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Application of Golden Nanoparticles against *Streptococcus Mutans* for Prevention of Caries Lesions

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Received: 22 July 2020 **Revised:** 5 September 2020 **Accepted:** 6 October 2020

ARTICLE INFO

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Keywords:
Dental caries;
Nanoparticles;
Streptococcus mutans;
Antimicrobial;
Fluoride

ABSTRACT

Background: Dental caries also known as baby bottle tooth decay, is a critical public health problem around the world for which *Streptococcus mutans* (*S.mutans*) has been introduced as the main infectious etiology. In the past two decades, nanotechnology has permitted the development of new materials with antimicrobial properties. The aim of this study was to compare the bactericidal and bacteriostatic effects of three golden nanoparticles (SP, NR, and CS) on *S.mutans*.

Methods: To determine the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs), a liquid dilution method was applied.

Results: All golden nanoparticles (GNPs) showed antimicrobial activity with no statistically significant differences (> 0.05) in MIC or MBC.

Conclusion: Our findings revealed that the size and shape of the nanoparticles did not significantly affect the antimicrobial properties of the GNPs. This finding might be useful for achieving important clinical effects with reduced toxicity in the management of early childhood caries in future in vivo studies.

Introduction

Early childhood caries (ECC) has become a major health problem, especially in the poor social population. It is characterized as the presence of one or more decayed, missing, or filled primary teeth in children aged 71 months or younger. It has several unique clinical features, such as the rapid growth of caries, which will soon affect several teeth after appearing in the oral cavity. These lesions include dental surfaces that are less susceptible to caries development.[1,2] The etiology of ECC is multifactorial and is mostly associated with the specific interaction of microorganisms with sugars on a tooth surface. Other main causes of the ECC development include poor oral hygiene, lack of fluoride exposure, and enamel defects.[3]

Streptococcus mutans (*S.mutans*), *Streptococcus subbrinus* and Lactobacillus are the most commonly associated microorganisms in the development of caries lesions. *S.mutans* is the main pathogen in dental caries isolated from human heart valves and the blood in patients with endocarditis.[4,5] The prevalence of caries in preschool children with high levels of S.mutans in the oral cavity is higher. They also have a greater risk for development of new lesions.[6,7] *Streptococcus mutans* is usually transmitted to young children through their mothers and high maternal salivary levels of *S.mutans* increases the risk of transmission. The prevention or delay of early mutans streptococci colonization in children is associated with lower caries prevalence.[8,9]

Recent evidences have revealed increases in resistance of various pathogenic bacteria against numerous synthetic drugs. Recently, applying golden nanoparticles (GNPs) as a delivery vehicle of antibiotic agents to improve their capacity for targeting a wide range of bacteria emerged as a highly demanding topic of research in the field of nanotechnology. To overcome this problem different reports on using Au (I) and Au (III) complexes for their antimicrobial activity against a wide variety of microorganisms have released.[10,11] Moreover, different classification of GNPs are available for instance Au (I) complexes categorized into three categories based on the ligand types a) phosphine ligands b) N-heterocyclic carbene ligands c) other Au (I) complexes. The purpose for the development of gold complexes is their solubility in water and potential application as therapeutic agents.[12]

One main challenge is assessing nanoparticle toxicity to develop them for imaging, and drug delivery. It should be mentioned that if nanoparticles do not enter cells, they have less side effects such as killing cells or changing cellular function so considering dimensional limitations in designing nanoparticles for targeting cells needs more investigations on different cells. It should also take into account that gold nanoparticles have relatively good biocompatibility and low toxicity in comparison to other kinds of inorganic nanoparticles.[13,14]

Specific surface area and the reactivity of GNPs' facets affect their antibacterial activity; particles with larger effective contact area present stronger antibacterial activity.[14] Thus, gold nanowires show the weakest antibacterial activity compared with silver nanocubes and nanospheres because of their weak contact with bacteria. However, the antimicrobial activities of nanoparticle colloids of different shapes are not yet clear.[15] The purpose of this study is therefore to determine whether the size and morphology of golden nanoparticles in colloidal solution alter their antimicrobial activity. We characterized the shape and size of three different GNPs in this study [gold nanospheres, nanorods, and core/shell silica/gold nanocrystals], which were coated with monocarboxy (1-mercaptoundec-11-yl) hexaethylene glycol and their antimicrobial activity against reference stocks of *Streptococcus mutans* ATCC 25175 were analyzed as well.

