Republic of Iraq
Ministry of higher Education
and Scientific Research
Ashur University
College of dentistry

Nanoparticles in oral infection

A project submitted.

To the college of Dentistry, Ashur University in partial fulfillment of the requirements for the Degree of bachelor's in dental surgery (BDS).

By student

Batoul Qasim Abbas

Aya munther noman

Supervised by

Dr. Fatima Riad Badai

2025 A.D ***1446 A.H***

بسم الله الرحمن الرحيم

وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ
وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى
عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ
تَعْمَلُونَ

التوبة **(501)**

Certification of the Supervisor

I certify that this project entitled '**Nanoparticles in oral infection** 'was prepared by the fifth-year student **[Batoul Qasim Abbas, Aya munther noman]** under my supervision at the College of Dentistry/Ashur University in partial fulfilment of the graduation requirements for the bachelor's degree in Dentistry.

Supervisor's Name: ***Fatima Riad Badai***
Date

إهداء

واخر دعواهم ان الحمد لله رب العالمين

حمد لله الذي ما تم جهدا ولا ختم سعي إلا بفضلة وما سلكنا البدايات الا بتيسيره وما بلغنا النهايات الا بتوفيقة وما حققنا الغايات الا بفضله فالحمد الله حباً وشكراً وامتناناً الحمد لله على البدء والختام .

بكل ما أتينا من مشاعر الحب نهدي بحث تخرجنا ،

الى من احمل اسمه بكل فخر الى من دعمني منذ الصغر وأنار دربي لتحقيق حلمي الى من رباني وكافح من اجلي ستبقى كلماتك نجوم اهتدى بها اليوم وفي الغد والى الأبد ..

والدي العزيز

الى من غمرتني بالحب والحنان واشعرتني بالأمان الى من علمتني المبادئ والقيم قبل الاضيات الى من كان دعاؤها سر نجاحي الى من ارشدتني وساعدتني في النهوض كلما وقعت.

امي الغالية

الى تلك النجوم التي تنير طريقي دوما الى ملهمي نجاحي وصناع قوتي وصفوة ايامي الى من انتظروا قطاف ثمرة جهدي طويلاً وكانوا شركاء كل بسمة ودمعة وحسره الى احباب قلبي.

اخوتي

Acknowledgement

I thank God for His guidance, strength, and blessings in completing this research. I am deeply grateful for the support and encouragement from my mentors, whose valuable insights have greatly contributed to this work. I also appreciate my colleagues for their collaboration and assistance throughout this journey.

Finally, my heartfelt thanks go to my family and friends for their unwavering support, patience, and encouragement, which have been a constant source of motivation.

Table of Contents

The aim of study

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.

CHAPTER ONE

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 micro-organisms 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 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.

Biofilms and Oral Infections

Biofilms of oral bacteria and yeasts can cause a number of localised diseases in the oral cavity, including dental caries, periodontal disease, candidosis (‘oral thrush’), endodontic (‘tooth root and pulp disease’), orthodontic (‘dental braces’) and dental implant (‘titanium root’) infections [14].

1.2. Oral Biofilms and Disease

1.2.1. Dental Caries and Periodontal Disease

Dental caries is a destructive condition of the dental hard tissues that can progress to inflammation and death of vital pulp tissue, and if untreated it may lead to the eventual spread of infection to the periapical area of the tooth and beyond. The disease process involves acidogenic plaque bacteria, including *Streptococcus mutans*, *S. sobrinus* and *Lactobacillus* spp [15]. whereas periodontal diseases can involve both the soft and hard tissues and are initiated by components of the plaque biofilm that develop on the hard root surface adjacent to the soft tissues of the supporting periodontium. Periodontal disease may be

confined to the gingiva (gingivitis) or extend to the deeper supporting structures with destruction of the periodontal ligament and the alveolar bone that supports the teeth (periodontitis). This loss of attachment, with associated periodontal pocket formation, may ultimately lead to loosening and loss of the affected teeth. *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are now regarded as the major pathogens in advancing periodontitis [16]. The prevention of dental caries and periodontal diseases is traditionally targeted at mechanical or non-specific control of the plaque biofilm because this is the precipitating factor. The use of antimicrobial agents represents a valuable complement to mechanical plaque control [17]. 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 [18].

