces treated with cationic antibacterial nanoparticles such as ZnO alone and a combination of ZnO and chitosan nanoparticles are able to significantly reduce *E. faecalis* adherence to dentine [80]. Further in vivo studies are required to determine whether such surface treatment could prevent bacterial recolonisation and biofilm formation.

4.3. Hydroxyapatite and other calcium phosphate-based systems

The application of nano-scaled HA particles has been shown to impact on oral biofilm formation and can also provide a remineralisation capability. Biomimetic approaches, based upon HA nanocrystals that resemble the structure at the nano-scale of abraded dental enamel crystallites, in theory should allow adsorbed particles to interact with bacterial adhesins, reduce bacterial adherence and hence impact on biofilm formation [81].

A number of oral healthcare products, including toothpastes and mouth rinses, have been developed containing nano-sized apatite particles with and without protein-based additives [82,83]. 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 stabilised by CPP [84]. Use of this technology has demonstrated anticariogenic activity both under in vitro and in vivo test 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 and promote remineralisation of enamel subsurface lesions [83]. Analysis of plaque samples demonstrated CPP-ACP nanocomplexes to be localised in plaque on the surface of bacterial cells and confirm earlier studies [85,86] that 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 colonisation as shown with CPP-ACP germanium-treated surfaces [82].

5. Incorporation of nanoparticles into polymeric materials for possible oral use

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. 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 nanoparticles to a polymer matrix to enhance its functionality [87]. This can be particularly effective in producing high-performance composites when optimum dispersion of the nanofiller is achieved, and the properties of such a filler can markedly enhance those of the matrix, e.g. 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 antimicrobial properties of dental polymeric materials, e.g. in denture materials [4] and orthodontic adhesives [69]. Lackovic et al. (unpublished observations) 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 <1% (w/v) was found not to alter the physical properties of the cement tested. However, no significant effect on either the attachment or growth of the cariogenic bacterium *S. 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 by

Ahn et al. clearly demonstrated that ECAs had rougher surfaces than conventional adhesives owing to the addition of silver nanoparticles [69]. 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 with regard to bond shear strength.

It is possible to enhance the properties of certain materials by encapsulation in a polymeric film, e.g. by encapsulating and modifying the surface properties of denture acrylic polymers with an inorganic silicone polymeric film [88] to prevent diffusion of food contaminates and bacteria as well as the ingrowth and adherence of *Candida* spp. hyphae that may lead to failure. 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 [89].

6. Photodynamic therapy and the use of nanoparticles to control oral biofilms

Photodynamic therapy is very well suited for the control of bacteria in oral plaque biofilms where there is relatively easy access for application of the photosensitising agent and light sources to areas requiring treatment [90]. Killing of micro-organisms with light depends upon cytotoxic singlet oxygen and free radical generation by excitation of a photoactivatable agent or sensitiser. The result of excitation is that the sensitiser moves from an electronic ground state to a triplet state, which then interacts with microbial components to generate cytotoxic species [91]. One of the advantages of light-activated killing is that resistance to the action of singlet oxygen is unlikely to become widespread in comparison with that experienced with more traditional chemical antimicrobial agents. The most commonly tested sensitisers on bacteria have been tricyclic dyes (e.g. methylene blue, erythrosine), tetrapyrroles (e.g. porphyrins) and furocoumarins (e.g. psoralen). Use of nanoparticles within this area is now receiving attention. 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 [92]. In theory, gold nanoparticle conjugates should have improved binding and cell wall penetration properties and so deliver a higher concentration of photoactive molecules. It remains to be fully established whether such conjugates will show increased antibacterial activity compared with more conventional treatments.

Most work on light-activated killing has been performed using suspensions of planktonic bacteria, with relatively few studies using micro-organisms grown as a biofilm. In vitro biofilm-grown *S. mutans* cells demonstrated a 3 log reduction when treated with erythrosine and white light (500–650 nm) [93], whilst an approach using antibody- and erythrosine-labelled nanoparticles has shown the potential for targeting specific bacterial species in oral plaque biofilms (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 caries-associated organisms in mixed biofilm cultures.

