International Journal of Nanomedicine

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REVIEW

Toxicology of antimicrobial nanoparticles for prosthetic devices

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
20 August 2014
Number of times this article has been viewed

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Abstract: Advances in nanotechnology are producing an accelerated proliferation of new nanomaterial composites that are likely to become an important source of engineered health-related products. Nanoparticles with antifungal effects are of great interest in the formulation of microbicidal materials. Fungi are found as innocuous commensals and colonize various habitats in and on humans, especially the skin and mucosa. As growth on surfaces is a natural part of the *Candida* spp. lifestyle, one can expect that *Candida* organisms colonize prosthetic devices, such as dentures. Macromolecular systems, due to their properties, allow efficient use of these materials in various fields, including the creation of reinforced nanoparticle polymers with antimicrobial activity. This review briefly summarizes the results of studies conducted during the past decade and especially in the last few years focused on the toxicity of different antimicrobial polymers and factors influencing their activities, as well as the main applications of antimicrobial polymers in dentistry. The present study addresses aspects that are often overlooked in nanotoxicology studies, such as careful time-dependent characterization of agglomeration and ion release.

Keywords: cytotoxicity, oxidative stress, genotoxicity, antifungal effect, denture bases, dentistry

Nanotechnology and health

Nanotechnology has become a major focus in scientific research efforts. Nanotechnology has been used in various fields of science and technology, including physics, electronics, medicine, and chemistry, among other areas of interest to the scientific community. Interest in nanotechnology is growing and continues to change the way we perceive and execute things, has a pronounced effect on therapeutics, and is shaping our ever-evolving society and influencing our daily lives.[1–4]

Nanotechnology has contributed to the improvement in materials used in medicine, as it can provide better functionality, mainly due to the nanometric sizes involved (eg, silver nanoparticles [AgNPs] that exhibit different properties once they are applied to biological systems, compared with traditional systems of treatment). The nanoscale endows the materials with the ability to penetrate into different biological membranes, such as bacterial cell walls, thereby increasing bactericidal effects; there are numerous examples of such applications in gene and drug delivery.[5–8]

The nanoscale range (1–100 nm) used to describe nanoparticles should not be considered strict, due to the variations that may exist in the nanoparticle shape or the appearance of nanoscale properties in particles slightly above or below the nanoscale limits. This can include other important properties, such as shape, surface area-to-mass ratio, and composition.[9]

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Nanoparticles are being explored extensively because of their size-related chemical and physical properties. The size of nanoparticles is similar to that of most biological molecules and structures, making them interesting candidates for applications in both in vivo and in vitro biomedical research. The results of their integration into the field of medicine have led to their applications, mainly in targeted drug delivery, imaging, sensing, and artificial implants. Another interesting opportunity within the field of medicine is the use of nanoparticles as carriers for antimicrobials to target highly pathogenic and drug-resistant microorganisms. However, for the application of nanoparticles in biology, biocompatibility is a greatly desired characteristic. Biocompatibility is a material's ability to perform medically without producing undesired local or systemic effects.[10]

The flip side of the benefits of nanoparticle use in medicine is their potential toxicity. Nanoparticles react differently when administered in various environments.[11] The observation of dose-related responses is important, because these responses help to determine the appropriate amount of nanoparticles to administer, in terms of median toxicity and the limits for human exposure in order to prevent any side effects.[12]

Nanoparticles may have different effects on human health relative to the bulk material from which they have been produced.[13] Some nanoparticles are small enough to be able to access skin, lungs, and the brain. Currently, no sufficient information is available on the adverse effects of nanoparticles on human health.[14–16]

Toxicity of nanoparticles

The in vitro testing methods used to evaluate nanoparticle toxicity have revealed the general and biological properties of the materials as they acquire a nanoscale structure and result in the formation of nanoparticles, thereby leading to tremendous applications in therapeutics.