1.2.2. Peri-implantitis

Implant systems are increasingly being used to replace missing teeth, and most integrate with bone without complications. Small amounts of plaque consisting mainly of streptococci and *Actinomyces* spp. will accumulate on successful implants. However, in peri-implantitis, anaerobic Gram-negative organisms predominate [19]. This infection is a key cause of dental implant failure whereby the induced inflammatory changes in the soft tissues surrounding oral implants lead to a progressive destruction of the supporting bone (classified as peri-implantitis and seen in up to 43% of implant-treated subjects) or soft tissues (classified as peri-implantitis mucositis and seen in up to 50% of implant-treated subjects) [20]. Current forms of treatment are often inadequate and may result in chronic

infection requiring implant removal and costly resective and regenerative procedures in an attempt to restore and reshape the implant support [20]. The incorporation of nanoparticles into implant coatings may well offer useful osteoconductive and antimicrobial functionalities to prevent dental implant failure.

1.2.3. Candidosis

The development of candidosis, including denture stomatitis (chronic atrophic candidosis), which can affect up to 65% of edentulous individuals [21], involves the formation of a biofilm. Despite the use of antifungal drugs to treat denture stomatitis, infection can often become re-established. [21], using a poly (methyl methacrylate) (PMMA) biofilm model, demonstrated, that *C. albicans* biofilms are potentially highly resistant to the currently used antifungal agents, with resistance developing with time and showing a correlation with biofilm maturation.

1.3. Control of Oral Biofilms

Issues surrounding the uptake and penetration of antimicrobial agents into biofilms are key considerations in the administration of therapeutics [22] .This is of particular importance within the oral cavity when these agents have to reach fewer accessible stagnation sites or through plaque to the enamel. Thus, there remains an interest in the development of plaque control measures that require a minimum of public compliance and professional health care intervention [23]. Within this context, antimicrobial nanoparticles may be of particular value if retained at approximal teeth surfaces and below the gum margin. The anti-caries potential of fluoride and other more conventional antimicrobial/antiplaque agents, which are mostly deployed in mouthwashes and toothpastes, have been well characterised [24].

However, the potential of nanoparticles as constituents of topical agents to control oral biofilms through either their biocidal or anti-adhesive capabilities is now emerging as an area worthy of serious consideration. The studies by Robinson and co-workers using the 'Leeds in situ model', a device that allows dental plaque to develop in situ on a removable human enamel surface, have helped in the assessment of novel antimicrobial agents and take into account the very complex microbial composition and architecture of plaque biofilms [25]. The use of such intact biofilms on natural tooth surfaces would be of particular value to a study of the penetration of nanoparticles and released ions in situ. This model has indicated that plaque contains voids and channels, sometimes extending completely through the biomass to the underlying enamel [26].

1.4. Nanometals and the Control of Oral Infections

1.4.1. Nanometals as Antimicrobial Agents

Metals have been used for centuries as antimicrobial agents. Silver, copper, gold, titanium and zinc have attracted particular attention, each having different properties and spectra of activity. Many oral products, including toothpastes, now incorporate powdered (micron-sized) zinc citrate or acetate to control the formation of dental plaque [27]. Powdered titanium dioxide is also commonly used as a whitener in toothpastes.With respect to nanoparticles, the antimicrobial properties of silver [28] , and copper [29] including PMMA [30]and hydrogels [31].An inverse relationship between nanoparticle size and antimicrobial activity has been clearly demonstrated, where nanoparticles in the size range of 1–10 nm have been shown to have the greatest biocidal activity against bacteria [32-33].Indeed, it has been shown that smaller silver nanoparticles are

more toxic than larger particles, more so when oxidised [34].At the nanoscale, Ag+ ions are known to be released (leached) from the surface [35].

As a result of their small size, particular nanoparticles 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-target 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 microorganisms to resist their antimicrobial activity. Shape may also affect the activity of nanoparticles. Indeed, it has been demonstrated that the shape of silver nanoparticles can influence antimicrobial activity, as has been shown in the case of *Escherichia coli* [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, in particular those that produce reactive oxygen species under UV light, such as titanium dioxide and zinc oxide are finding increasing 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 bacteriocidal properties in filters and coatings on substrates such as polymers, ceramics, glasses and alumina [39]. Significant activity using nanoparticles and their compound clusters (as produced by thermal plasma technology) against fungal and bacterial

