Bactericidal Capacity of Silver Nanoparticles Associated with Gantrez S-97 on *Streptococcus Mutans*

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Dental caries is a worldwide public health problem. S mutans plays an important role in the etiology of caries. There have been studies that showed the antimicrobial properties of silver nanoparticles are an effective agent to diminish S. mutans. The ***objective*** *of this study was to evaluate the bactericidal and bacteriostatic effects of silver nanoparticles in addition to the Gantrez S-27 copolymer, on S mutans.* ***Method****: We performed an in vitro experimental study using the liquid microdilution method in order to find the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) with the subcultures obtained. The mixture was obtained by preparing 98 µg/mL of silver nanoparticles (10^{-3}mol) with Gantrez S-27 2%, in distilled water. The readings were performed 24 hours after incubation and on 3 consecutive days. The* ***results*** *showed an average MIC of 6.12 µg /mL and MBC of 6.12 µg /mL.* ***Conclusion****: The addition of Gantrez 2% to silver nanoparticles does not alter its antimicrobial effect.*
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INTRODUCTION

Dental caries, a carbohydrate-modified, local infection that destroys the hard tissues of teeth, has a multifactorial etiology in which there is an interaction of various factors including the host, agent, substrate, and time.[1] It is characterized by the destruction of hard dental tissue as it is demineralized by the acids generated by the bacterial plaque, which form during degradation of the carbohydrates of the diet.[2] *S mutans* has been implicated as the principal pathogen involved in the development of dental caries, which is the reason many epidemiological studies in dentistry have been focused on isolating this microorganism to determine its pathogenicity.[3-5]

The recognition of *S mutans* as the most important microorganism in the initiation of dental caries makes it feasible to design preventive measures toward eliminating or diminishing its number in the oral cavity. *S mutans* forms a biofilm over the dentral stucture in the presence of sucrose, which produces lactic acid and participates in the demineralization of the dental surface.[6-9]

Previously we reported on the bactericidal and bacteriostatic effects of nanoparticles of silver, zinc oxide, and gold on *S mutans*. For silver, the results showed an average minimum inhibitory concentration (MIC) of 4.86 ± 2.71 µg/mL and minimum bacteriostatic concentration (MBC) of 6.25 µg/mL; for zinc, the MIC was 500 ± 306.18 µg/mL and the MBC was 500 µg/mL; the gold nanoparticles demonstrated an effect only at an initial concentration of 197 µg/mL.[10]

It has been reported that the addition of a Gantrez S-97 copolymer to toothpaste increases its adhesion to the enamel surface, giving the paste more time to act against the agents that promote plaque and caries.[11-13]

The objective of this study was to evaluate the bactericidal and bacteriostatic effects of nanoparticles of silver (with

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added Gantrez S-97 copolymer to adhere solutions to dental surfaces) on *S mutans,* using the liquid microdilution method.

METHOD

Design

This was an experimental *in vitro* study, performed in the installations of the Universidad Autónoma de San Luis Potosí. The Materials Laboratory of the Facultad de Ciencias elaborated the nanoparticles, and the antibacterial tests were developed in the Microbiology Laboratory of the Facultad de Ciencias Químicas and Basic Sciences Laboratory of the Facultad de Estomatología.

Manufacturing of Nanoparticles

The procedure involved reactions of colloidal solutions with oversaturation of salts, and the creation of a precipitate by homogeneous or heterogeneous nucleation. After nucleation, the nanoparticles' growth took place by diffusion. The concentration gradient, temperature changes, agitation, and surfactants allowed modification of the size increment. We produced monodispersed, nonagglomerated particles with a uniform size.

First, we prepared a solution of silver with sodium tetrahydroborate (NaBH4) included as a reducing agent. The ratio between silver and the reducing agent was greater than one. After the reaction, we added a stabilizer for controlling size and uniformity.

For the experiment, the mixture was prepared with 98 µg/mL of silver nanoparticles (10^{-3}mol) with Gantrez S-97 2%, suspended in distilled water. The size of the nanoparticles was between 40 nm to 80 nm.

Strains of *S Mutans*

For standardization and quality control of minimum inhibitory concentrations (MICs) before the experiment, we used a reference strain of *S aureus* (25923) derived from the American Type Culture Collection (ATCC; Manassas, Va, USA). This was exposed to the antibiotic oxycillin and the MIC determined.

The strains were prepared in 85% saline solution at a concentration of 0.5 on the McFarland scale, and it was verified by colorimetric measurement (Vitek; bioMérieux, Mexico City, Mexico). This concentration is equivalent to 1.5 x 10^8 bacteria. Then we added a volume of 10 µL of solution in 10 mL of phosphate buffer, for a final concentration of 1:1000 microorganisms. The strains were stored by freezing in Mueller-Hinton/glycerol solution 20% (vol/vol), and they were maintained at –20°C until onset of the tests.