7. Biocompatibility of nanoparticles within the oral cavity

Although the development and application of nanotechnology are of considerable interest, knowledge regarding the possible toxicity of nanotechnology products to humans is limited [94]. To fully 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 [95]. In theory, certain nanoparticles may accumulate within the body 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. In vitro studies with lung epithelial cells, enterocytes and skin keratinocytes indicate marked cell-specific differences in susceptibility to metallic nanoparticles according to the cell type tested [96]. However, the surface chemistry of a particle, which in some cases can be modified, can determine whether it should be considered further for biomedical applications [21].

Toxicology and biodynamic studies suggest that silica, silicon and chitosan nanoparticles are relatively safe if introduced via the oral route [94]. 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 [77]. Similarly, QA-PEI nanoparticles incorporated into composite resins at 1% (w/w) demonstrated no additional toxic effects on cultured cells or experimental animal tissue in comparison with unmodified composites [73]. In comparison with other metals, silver is less toxic to human cells and is only ever used at very low concentrations in vivo [24].

The safe use of nanotechnology and the design of nanomaterials for biological applications involve a thorough understanding of the interface between these materials and biological systems [21]. 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 with regard to interaction with biological systems, whether mammalian or microbial, are chemical composition, surface function, shape and number of sides, porosity and surface crystallinity, size heterogeneity, roughness, and hydrophobicity or hydrophilicity [97].

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 both to mammalian and microbial interactions has been illustrated using surfactant-coated nanoparticles [98]. Anti-adherent and antifungal effects were shown using buccal epithelial cells treated with non-drug-loaded poly(ethyl cyanoacrylate) nanoparticles. Nanoparticles were prepared using emulsion polymerisation and were stabilised with cationic, anionic or non-ionic surfactants. Cationic surfactants, e.g. cetrimide, which are known antimicrobial agents, were the most effective in reducing *C. albicans* blastospore adhesion and demonstrated a growth-inhibitory and biocidal effect against the yeast. Production of nanoparticles with an anionic surfactant gave lower yields and wide particle size distributions, with no evidence of killing against *C. albicans*, whilst non-ionic surfactant-coated nanoparticles produced intermediate kill rates. Such 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.

In vivo screening of approximately 130 nanoparticles intended for therapeutic use has allowed detailed assessments with regard to biocompatibility [21]. It was shown that the main independent particle variables that 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 would be

Table 1
Nanoparticle cytotoxicity to mammalian cells.

Nanoparticle	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 [21].
ROS, reactive oxygen species.

rapidly removed by the reticuloendothelial system, were shown to be less toxic. Karlsson et al. [54] have shown that metal oxide nanoparticles are more toxic than at first envisaged at concentrations down to 40 μg/mL and show marked variation with regard to different nanoparticle species to cause cytotoxicity, DNA damage and oxidative DNA lesions. 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 to mammalian cells are listed in Table 1.

To help prevent aggregation of nanoparticles, stabilising (capping) agents that bind to the entire nanoparticle surface can be used; these include water-soluble polymers, oligosaccharides and polysaccharides, sodium dodecyl sulphate, PEG and glycolipids. The specific impact of surface capping, size scale and aspect ratio of ZnO particles upon antimicrobial activity and cytotoxicity has been investigated [99]. PEG-capped ZnO nanoparticles demonstrated an increase in antimicrobial efficacy with a reduction in particle size. However, such 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 hypothesised that the toxicity to eukaryotic cells is related to nanoparticle-enhanced apoptosis by upregulation of the Fas ligand on the cell membrane [99].

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 starting to be identified [21], 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 infections.

8. Conclusions

Application of nanoscaled 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. 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.

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