pathogens such as meticillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* has recently been demonstrated. These have also shown the capability to inactivate viruses, including SARS, H1N1 Influenza and H5N1 Bird Flu. For example, new broad-spectrum materials (5–60 nm) can reduce virus levels by 80–100% through direct or indirect contact. Nanoparticle preparations, including those based on nickel (Ni, NiO), zirconium (ZrO_2), copper (Cu, CuO, and Cu_2O), titanium (TiO_2), zinc (ZnO), aluminum (Al_2O_3), silicon(IV) nitride (Si_3N_4), silver (Ag), and tungsten carbide (WC) have been compared as regards their antimicrobial potential. Significant activity with Ag, ZnO, TiO_2 (in the presence of UV light), SiO_2, Cu, Cu_2O, and CuO against bacterial pathogens, including MRSA and *Pseudomonas aeruginosa*, has been demonstrated. Minimum bacteriocidal concentrations (MBC) were found to be in the range of 0.1–5 mg mL^{-1}. In comparison, traditional antibiotics are effective at concentrations 1,000-fold lower. NiO, Ni, Al_2O_3, TiO_2 (in the absence of UV light), Si_3N_4, WC, and ZrO_2 were found to lack antimicrobial activity at the concentrations tested. Ag and CuO nanoparticles also proved sensitive to the oral infections Streptococcus intermedius, Porphyromonasgingivalis, Fusobacterium nucleatum, Prevotella intermedia and Aggregatibacter [40].

1.4.2. Silver (Ag)

The antibacterial and antiviral actions of elemental silver, Ag+ ions, and silver compounds have been extensively investigated [41]. 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]. The use of silver salt nanoparticles instead of elemental silver or complex silver compounds to prevent biofilm formation on surfaces for both biomedical and more general use has been investigated. Using silver bromide precipitation to synthesise polymer–nanocomposites, surfaces were shown to resist biofilm formation. It was also shown to be possible, through controlling the size of the embedded AgBr, to modify the release of biocidal Ag+ ions [43].In comparison to conventional antimicrobials, surprisingly little is known about how nanoparticles behave in relation to microorganisms, particularly at the cellular level. The mechanism of the antimicrobial activity of silver is not completely understood, but is likely to involve multiple targets in comparison to the more defined targets of antibiotics. Studies have shown that the positive charge on the Ag+ ion is critical for antimicrobial activity, allowing the electrostatic attraction between the negative charge of the bacterial cell membrane and positively charged nanoparticles [44].In terms of the molecular mechanisms of inhibitory action of Ag+ ions on microorganisms, it has been shown that DNA loses its ability to replicate [45],and the expression of ribosomal subunit proteins and other cellular proteins and enzymes necessary for ATP production becomes inactivated [46].However, the precise mechanism(s) of biocidal activity of silver nanoparticles against bacteria remains to be fully elucidated [47]. demonstrated structural changes and damage to bacterial membranes resulting in cell death. These particular studies suggest that sulphur-containing proteins in the membrane or inside the cells and phosphorus-containing elements, such as DNA, are likely to be the preferential binding sites for silver nanoparticles. The contribution of Ag+ ion release from nanoparticles to the overall antimicrobial activity remains unclear. It is suggested that a bacterial cell in contact with silver nanoparticles will take up Ag+ ions, which possibly

in turn will inhibit respiratory enzymes and so help to generate free radicals, and subsequent free radical-induced damage to the cell membrane. In order to determine the relationship between free radical formation and antimicrobial activity, the use of antioxidants does suggest that free radicals may be derived from the surface of silver nanoparticles [48].

1.4.3. Copper (Cu)

Alongside silver, copper is a traditionally well-known antimicrobial material. In comparison to silver, relatively few studies have reported the antimicrobial properties of copper. It is suggested that copper may well have a similar mode of action to that of silver. However, it remains unclear as to the precise mechanism by which copper nanoparticles exert their antimicrobial activity. As with silver, it is thought that copper partly elicits its antimicrobial activity by combining with the –SH groups of key enzymes [49]. demonstrated superior antimicrobial activity with copper nanoparticles against *E. coli* and *Bacillus subtilis* when compared to silver nanoparticles. However, in the author's laboratory, silver consistently demonstrated superior activity to copper with a wide range of different species and strains [50].The antimicrobial properties of both silver and copper nanoparticles were also investigated [51]. using strains of *E. coli*, *B. subtilis*, and *S. aureus*. The bacteriocidal effect of the nanoparticles was compared using disc diffusion tests, and minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) determinations in batch cultures. Bacterial sensitivity was found to differ according to the species tested and the test system employed. For all strains of *S. aureus* and *E. coli*, the action of silver nanoparticles was found to be superior. Strain-specific variation for *S. aureus* was negligible, while some strain-specific variation was observed for *E. coli*.

