

Perspectives for the use of silver nanoparticles in dental practice

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Nanotechnology has been used for medical applications in several forms, including dental practice with the development of silver nanoparticles (Ag NPs) as a useful tool. The aim of this review was to identify the properties and appliances of Ag NPs in dental practice. Silver compounds and NPs have already been used as dental restorative material, endodontic retrofill cements, dental implants and caries inhibitory solution. Despite the effectiveness that Ag NPs has showed in dental practice, Ag NPs remain a controversial area of research with respect to their toxicity in biological and ecological systems. Therefore any application of Ag NPs in dentistry requires more studies. In order to avoid the toxicity of these materials Ag NPs can be temporarily used in dentistry.

Key words: Silver nanoparticles, dental practice, applications of Ag NPs

INTRODUCTION

Nanotechnology, which concerns structures at the nanometer scale (1–100 nm), is considered as a vital current technology of the 21st century based on its economic and scientific potential. In 2008, the public expenditure of nanotechnology was 430 mmd compared to 25 mmd in 1997^{1,2}. Nanoparticles (NPs) have a greater surface-to-volume ratio (per unit mass) than non-nanoscale particles of the same material, and therefore are more reactive. Particles smaller than 50 nm are subject to the laws of quantum physics³.

In 2008 and 2009, silver production was 21 300 and 21 400 tons, respectively, according to United States Geological Survey (USGS)⁴. Over the years, silver compounds and NPs (Figure 1) have exhibited antibacterial activity resulting in the widespread use of silver nanoparticles (Ag NPs) in bedding, washing machines, water purification, toothpaste, shampoo and rinse, nursing bottles, fabrics, deodorants, filters, kitchen utensils, toys and humidifiers⁵. Furthermore, silver compounds and NPs⁶ have been studied for dental applications including dental restorative material⁷, endodontic retrofill cement⁸, dental implants⁹, and caries inhibitory solution¹⁰. The aim of this brief review was to

identify the properties and appliances of Ag NPs in dentistry practice.

ANTIMICROBIAL PROPERTIES

Most of the studies have indicated that silver interacts with sulfhydryl groups of proteins and with DNA, altering hydrogen bonding, respiratory processes, DNA unwinding, cell-wall synthesis and cell division^{11,12}. At the macro level, these interactions effectively produce bacterial death¹³.

It is recognized that Ag NPs have antimicrobial activity against Gram-negative bacteria performing 'pits' in the cell wall of the bacteria. Clearly, a membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell. Overall, silver mainly induces denaturation and oxidation for cell wall which lead to rupture of the internal cell organelles, resulting in bacterial death^{14,15}. Although bacterial cell lysis could be one of the reasons for the observed antibacterial property, NPs also modulate the phosphotyrosine profile of putative bacterial peptides, which could affect bacterial signal transduction and inhibit the growth of the organisms¹⁶.

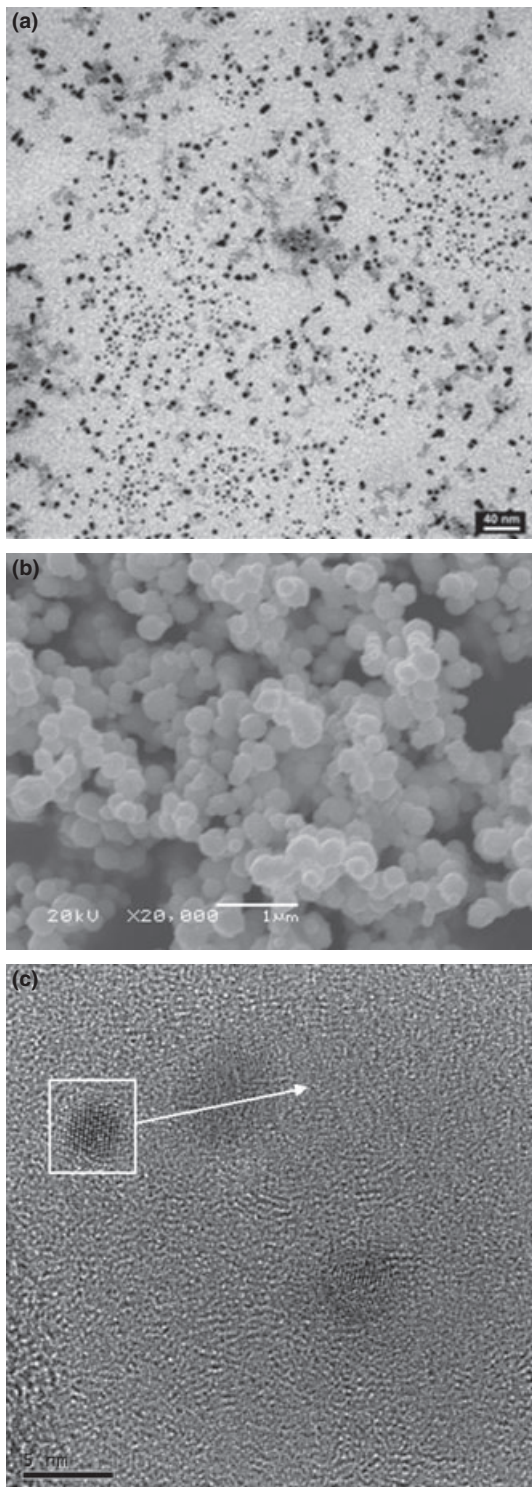


Figure 1. Scanning Electron Microscope views of Ag NPs. Illustration provided by Professor Raúl Alberto Morales Luckie, Faculty of Chemistry, UAEMex.