Nanoparticles can cross membrane barriers through transcytosis, which facilitates the function of a drug that is applied to these nanoparticles, by means of hydrophilic surfactants like Tween® 80 for targeted actions.[17] Several studies have also shown that the interaction between cells and nanoparticles results in DNA damage, causing cancer and developmental toxicity that leads to further growth retardation, malformation, or death of embryos.[18]

Traditional in vitro assays of nanoparticles can lead to misrepresentation of cellular uptake data, and the results are not always dependable. The shape of nanoparticles is an important characteristic that must be considered when assessing toxicological effects. Some particles may exist in different shapes, eg, carbon nanotubes are considered potentially toxic due to their resemblance to asbestos and other carcinogenic fibers; they are also graphitic and therefore are expected to be biologically persistent in the body.[12,19,20] Several factors such as dose, exposure time, size, shape, surface chemistry, and cell type play important roles in mediating cellular responses when nanoparticles are administered to cells.[21] One study evaluated the cell toxicity effect after nanoparticle administration, taking into account the size and dose administered, significant cell toxicity was only evident for 10 nm citrate-coated ($P \leq 0.05$) and 10 nm polyvinylpyrrolidone-coated ($P \leq 0.01$) AgNPs after 24 hours at their highest doses (50 μg/mL); however, no significant alterations of the mitochondrial activity of the BEAS-2B cells (human lung cells) were observed for any of the lower doses (5 and 10 μg/mL) of the AgNPs.[22] Liu et al found that 5 nm AgNPs were more toxic than 20 and 50 nm AgNPs in four cell lines (A549, HepG2, MCF-7, SGC- 7901), indicating a size-dependent effect on cell viability.[23]

Oxidative stress

Previous studies on the toxicity of nanoparticles have related their effects to oxidative stress[24,25] and shown that nanoparticles can provoke oxidative stress and inflammatory responses in the nervous system, as they travel along dendrites and axons.[18]

Specifically, in vitro studies of AgNP toxicity have shown an increase in the production of reactive oxygen species (ROS), oxidative stress, mitochondrial damage, DNA damage, and cytotoxicity in BRL 3A rat liver cells, human liver cells, THP-1 monocytes, human alveolar epithelial cells (A549), human mesenchymal stem cells, human fibroblasts, and glioma cells.[16,26–32]

Recent reports have suggested that many nanomaterials, especially nanometal oxides, generate O_2^-, a ROS in biological systems.[15,33–36]

Cell viability (cytotoxicity)

Viability and proliferation assays are based on the ability of mitochondrial dehydrogenase enzymes, present in living cells, to reduce tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to an insoluble, purple formazan product or 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) to a water-soluble, orange formazan product.[37,38]

Toxicity testing of engineered nanomaterials, including nano-TiO_2, has generated many publications in the last

several years. Conflicting cell viability and proliferation results have been reported from these studies when based on the MTT assay.[39–42] Nano-TiO_2 increases the formation of ROS in different mammalian cells.[43,44] It is known that the redox potential of tetrazolium salts is highly dependent on their structure and substituents.[45,46] In this sense, MTT and XTT assays may underestimate a compound's cytotoxicity by overestimating cell viability. Thus, it is safe to assume that MTT, XTT, water-soluble tetrazolium salts, and other tetrazolium dyes currently used for toxicity testing may have different reaction rates with superoxide. Depending on the concentration of superoxide dismutase or other molecules that also react with superoxide in cells, these competitive reactions may well influence estimates and interpretations of viability and proliferation. Therefore, tetrazolium dye assays for determination of cell viability and cell proliferation may be affected under experimental conditions that influence the level of O_2 in a biological system.

Acosta-Torres et al summarized common in vitro biologic assays that are used to evaluate a nanocompound material's influence on proliferation, viability, genotoxicity, and oxidative stress.[47] Two or more assays should be used to analyze possible toxicological effects of nanocompounds.

Antimicrobial nanoparticles

In general, various salts of silver and their derivatives have been used as antimicrobial agents; these materials have also been studied as media for antibiotic delivery and use in synthesis of composites for use as disinfecting filters and coating materials.

Previously we described reports by some authors who described that, under certain conditions, nanoparticles were toxic in a diverse variety of cells. Recent studies have reported that both AgNPs and metal oxide alginate forms (eg, TiO_2 and Fe_2O_3) exhibit antifungal properties when they are joined into the polymer formulation, eg, polymethyl methacrylate (PMMA), a versatile polymer used in dentistry and for which biocompatibility and non-genotoxicity have been demonstrated.[48–50] Some other dental applications of TiO_2 nanoparticles have been reported in addition to a conventional glass ionomer (3% [weight/weight]) to improve mechanical and antibacterial properties. These new materials will be used for higher stress-bearing site restorations.[51]

Growing evidence indicates that *Candida albicans* and other *Candida* spp. are able to adhere to acrylic resin dentures (PMMA); this capability predisposes some denture wearers to health problems and can also require a prolonged treatment period for candidiasis.[52]