A higher sensitivity, as shown with *B. subtilis*, may be attributed to more amine and carboxyl groups (in comparison to other species) on the cell surface, these groups having a greater affinity for copper [52].Released copper ions within the cell may then disrupt nucleic acid and key enzymes [53].In theory, a combination of silver and copper nanoparticles may give rise to a more complete bacteriocidal effect, especially against a mixed population of bacteria [54].

1.4.4. Gold (Au)

In comparison to silver and copper, gold generally shows a weak antimicrobial effect. However, gold nanoparticles are employed in multiple applications involving biological systems. The binding properties of gold are exceptional, and this makes it particularly suitable for attaching ligands to enhance biomolecular interactions. Gold nanoparticles also exhibit an intense colour in the visible range and contrast strongly for imaging by electron microscopy [55]. Despite all the current and potential applications for gold nanoparticles, there remains little information as to how these particles affect microorganisms. Growth inhibition studies, to measure the effect of gold nanoparticles (polyethylene glycol-coated to allow dispersion) on *E. coli* at various concentrations, demonstrated no significant activity [56].

1.5. 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 [57].However, certain metal oxides are now coming under close scrutiny because of their potential toxic effects [58].

1.5.1. Copper Oxide (CuO)

Copper oxide (CuO) is a semi-conducting compound with a monoclinic structure. CuO has attracted particular attention because it is the simplest member of the family of copper compounds and exhibits a range of potentially useful physical properties, such as high temperature superconductivity, electron correlation effects and spin dynamics[59-60].Limited information on the possible antimicrobial activity of nano CuO is available. Copper oxide is relatively cheap, easily mixed with polarised liquids (i.e. water) and polymers, and relatively stable in terms of both chemical and physical properties. Highly ionic nanoparticulate metal oxides, such as CuO, may be particularly valuable antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies [61].Copper oxide nanoparticles have been characterised, both physically and chemically, and investigated with respect to potential antimicrobial applications [62].It was found that nano-scaled CuO, as generated by thermal plasma technology, demonstrated particle sizes in the range 20–95 nm with a mean surface area of 15.7 $m^2 g^{-1}$. CuO nanoparticles in suspension showed activity against a range of bacterial pathogens, including MRSA and *E. coli*, with minimum bacteriocidal concentrations ranging from 0.1 to 5.0 mg mL^{-1}.

As with silver, studies of CuO nanoparticles incorporated into polymers suggest that release of ions may be required for optimum killing [62].

1.5.2. Zinc Oxide (ZnO)

The antimicrobial mechanisms of zinc are not completely understood. In recent years, nano-zinc oxide has received increasing attention, partly because it is stable under harsh processing conditions but also because it is generally regarded as safe to man [63].The proposed mechanisms of antibacterial activity include induction of reactive oxygen species [64-65] and damage to the cell membrane with subsequent interaction of the nanoparticle with the intracellular contents [66].

1.5.3. 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 [67].TiO_2 is widely used in a number of applications, as a powder and increasingly in a nanoparticulate form, and is generally considered to be non-toxic at the concentrations normally employed. However, there are recent concerns that nano-titanium oxide may present a hazard to health through inflammation as generated by release of IL-1α [68].The anatase form of nano TiO_2 and UV light excitation are required to ensure maximum antimicrobial activity. Such TiO_2 photocatalysis is able to promote the peroxidation of the polyunsaturated phospholipid component of the microbial lipid membrane, induce loss of respiratory activity, and elicit cell death [69].

1.6. Oral Applications of Nanoparticulate Metals and Metal Oxides

In order to reduce bacterial and fungal adhesion to oral materials and devices, silver nanoparticles are being investigated for a range of possible applications, for example, incorporation into denture materials {70] and orthodontic adhesives [71].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 [71] . demonstrated that experimental composite adhesives (ECAs) had rougher surfaces than conventional adhesives due to the addition of silver nanoparticles, although bacterial adhesion to ECAs was shown to be less than that to conventional adhesives and was not influenced by saliva coating. However, no significant difference between ECAs and conventional adhesives was shown as regards bond shear strength. Scanning electron micrograph of a fractured polymethyl methacrylate PMMA/Ag nanocomposite containing approximately 0.04% w/w silver. Distribution of silver particles in the PMMA acrylic resin shown. (**a**) *White areas* are agglomerated silver nanoparticles distributed in the PMMA (×828 magnification). (**b**) Silver nanoparticles (*white dots*) with approximate mean size 88 nm distributed in the PMMA matrix. (×50,000 magnification) [72].