It is worth noting that the antibacterial activity of nanosilver is dominated by silver ions (Ag^+ ions) when fine Ag NPs (less than about 10 nm in average diameter) are employed that release high concentrations of these ions. In contrast, when relatively larger

Table 1 Antimicrobial properties of Ag NPs

Gram-positive bacteria	<i>Streptococcus sanguis</i> ⁶ <i>Bacillus subtilis</i> ³⁵ <i>Enterococcus faecalis</i> ³⁵ <i>Staphylococcus aureus</i> ³⁶
Gram-negative bacteria	<i>Escherichia coli</i> ³⁶ <i>Salmonella typhimurium</i> ³⁵ <i>Shigella dysenteriae</i> type 1 ³⁸ <i>Citrobacter sp</i> ³⁷ <i>Pseudomona aeruginosa</i> ³⁷ <i>Pseudomona fluorescens</i> ³⁸
Fungi	<i>Candida albicans</i> ³⁹ <i>Fusarium oxysporum</i> ³⁷
Virus	<i>Arenavirus</i> ⁴⁰ <i>Human immunodeficiency virus-1</i> ⁴² <i>Murine norovirus</i> ⁴¹ <i>Hepatitis B virus</i> ⁴²

Ag NPs are used, the concentration of the released Ag^+ ions is lower¹⁷. Likewise, Ag NPs with average size 14 ± 6 nm and Ag^+ ions such as AgNO_3 , inhibit the growth of *Escherichia coli* $55 \pm 8\%$ and 100%, respectively (Table 1 shows the antimicrobial effect of Ag NPs).

Furthermore, silver particles are also used as alternative radiopacifier to get the necessary radiopacity to calcium silicate cements (CSC) and assess the purity of the radiopacifying agents¹⁸. These nanomaterials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials¹³.

CARIES INHIBITORY PROPERTIES

The most common worldwide oral diseases are dental caries and periodontal diseases, 60–90%, according to the World Health Organization (WHO)¹⁹. In Mexico, some authors estimate that such diseases affect 90% and 70% of the population, respectively²⁰. In this regard, the use of silver solution, specifically, silver diamine fluoride ($\text{Ag}[\text{NH}_3] 2\text{F}$) has been used as caries inhibitor. In context, fluoride and silver interact synergistically to form fluorapatite. The first step is the formation of calcium fluoride (CaF_2) and silver phosphate (Ag_3PO_4) in a basic environment, the second reaction is the subsequent dissociation of calcium and fluoride²¹.

Experimental composite adhesives (ECAs) showed slower bacterial growth than those containing conventional adhesives, suggesting that ECAs can help prevent enamel demineralization around their surfaces without compromising physical properties²².

RESTORATIVE MATERIALS

Whereas restorative materials with a silver polymer compound have showed effective antimicrobial properties on implant components against *Streptococcus*

*sanguis*⁶, silver has been incorporated into glass ionomer cements in order to improve the antibacterial properties, including also, compressive, tensile strength and creep resistance.

Biofilms are surface-adherent populations of microorganisms consisting of cells, water and extracellular matrix material. Nanotechnology is a promising field of science which can guide our understanding of the role of interspecies interaction in the development of biofilm. *Streptococcus mutans* with other species of bacteria has been known to form dental biofilm. The correlation between genetically modified bacteria *Streptococcus mutans* and nanoscale morphology has been assessed using atomic force microscopy (AFMi). Occasionally, silver nanofibers have been attached to the implant surfaces to reduce the need of using high doses of antibiotics during the healing period, giving self-cleaning against plaque biofilm⁹.

THERAPEUTICS

Nanostructures of different sizes, shapes and material properties have many applications in biomedical imaging, clinical diagnostics and therapeutics. In spite of what has been achieved so far, a complete understanding of how cells interact with nanostructures of well-defined sizes, at the molecular level, remains poorly understood.

Gold and Ag NPs coated with antibodies can regulate the process of membrane receptor internalization. The binding and activation of membrane receptors and subsequent protein expression strongly depend on nanoparticle size. Although all NPs within the 2–100 nm size range alter signaling processes essential for basic cell functions (including cell death) 7, 40- and 50-nm NPs demonstrate the greatest effect. These results show that NPs should no longer be viewed as simple carriers for biomedical applications, but can also play an active role in mediating biological effects. These findings may assist in the design of nanoscale delivery and therapeutic systems and provide insights into nanotoxicity²³.