Materials and Methods

AuNPs: We applied four available models of morphologically different GNPs including spherical (SP), rodlike (NR), and core/shell silica/gold (CS). All GNPs were stabilized with monocarboxy (1-mercaptoundec-11-yl) hexamethylene glycol (OEG) which improves the stability of NPs for aggregation and decreases the possibility of nonspecific interactions with biological molecules such as serum proteins. Additionally, the negative charge of the OEG GNPs lowers their cellular toxicity. The morphology, size, and shapes of GNPs were identified using transmission electron microscopy (TEM), zeta potential measurement and spectrophotometer. TEM measurements were done on JEOL and JEM-1400 at an elevated voltage of 120 KV. A drop of colloidal gold solutions from 10 mM and 20 mM was put on TEM copper lace. After 5 minutes of drying and removing extra solution by a blotting paper, size diffusion of GNPs was measured on images of TEM. The hydrodynamic diameter of GNPs was calculated using zeta sizer nano-ZS (Malvern instrument, Malvern, UK) following the data analysis in an automated way. The UV- visible spectroscopic measurement was observed using single beam spectrophotometer (Systronics 169) at distinct wavelength (300-600 nm).

Antimicrobial tests

Strain and medium: *Streptococcus mutans* ATCC 25175 was utilized as the microbial strain which was precultivated in brain heart infusion (BHI) broth (Himedia).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The minimum inhibitory concentration (MIC) was assessed utilizing a spectrophotometric microdilution method (SMM) and turbidity. Then resazurin (Sigma, St Louis, USA) as oxidation–reduction indicator to investigate viable antimicrobial activity was added. The three doses were determined for the GNPs solution. The 20 µL (low dose) solution included 1.35 µg Au NPs, 30 µL (medium dose) contained 2.03 µg Au NPs and 40 µL (high dose) contained 2.70 µg Au NPs. Four tubes of each dose were made and incubated at 37 °C for 24 h. The wells were supplied with 100 µl BHI, 100 µl test solution, and 10 µl exponentially growing bacterial culture (about 108 colony-forming units/mL). A 200 µL from each tube was distributed in each micro-well plate and seen by spectrophotometer. The optical density (OD) values from ELISA reader were used in absorption mode which covered the bacterial growth in each sample. Three values of OD for each sample and their mean were calculated with standard deviation (SD). Four tubes of each dose were made ready and incubated at 37°C for 24 h. The minimum bactericidal concentration (MBC) was discovered from the MIC value; 10 µl of the solution was pipetted from wells, located on BHI agar, and incubated at 37°C for 18 h anaerobically. For each GNP, four concentrations were examined: the MIC value, two concentrations above, and one below. The MIC values were indicated as the lowest concentration effective in suppressing bacterial growth. The MBC was defined at the point when aliquots from the MIC wells did not show visible bacterial growth on agar plates. All the experiments were done in triplicate.

Results

Characterization of nanoparticles: Figure 1 displays TEM pictures of all the different types of nanoparticles.

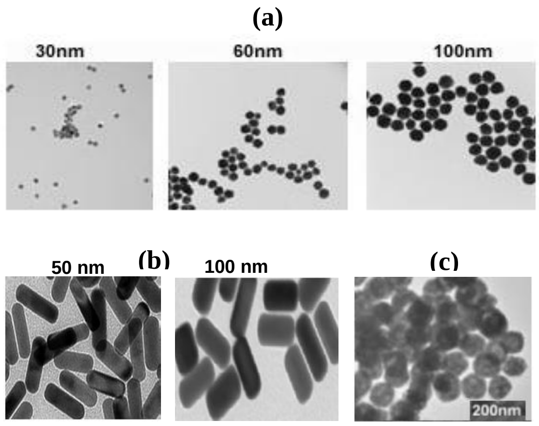

Figure 1. TEM images of gold nanoparticles: a) gold nanospheres, b) gold nanorods, c) hollow gold nanoparticles

The average size of each type AuNPs was measured by TEM images. All GNPs were stabilized with monocarboxy (1-mercaptoundec-11-yl) hexaethylene glycol (OEG). The OEG capping layer enhances the stability of NPs against aggregation and minimizes the feasibility of nonspecific interplays with biological molecules such as serum proteins. Furthermore, the negative charge of the OEG GNPs decreases their possible cellular toxicity, whereas the ethylene glycol units may enhance the retention times of the particles in the blood. The nanoparticles were identified with TEM, and zeta potential measurements. The nanoparticles were robust in solution and did not display any signs of aggregation. Moreover, the zeta potential measurements showed a strong negative charge for all the types of OEG GNPs. The average number of OEGs attached to each nanoparticle was detected with Ellman's method. Ten independent measurements were performed for each type of NP (Table 1). The number of capping ligands was adequate to provide the appropriate surface stabilization.

Table 1. Physicochemical characterization of OEG-capped gold nanoparticles of different morphologies

OEG NSP	core (nm)	Diameter (nm)	Charge (Mv)
SP	14 ± 1	27 ± 1	-26 ± 2
NR	18 ± 1/43 ± 1	-	-29 ± 1
CS	26 ± 1/7 ± 2	45 ± 4	-31 ± 2

Antimicrobial activity of AuNPs: The bactericidal property of metals depends on the contact surface with bacterial cells, so using GNPs against *Streptococcus mutans* appears to be an alternative for stopping bacterial growth, since the large surface area of nanoparticles allows a wide range of interactions with organic and inorganic molecules. The MIC and MBC values were 3.1 ± 1.1 and 2.1 μg mL^{-1} for SP, 3.2 ± 1.7 and 4.2 μg mL^{-1} for NR, 3.4 ± 1.3, and 2.2 μg mL^{-1} for CS, respectively. The results were not significantly different (P = 0.32) for both MIC and MBC (Table 2).