1.7. Photodynamic Therapy and the Use of Nanoparticles to Control Oral Infections

Photodynamic therapy (PDT) is very well suited for the control of bacteria in oral plaque biofilms where there is relatively easy access for the application of the photosensitising agent and light sources to areas requiring treatment [73].This approach is now being utilised within the

clinical setting in some countries. The killing of microorganisms with light depends upon cytotoxic singlet oxygen and free radical generation by the excitation of a photo-activatable agent or sensitiser. The result of excitation is that the sensitiser moves from an electronic ground state to a triplet state that then interacts with microbial components to generate cytotoxic species [74].One of the advantages of light-activated killing is that resistance to the action of singlet oxygen is unlikely to become widespread in comparison to that experienced with more traditional chemical antimicrobial agents. A sensitiser ideally should absorb light at red to near-infrared wavelengths because these wavelengths are able to penetrate more. The most commonly tested sensitisers on bacteria have been tricyclic dyes (for example methylene blue, erythrosine), tetrapyrroles (for example porphyrins) and furocoumarins (for example psoralen). The use of nanoparticles within this area is now under investigation. For example, a complex of biodegradable and biocompatible poly(lactic-co-glycolic acid) (PLGA) and colloidal gold nanoparticles, loaded with methylene blue and exposed to red light at 665 nm, have been tested against planktonic *E. faecalis* and in experimentally infected root canals [75].In theory, gold nanoparticle conjugates should have improved binding and cell wall penetration properties, and so should deliver a higher concentration of photoactive molecules. It remains to be fully established whether such conjugates will show an increased antibacterial activity when compared to more conventional treatments.Most work on light-activated killing has been performed using suspensions of planktonic bacteria, with relatively few studies observing biofilm-grown microorganisms. In vitro biofilm-grown *Streptococcus mutans* cells demonstrated a 3-log reduction when treated with erythrosine and white light (500–650 nm) [76] ,These in vitro studies, employing constant-depth film fermenters with gold

nanoparticles conjugated to erythrosine and antibody to either *Streptococcus mutans* or *Lactobacillus casei*, have shown specific killing of target organisms in mixed-biofilm cultures.Considerations in relation to the therapeutic use of light-activated killing of biofilms on host surfaces include: (1) direct toxicity of the sensitiser, (2) indirect toxicity of the sensitiser in terms of 'by-stander' damage to adjacent host cells, (3) penetration into the biofilm, (4) light exposure time required to kill bacteria within in vivo biofilms and (5) widespread relatively non-specific bacterial killing [73].

1.8. Biocompatibility of Nano-Antimicrobials Within the Oral Cavity

Although the development and application of nanotechnology are of major importance in both industrial and consumer areas, knowledge regarding the possible toxicity of nanotechnology products to humans is limited. Whereas it is well known that copper in a non-nanoparticulate form is actively excreted from the normal body, non-nanoparticulate silver can accumulate within it. However the threat posed by these metals in a nanoparticulate form is far from clear [77].In order to understand the mechanism of toxicity, a thorough knowledge of the toxico-kinetic properties of nanoparticles is required. This includes information on the absorption, distribution, metabolism and excretion (ADME) of nanoparticles [78]. In theory, certain nanoparticles may be retained within the body for longer than is desirable, and thus the safety profile becomes a matter of overriding significance. Nanomaterials are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot. Nanomaterials can enter the blood stream following inhalation or ingestion, and some can even penetrate the skin. In vitro studies with lung epithelial cells, enterocytes and skin

keratinocytes indicate marked differences in susceptibility to metallic nanoparticles according to cell type tested (R.P. Allaker and M.A. Vargas-Reus, 2010). However, a particle's surface chemistry, which in some cases can be modified, can govern whether it should be considered further for biomedical applications [79].

1.9. Toxicity to Cells in the Oral Cavity

Toxicology and biodynamic studies suggest that silica, silicon, and chitosan nanoparticles are relatively safe if introduced via the oral route [80].Testing of NO-releasing silica nanoparticles (at the highest concentration tested of 8 mg mL^{-1}) with fibroblasts demonstrated that cell proliferation was inhibited to a lesser degree than with chlorhexidine [81].Likewise, quaternary ammonium poly(ethylene imine) (QA-PEI) nanoparticles incorporated into composite resins to restore teeth at 1% w/w demonstrate no additional toxic effects on cultured cells or experimental animal tissue in comparison to unmodified composites [82].In comparison to other metals, silver is less toxic to human cells, and is only ever used at very low concentrations in vivo [83]. For example, silver nanoparticles have been shown to inhibit *Candida* spp. at a concentration of 0.2 μg mL^{-1}, which is markedly less than the concentration (30 μg mL^{-1}) required to demonstrate a toxic effect against human fibroblasts [84].