Antimicrobial Test

MICs and MBCs of *S. mutans* were determined from a known concentration of nanoparticles in micrograms per milliliter, using the liquid microdilution method. The cutoff points were compared with those described by the National Committee for Clinical Laboratory Standards (NCCLS) at the Clinical and Laboratory Standards Institute. We placed 50 µL of microorganisms diluted 1:1000 into cells on a microplate with the various concentrations and types of nanoparticles. These were incubated in a closed chamber at 37°C and a microaerophilic environment for 24 hours. The determination of MIC (NCCLS) was based on turbidity. The MBC was achieved from subcultures of sheep's blood agar from the dilution plaques, after incubation for 24 hours at 37°C in a microaerophilic environment. All the experiments (MIC and MBC) were triplicated on three different days. The Research Ethics Committee of the Faculty of Dentistry authorized the study.

Statistical Analysis

Measures of central tendency and dispersion for MIC and MBC were calculated.

RESULTS

For three consecutive days, we used an initial concentration of silver nanoparticles of 98 µg/mL with Gantrez S-97 2%, and from that concentration we diluted until we obtained the MIC and MBC against *S mutans.* Table 1 indicates the day-to-day results obtained for the MBC and MIC.

The optimal concentration of nanoparticles associated with Gantrez S-97 2% was found to be 6.12 µg/mL. This concentration was chosen because it showed the antibactericidal effect. Figure 1 shows silver nanoparticles adhering to the Gantrez S-97. It can be observed that some nanoparticles are inside the Gantrez S-97, while others are on the surface.

Table 1. MBC and MIC of silver nanoparticles 98 mg/mL with Gantrez S-97 2% / day, in mg/mL.

DAY	MBC	MIC
1	6.12 mg/mL	6.12 mg/mL
2	6.12 mg/mL	6.12 mg/mL
3	3.06 mg/mL	3.06 mg/mL

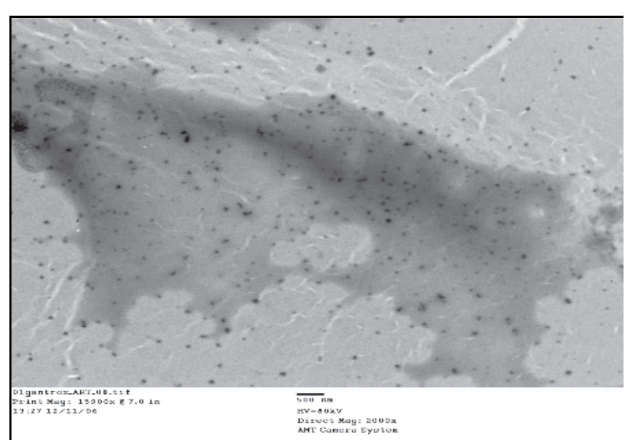

Figure 1. Silver nanoparticles adhering to Gantrez S-97, as viewed by electron transmission microscopy.

DISCUSSION

Dental caries continues to be the most common infectious disease of the oral cavity. The mainstay of caries prevention and remineralization has been the delivery of fluoride, either in systemic or topical form. However, antimicrobial agents specific for microorganisms associated with dental caries such as *S mutans* may be beneficial.

In order to reduce dental caries, it is necessary to create new strategies aimed at prevention.[14] There are actually a variety of substances that can inhibit the growth of caries-producing microorganisms, The most frequently used are chlorhexidine and triclosan, which have been added to toothpaste, mouth rinses, and dental varnish.[15-17] However, it is essential to determine the susceptibility of *S mutans* to these substances so that we can detect possible bacterial resistance such as recently seen toward triclosan.[18-19] Diverse studies have demonstrated the efficacy of chlorhexidine, and this effect has been proposed as a preventive measure against the development of dental caries by reducing the population of *S mutans* in the oral cavity;[20] however, other studies have failed to demonstrate it.[21] Also, the prolonged use of chlorhexidine is associated with side effects such as pigmentation of the teeth and oral mucosa, including the tongue, and taste alterations.[22-23]

In a previous study, it was determined that the MIC of nanoparticles against *S mutans* was 4.86 µg/mL and the MBC was 6.25 µg/mL.[10] In this study we found similar results.

CONCLUSIONS

The addition of Gantrez S-97copolymer does not diminish the bactericidal properties of silver nanoparticles. It is important to continued studies of toxicity in tissue culture, animal models, possible color modification, and adherence to dental structure, that will allow us to evaluate whether the solution stays adhered to the dental surface sufficiently to permit the destruction of *S mutans* and to find the most efficient way for human administration.

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