Microbial adhesion on biomaterial surfaces depends on the surface structure and the composition of biomaterials, as well as on the physicochemical properties of the microbial cell surface; so that, AgNPs, have been proposed as antimicrobial agents in polymeric materials. Antibacterial, antiviral, and antifungal effects have been reported for AgNPs, which are broadly used as antimicrobial agents in polymeric nanocomposites.[49,52]

Other nanoparticles used for *C. albicans* are the ZnO nanoparticles. A concentration-dependent effect of ZnO on the viability of *C. albicans* has been observed.[15] The minimal fungicidal concentration of ZnO was found to be 0.1 mg/mL − 1 ZnO; this concentration caused an inhibition of over 95% in the growth of *C. albicans*.[15]

Factors related to development of *Candida*

Commonly used biomaterials exhibit significant differences in surface free energy, ie, the interaction between the forces of cohesion that determine whether or not wetting will occur. Data have shown that the higher the adherence tendency to various base resin materials, the lower the value for the free energy change in tests with both *C. albicans* and *Candida tropicalis*.[53] Surface roughness directly influences initial microorganism adherence to surfaces, biofilm development, and Candida spp. colonization.[54] Surface roughness is calculated as the arithmetic average deviation of the surface valleys and peaks of a given surface. It directly influences initial microorganism adherence to surfaces, biofilm development, and *Candida* spp. colonization.[54] Materials with rougher surfaces usually exhibit higher yeast counts. This happens because surfaces can serve as a reservoir, with surface irregularities providing an increased chance of microorganism retention and protection from shear forces, even during denture cleaning. In addition, these irregularities sometimes allow time for the entrapped microbial cells to attach irreversibly to a surface.

The antibacterial activity of different metal nanoparticles, such as silver colloids, is closely related to their size; that is, the smaller the silver nuclei, the higher the antibacterial activity. Specific controls of shape, size, and size distribution are often achieved by varying the methods and reducing agents and stabilizers used during synthesis.[55]

The interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. The interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research focused on the bactericidal effects; due to the rapid increase in microbes that are resistant to conventionally used antibiotics.[56]

Inorganic nanoparticles (ZnO, iron oxide, TiO_2) and metal nanoparticles (silver, gold, iron, copper, magnesium) have good antibacterial activities. The size of a metallic nanoparticle ensures that a significantly large surface area of the particle is in contact with the bacterial effluent. Synthesis and characterization of nanoscale materials in terms of novel physicochemical properties are of great interest in the formulation of bactericidal materials. Additionally, it appears that bacteria are far less likely to acquire resistance to metal nanoparticles compared to other conventional and narrow-spectrum antibiotics.

Nanoparticles in dental applications

There are major challenges to overcome in the area of preventive dentistry, such as tooth decay, treatment of injuries, and cavities in teeth. Nowadays, different research laboratories have developed solutions and pastes that are composed of nanometric materials for tissue regeneration in healing these dental lesions.[57–59]

Beyth et al described the incorporation of quaternary ammonium polyethyleneimine nanoparticles in a resin composite with antimicrobial effect against a wide range of bacteria with no apparent negative effect on biocompatibility.[60] Quaternary ammonium polyethyleneimine nanoparticles have a strong bactericidal activity against *Streptococcus mutans*, and a wide variety of microorganisms rapidly killing bacterial cells when incorporated at small concentrations into restorative composites.[60]

AgNPs are well known for their use in wound dressings and catheters, as well as in various processes involving their antimicrobial potential; nevertheless, the size-dependent toxicity of AgNPs is an expanding field of research. The size of an AgNP is decided upon the basis of its biomedical application. The cytotoxic effects of AgNPs were observed when administered directly in several cell lines, including MC3T3-E1 and PC12 cells, as they induce cellular death when the AgNPs are 10 nm in size, and the toxicity is greater than that seen with AgNPs that are 50–100 nm.[61,62] Additionally, several studies have indicated primary size- and agglomeration-dependent toxic effects of engineered nanoparticles. A comparison of large and small particles/agglomerates revealed greater toxicity for smaller particles/agglomerates that were at least partly due to differences in cellular uptake.[63–66]

With the myriad of postulated physicochemical characteristics of engineered nanoparticles that may impact toxicity, it is paramount for risk assessments to determine the concentration range at which well-characterized nanoparticles of varying composition, size, and surface coatings can cause early cellular perturbations, such as an oxidative stress response. Particularly for AgNPs, for which it has been widely shown that silver ions (Ag+) can induce toxicity, a comparison between the insoluble nanoparticle form and soluble ionic form of silver should focus on characterizing the biological effects of exposure and the impacts of differences in solubility, cellular uptake, particle size, and particle coating.