1.10. Alteration of Biocompatibility and Desired Function

The safe use of nanotechnology and the design of nanomaterials for biological applications, including the control of oral biofilms, involve a complete understanding of the interface between these materials and biological systems [79]. The interface comprises three interacting components: (1) the surface of the nanoparticle, (2) the solid–liquid interface and the effects of the surrounding medium and (3) the contact zone with biological substrates , 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 [85].

1.11. 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 [85].

CHAPTER TWO

2.1. 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

References

1.Agarwal A, Weis TL, Schurr MJ, Faith NG, Czuprynski CJ, McAnulty JF, Murphy CJ, Abbott NL. Surfaces modified with nanometer-thick silver-impregnated polymeric films that kill bacteria but support growth of mammalian cells. Biomaterials. 2010;31:680–690. doi: 10.1016/j.biomaterials.2009.09.092.

2.Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater. 2009;25:206–213. doi: 10.1016/j.dental.2008.06.002

3.Allaker RP, Douglas CWI. Novel anti-microbial therapies for dental plaque-related diseases. Int J Antimicrob Agents. 2009;33:8–13. doi: 10.1016/j.ijantimicag.2008.07.014.

4.Allaker RP, Hardie JM. Topley and Wilson's Microbiology and Microbial infections. 9. London: Arnold; 1998. Oral Infections; pp. 373–390.

5.Allaker RP, Ren G. Potential impact of nanotechnology on the control of infectious diseases. Trans R Soc Trop Med Hyg. 2008;102:1–2. doi: 10.1016/j.trstmh.2007.07.003.

6.Almaguer-Flores A, Ximenez-Fyvie LA, Rodil SE. Oral bacterial adhesion on amorphous carbon and titanium films: Effect of surface roughness and culture media. J Biomed Mater Res B Appl Biomater. 2010;92:196–204. doi: 10.1002/jbm.b.31506

7.Aydin Sevnic B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. J Biomed Mater Res B Appl Biomater. 2010;94:22–31. doi: 10.1002/jbm.b.31620.

8.Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Disk. 2003;9(Suppl 1):23–29. doi: 10.1034/j.1601-0825.9.s1.5.x.

9.Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol. 2008;42:4133–4139. doi: 10.1021/es7032718.
10.Beveridge TJ, Murray RGE. Sites of metal deposition in the cell wall of Bacillus subtilis. J Bacteriol. 1980;141:876–878. doi: 10.1128/jb.141.2.876-887.1980.
11.Blake DM, Maness P-C, Huang Z, Wolfrum EJ, Jacoby WA, Huang J. Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. Separation and Purification Methods. 1999;28:1–50. doi: 10.1080/03602549909351643.
12.Boldyryeva H, Umeda N, Plaskin OA, Takeda Y, Kishimoto N. High-fluence implantation of negative metal ions into polymers for surface modification and nanoparticle formation. Surf Coat Tech. 2005;196:373–377. doi: 10.1016/j.surfcoat.2004.08.159 .
13.Bragg PD, Rainnie DJ. The effect of Ag+ ions on the respiratory chain of E. coli. Can J Microbiol. 1974;20:883–889. doi: 10.1139/m74-135.
14.Marsh PD, Martin MV. Oral Microbiology. 5. Edinburgh: Churchill Livingstone; 2009.

15.Hardie JM. Oral microbiology: current concepts in the microbiology of dental caries and periodontal disease. Brit Dent J. 1992;172:271–278. doi: 10.1038/sj.bdj.4807849.
16.Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol. 2000;27:648–657.
17.Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Disk. 2003;9(Suppl 1):23–29.

18.van der Ouderaa FJ. Anti-plaque agents. Rationale and prospects for prevention of gingivitis and periodontal disease. J Clin Periodontol. 1991;18:447–454.

19. Allaker RP, Hardie JM. Topley and Wilson‟s Microbiology and Microbial infections. 9. London: Arnold; 1998. Oral Infections; pp. 373–390.

20.Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol. 2008;35:286–291.

21.Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol. 2001;183:5385–5394.

22.Stewart PS. Diffusion in biofilms. J Bacteriol. 2003;185:1485–1491. doi: 10.1128/JB.185.5.1485-1491.2003.

23.Wilson M. Susceptibility of oral bacterial biofilms to antimicrobial agents. J Med Microbiol. 1996;44:79–87.

24.Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Disk. 2003;9(Suppl 1):23–29.