ADVERSE EFFECTS

Metal ions are released from casting alloys and cause damage to cell structures and local inflammation. Ag(NH₃)₂F in contact to Human Gingival Fibroblast (HGF) for only one hour induced irreversible necrosis cell death, whereas longer duration of contact with AgCl was necessary to induce this same effect. These data suggest the importance of cautious application of Ag (NH₃)₂F into the oral cavity²⁴. Ag(NH₃)₂F shows much more sensibility at dose-dependent ion against three normal cells and three cancer line cells than AgCl (Figure 2a,b).

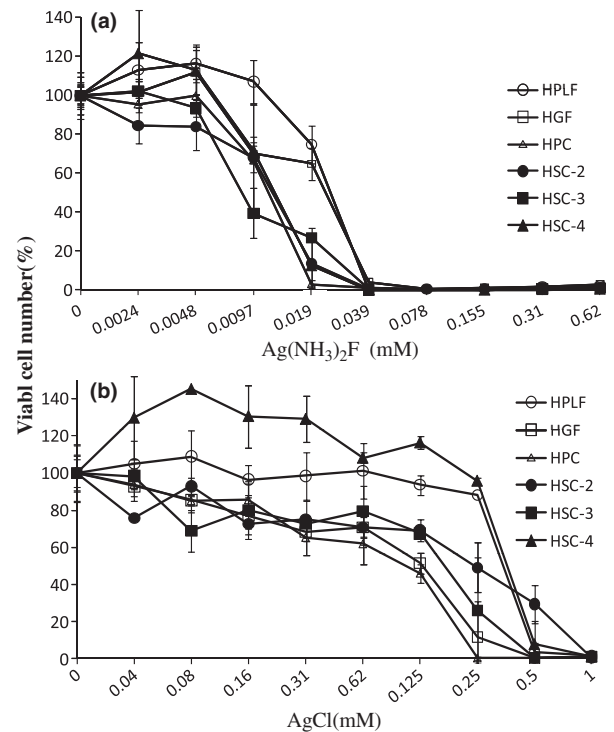


Figure 2. Cytotoxicity of Ag(NH₃)₂F and AgCl against six cell lines, respectively. HPLF, human periodontal ligament fibroblast cells; HGF, human gingival fibroblast cells; HPC, human pulp cells and human oral squamous cells carcinoma obtained from different patients (HSC-2, 3, 4) were incubated for 24 h with the indicated concentrations of metal and then the relative viable cell number was determined by MTT method. Each value represents the mean \pm S.D. of triplicate assays. Reproducible results were obtained in further two independent experiments.

Nanotoxicity is the toxicity imposed by nanomaterials²⁵. The toxic effects of Ag NPs are proportional to the activity of free Ag⁺ ions released by the NPs²⁶. Although NPs have tremendous potential for a host of applications, their adverse effects on living cells have raised serious concerns for their use in the healthcare and consumer sectors. For example, NPs may be taken up directly into the brain by trans-synaptic transport and Ag NPs can enter via the blood-brain barrier and accumulate in different regions of the brain and this may be beneficial for drug delivery, increasing a risk to the patient. It has also been reported that nanoparticle exposure can induce impairments to normal neuron-microglia microenvironment and even aggravate the process of brain pathology²⁷.

In support of the damage notion, *in vitro* cell line studies have shown decreased mitochondrial function after exposure to Ag NPs in murine neuroblastoma cells²⁸, hepatic cells²⁹, germline stem cells³⁰, human skin carcinoma³¹ and human epidermal keratinocytes and fibroblasts³², while *in vivo* studies showed that exposure to NPs could result in an inflammation, oxidative stress, myocardial infarction and thrombosis³³.

As above mentioned, NPs could alter the permeability of blood brain barrier³⁴.

Exposure to Ag NPs has been associated with tissue damage especially in liver. In rats, it has been determined for Ag NPs a No Observable Adverse Effect Level (NOAEL) of 30 mg/kg and the Lowest Observable Adverse Effect Level (LOAEL) of 125 mg/kg³⁵.

NPs could also damage DNA causing deletions, mutations, single and double strand breakages, adduct formation, and cross linking with proteins. Some studies have confirmed DNA adducts and oxidation and induced DNA fragmentation following exposure to metal oxide NPs. In response to DNA insult, cells attempt to repair damage DNA but repair failure may lead to cell death (apoptosis) or cell transformation. In the case of severe damage to DNA, cells may die by either necrosis or apoptosis. In this regard, it has been published previously that exposure to certain metal oxide NPs induces apoptosis³⁶.

Corrosion and discoloration of dental materials in contact with Ag NPs may be a concern. On the other hand, antibacterial property carries with it a potential environmental risk once these NPs are discharged into the environment. Of particular concern, Ag⁺ ions from AgNO₃ inhibit the algae's photosynthesis around 18 times more than Ag NPs. However, over a long period, the NPs are even more toxic than the ions alone³⁷. These environmental concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted.

CONCLUSION

Despite the effectiveness that Ag NPs have showed in dental practice, Ag NPs remain a controversial area of research with respect to their toxicity in biological and ecological systems³⁸. Therefore any applications of Ag NPs in dentistry requires more study. Initially, in order to avoid the toxicity of these materials we think Ag NPs can be used for temporary periods in the dental field.

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