Table 2. MIC and MBC of golden nanoparticles for *Streptococcus mutans* ATCC 25175

GNPs	MIC (μg mL-1)	MBC (μg mL-1)
SP	3.1 ± 1.1	3.1
NR	3.2 ± 1.7	4.2
CS	3.4 ± 1.3	3.2

Discussion

S.mutans has been considered as the principal microbiological agent in the development of dental caries. The amount of *S.mutans* naturally in the oral cavity is almost 1 × 10^5 colony-forming units per milliliter of saliva. Thus, a number of methods such as DNA plasmids resistant to *S.mutans*, vaccines and antibodies have been applied to reduce colonization of *S.mutans*.[16,17] Gold nanoparticles have been broadly used in bionanotechnology based on their unique properties and multiple surface functionalities for example the ease of AuNP functionalization as a versatile platform for nanobiological assemblies with antibodies, proteins and oligonucleotides. Additionally, AuNPs serve as platforms for therapeutic agents, with their large surface area allowing a dense presentation of multifunctional moieties. AuNPs have also gained attention for the design and development of innovative biomedical tools.[18] Some critical characteristics of AuNPs such as facility of functionalization, and simple synthesis allowing the release of high drug at infected sites and an ability to penetrate biological membranes which make them promising candidates for the improvement of novel antibacterial agents.[19,20] Several methods for NP surface synthesis and functionalization with antimicrobial drugs through covalent or noncovalent interactions have been described, such as amoxicillin-coated AuNPs, vancomycin-capped AuNPs, and ampicillin- and, streptomycin- conjugated AuNPs. The aforementioned studies are compatible with the application of nanoparticles combined with other antimicrobial effects to decrease intrinsic toxicity of nanoparticles, improve the microbicidal effects, and reduce the probability development of resistance.[21-23]

The current study revealed that the three GNPs presented reasonable MIC and MBC values giving a new perspective for the development of antimicrobial compounds for control of caries especially in children.

Furthermore, the results shown herein demonstrate that variation in the shape and size of the gold nanoparticles do not affect their antimicrobial ability and the color of the solution has no direct association with the antimicrobial property. Although, it is not possible to compare all the MIC/MBC values described in different investigations because of the wide range of initial bacterial concentrations, microbial strains, and culture medium components used. Antibiotics also influence the oral flora and suppress certain categories of microbes. For example, penicillin removes oral bacteria or broad-spectrum antibiotics diminish gram-positive and gram-negative bacteria, and so, provide a suitable environment for fungi and yeast to grow as opportunistic agents creating. On the other hand, the rapid spread of resistant bacteria occurring worldwide is endangering the efficacy of antibiotics, which saved millions of lives.[24]

Early childhood caries management is expensive, and often needs comprehensive treatment for the repair and extraction of teeth at an early age. As young children are not able to cope with widespread methods of treatment, deep sedation or general anesthesia may be necessary.[25] Since 1940s using fluorides to prevent dental caries, including fluoride toothpaste, water fluoridation, or professional topical fluoride application, mostly by inhibiting mineral loss from the tooth has been considered as an effective option especially in childhood caries but the multiple pathways to the development of dental caries make it difficult to ascertain the contribution of fluoride ingestion to dental caries inhibition.[26] Given that fluoride activity is effective in preventing dental caries in a topical manner so, only topical fluoride products are likely to contribute to the claimed benefits of this chemical. In addition, fluoride exposure has a complex relationship concerning dental caries and may increase the risk of dental caries in children with malnutrition due to decreased calcium and hypoplastic enamel.[27,28]

Uses of probiotics chewable tablets or supplements containing GNPs also are some feasible options in controlling the caries in children which needs more in-vivo and in vitro studies. These findings lead us to consider that SP, NR and CS materials may be useful for controlling *S.mutans* and therefore caries. Improvement of a carrier to deliver GNPs locally, as well as determining the toxicity in vivo to use GNPs as fluoride alternative in ECC management is suggested for future studies.

Conclusion

Currently, an extensive research in nanotechnology and its utilizations in infectious or contagious diseases have led to the development of antimicrobial nanoparticle formulations that act as an effective bactericidal agent. In present study, we showed the potential application of three gold nanoparticles (SP, NR, and CS) in vitro, to diminish or eradicate *S.mutans*.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

The authors thank the editors and the anonymous reviewers for insightful suggestions on this study.

How to Cite: Daliri K, Seifi-Shalamzari N, Saeida-Ardekani M, Ekraminasab S, Neamatzadeh H. Application of Golden Nanoparticles against *Streptococcus Mutans* for Prevention of Caries Lesions. World J Peri & Neonatol 2020; 3(2): 56-61. DOI: 10.18502/wjpn.v3i2.6155

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