25.Watson PS, Pontefract HA, Devine DA, Shore RC, Nattress BR, Kirkham J, Robinson C. Penetration of fluoride into natural plaque biofilms. J Dent Res. 2005;84:451–455.

26. Wood SR, Kirham J, Marsh PD, Shore RC, Nattress B, Robinson C. Architecture of intact natural human plaque biofilms studied by confocal laser scanning microscopy. J Dent Res. 2000;79:21–27.

27.Giersten E. Effects of mouth rinses with triclosan, zinc ions, copolymer, and sodium lauryl sulphate combined with fluoride on acid formation by dental plaque in vivo. Caries Res. 2004;38:430–435.

28.Sondi I, Salopek-Sondi B. Silver nanoparticles as an antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004;275:177–182.

29.Cioffi N, Torsi L, Ditaranto N, Sabbatini L, Zambonin PG, Tantillo G, et al. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chem Mater. 2005;17:5255–5262.

30.Boldyryeva H, Umeda N, Plaskin OA, Takeda Y, Kishimoto N. High-fluence implantation of negative metal ions into polymers for surface modification and nanoparticle formation. Surf Coat Tech. 2005;196:373–377.

31.Lee WF, Tsao KT. Preparation and properties of nanocomposite hydrogels containing silver nanoparticles by ex situ polymerization. J Appl Poly Sci. 2006;100:3653–3661.

32.Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16:2346–2353.

33.Verran J, Sandoval G, Allen NS, Edge M, Stratton J. Variables affecting the antibacterial properties of nano and pigmentary titania particles in suspension. Dyes and Pigments. 2007;73:298–304.

34. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PKH, Chiu JF, Che CM. Silver nanoparticles: partial oxidation and antibacterial activities. J Biol Inorg Chem. 2007;12:527–534.

35.Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol. 2008;42:4133–4139.

36.Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3:95–101.

37. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. Appl Environ Microbiol. 2007;73:1712–1720.

38.Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ. Interaction of silver nanoparticles with HIV-1. J Nanobiotechnol. 2005;3:6.

39. Han J, Chen L, Duan S, Yang QX, Yang M, Gao C, Zhang BY, He H, Dong XP. Efficient and quick inactivation of SARS coronavirus and other microbes exposed to the surfaces of some metal catalysts. Biomed Environ Sci. 2005;18:176–180.

40.Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents. 2009;33:587–590.

41.Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents. 2009;34:103–110. doi: 10.1016/j.ijantimicag.2009.01.017.

42.Herrera M, Carrion P, Baca P, Liebana J, Castillo A. In vitro antibacterial activity of glass-ionomer cements. Microbios. 2001;104:141–148.

43. Sambhy V, MacBride MM, Peterson BR, Sen A. Silver bromide nanoparticle/polymer composites: dual action tunable antimicrobial materials. J Am Chem Soc. 2006;128:9798–9808. doi: 10.1021/ja061442z.

44.Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH.

Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3:95–101.

45. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TM, Kim JO. A mechanistic study of the antibacterial effect of Ag+ ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res. 2000;52:662–668. doi: 10.1002/1097-4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3.

46.Yamanaka M, Hara K, Kudo J. Bactericidal actions of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl Environ Microbiol. 2005;71:7589–7593. doi: 10.1128/AEM.71.11.7589-7593.2005. 47.Sondi I, Salopek-Sondi B. Silver nanoparticles as an antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004;275:177–182.

48. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3:95 .

49.Yoon KY, Byeon JH, Park JH, Hwang J. Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles. Sci Tot Environ. 2007;373:572–575. doi: 10.1016/j.scitotenv.2006.11.007.

50.Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents. 2009;33:587–590.

51.Ruparelia JP, Chatterje AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomaterialia. 2008;4:707–716. doi: 10.1016/j.actbio.2007.11.006.

52.Beveridge TJ, Murray RGE. Sites of metal deposition in the cell wall of Bacillus subtilis. J Bacteriol. 1980;141:876–878.

53.Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Rad Biol Med. 1995;18:321–336. doi: 10.1016/0891-5849(94)00159-H.

54.Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents. 2009;33:587–590. doi: 10.1016/j.ijantimicag.2008.12.004.

55.Lin C, Yeh Y, Yang C, Chen C, Chen G, Chen CC, Wu YC. Selective binding of mannose-encapsulated gold nanoparticles to type I pili in Escherichia coli. J Am Chem Soc. 2002;13:155–168. doi: 10.1021/ja0200903.