One subject of debate with respect to AgNPs is the mechanism of their toxicity, specifically, whether ionic silver is the only cause of the toxicity of AgNPs or whether nanoparticles have toxicities distinct from those of ionic silver.[67–72]

Sivolella et al reviewed potential clinical applications in alveolar bone and dental implant surgery of AgNP-based devices.[73] The authors suggested that AgNPs may be an alternative strategy for reducing bacterial adhesion and preventing biofilm formation, despite AgNPs having exhibited some toxic effects in in vitro and in vivo studies. They also proposed that clinical trials should be undertaken to determine suitability for AgNP applications.

Table 1 summarizes the different metal and metal oxide nanoparticle polymer formulations now in development and their demonstrated antimicrobial effects.

Future trends for antimicrobial denture products

Base materials have been developed in order to reduce and redistribute occlusal forces from dentures that might damage the underlying mucosal tissues; in this sense, the use of denture liners, either hard or soft, has increased.[84] Liners are needed in many clinical situations in which patients have thin, sharp, or badly resorbed residual alveolar ridges or chronic tissue irritation from dentures; even though, these liner materials exhibit excellent tissue tolerance, one associated problem is colonization by *Candida* spp. on and within the material.[85]

Fungal growth on the surface of a liner, may lead to irritation of the oral tissues; these negative effects form the rationale for attempts to incorporate antifungal agents in these materials.[86] The use of antimicrobial nanoparticles in dentistry is promising, but effective monitoring strategies still remain to be established concerning the toxicity of such materials.[87]

Acknowledgments

The authors thank the following DGPA-UNAM supporting programs: PAPIME-PE202214 and PAPIIT-TA200414. Victor M Castaño is on sabbatical leave at CIATEQ-Queretaro.

Table 1 Different metal and metal oxide nanoparticles added to polymer formulations increase their antimicrobial effect

Nanoparticle material	Antimicrobial characteristics	Applications	Toxicity	References
Titanium dioxide	Nanosized structured TiO_2 have exhibited antimicrobial properties, due to TiO_2-induced photocatalytic production of cytotoxic oxygen radicals. Previous reports have described the use of TiO_2 for water treatment and air purification. Recent reports have mentioned usage of TiO_2 nanoparticles to decrease *Candida albicans* adherence in an experimental PMMA for dentures.	Titanium nanoparticles have been applied in the pharmaceutical industry as drug delivery vehicles and in excipient formulations.	The composite material combines the high adsorption capability of apatite with the photocatalytic activity of titanium. Apatite coatings may thus become useful in the attenuation of the toxicological effects of inorganic metal oxide nanoparticles.	49,74–78
Iron oxide	Ferrite nanoparticles may be a source of cellular toxicity. The hematite crystalline phase of Fe_2O_3 nanoparticles decreased *C. albicans* adherence in an experimental PMMA for dentures. Transition metal-substituted cobalt ferrite nanoparticles formed a cubic spinel structure with a crystallite size in the range of 40–50 nm, that significantly improved antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.	Used in cellular therapy, such as cell labeling and targeting, and as a tool for cell biology research to separate and purify cell populations. Also used in: • tissue repair; • drug delivery; • magnetic resonance imaging; • hyperthermia; • magnetofection.	No toxicity reported.	49,79,80
Silver	PMMA/AgNPs have been proposed for dentistry applications as cytocompatible dental materials with antifungal properties. Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications; it has been shown also to prevent HIV binding to host cells. Modified tissue conditioner combined with AgNPs displayed antimicrobial properties against *S. aureus*, *Streptococcus mutans*, and *C. albicans* incorporated after a 24- or 72-hour incubation.	Used for covering urinary catheters, surgical instruments, and bone prostheses. Additionally, silver has been used in water and air filtration to eliminate microorganisms. AgNPs have been added to soft tissue conditioners for prosthetic devices.	Exposure of metal-containing nanoparticles to human lung epithelial cells generates ROS, which can lead to oxidative stress and cellular damage (AgNPs). Silver nanowires resulted in the strongest cytotoxicity and immunological responses, whereas spherical silver particles had negligible effects on cells when tested in human cells.	21,25,50, 81–83

Abbreviations: AgNPs, silver nanoparticles; PMMA, polymethyl methacrylate; ROS, reactive oxygen species.

Disclosure

The authors report no conflicts of interest in this work.

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