56.Williams DN, Ehrman SH, Pulliman Holoman TR. Evaluation of the microbial growth response to inorganic nanoparticles. J Nanobiotech. 2006;4:3. doi: 10.1186/1477-3155-4-3.

57.Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir. 2002;18:6679–6686. doi: 10.1021/la0202374.

58.Karlsson HL, Cronholm P, Gustafsson J, Moller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol. 2008;21:1726–1732.

59.Cava RJ. Structural chemistry and the local charge picture of copper oxide superconductors. Science. 1990;247:656–662.

60.Tranquada JM, Sternlieb BJ, Axe JD, Nakamura Y, Uchida S. Evidence for stripe correlations of spins and holes in copper oxide superconductors. Nature. 1995;375:561. 61.Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir. 2002;18:6679–6686. doi: 10.1021/la0202374.

62.Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents. 2009;33:587–590. doi: 10.1016/j.ijantimicag.2008.12.004.

63. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir. 2002;18:6679–6686. doi: 10.1021/la0202374.

64. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J Microbiol Methods. 2003;54:177–182.

65.Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Letters. 2008;279:71–76. doi: 10.1111/j.1574-6968.2007.01012.x.]

66. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fievet F. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Letters. 2006;6:866–870.

67.Blake DM, Maness P-C, Huang Z, Wolfrum EJ, Jacoby WA, Huang J. Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. Separation and Purification Methods. 1999;28:1–50.

68.Yazdi AS, Guarda G, Riteau N, Drexler SK, Tardivel A, Couillin I, Tschopp J. Nanoparticles activate the NLR pyrin domain containing 3 (NIrp3) inflammasome and cause pulmonary inflammation through release of IL-1α and IL-1β. PNAS. 2010;107:19449–19454. doi: 10.1073/pnas.1008155107. [DOI] [PMC free article] [PubMed] [Google Scholar]

69. Maness PC, Smolinski S, Blake DM, Huang Z, Wolfrum EJ, Jacoby WA. Bacteriocidal activity of photocatalytic TiO2 reaction: toward an understanding of its killing mechanism. Appl Envirol Microbiol. 1999;65:4094–4098.

70.Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents. 2009;34:103–110. doi: 10.1016/j.ijantimicag.2009.01.017.

71.Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater. 2009;25:206–213.

72.Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents. 2009;34:103–110.

73.Allaker RP, Douglas CWI. Novel anti-microbial therapies for dental plaque-related diseases. Int J Antimicrob Agents. 2009;33:8–13.

74.MacRobert AJ, Bown SG, Phillips D. What are the ideal photoproperties for a sensitizer? Ciba Found Symp. 1989;146:4–12.

75.Pagonis TC, Chen J, Fontana CR, Devalapally H, Ruggiero K, Song X, Foschi F, Dunham J, Skobe Z, Yamazaki H, Kent R, Tanner AC, Amiji MM, Soukos NS. Nanoparticle-based endodontic antimicrobial photodynamic therapy. J Endod. 2010;36:322–328.

76.Wood S, Metcalf D, Devine D, Robinson C. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. J Antimicrob Chemother. 2006;57:680–684.

77.Seetharam RN, Sridhar KR. Nanotoxicity: Threat posed by naoparticles. Curr Sci. 2006;93:769–770.

78.Hagens WI, Oomen AG, de Jong WH, Cassee FR, Sips AJ. What do we (need to) know about the kinetic properties of nanoparticles in the body? Reg Toxicol Pharmacol. 2007;49:217–229.

79.Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nature Mater. 2009;8:543–557.

80. Seetharam RN, Sridhar KR. Nanotoxicity: Threat posed by naoparticles. Curr Sci. 2006;93:769–770.

81.Seetharam RN, Sridhar KR. Nanotoxicity: Threat posed by naoparticles. Curr Sci. 2006;93:769–770.

82.Yudovin-Farber I, Beyth N, Nyska A, Weiss EI, Golenser J, Domb AJ. Surface characterization and biocompatibility of restorative resin containing nanoparticles. Biomacromolecules. 2008;9:3044–3050. doi: 10.1021/bm8004897.

83. Sondi I, Salopek-Sondi B. Silver nanoparticles as an antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004;275:177–182.

84.Panacek A, Kolar M, Vecerova R, Prucek R, Soukupova J, Krystof V, Hamal P, Zboril R, Kvitek L. Antifungal activity of silver nanoparticles against Candida spp. Biomaterials. 2009;30:6333–6340.

85.Nel A, Xia T, Madler I, Li N. Toxic potential of materials at the nanolevel. Science. 2006;311:622–627.