Action of silver nanoparticles towards biological systems: cytotoxicity evaluation, inhibition of biofilm formation, and clinical trial as anti-caries agent

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Introdução

Silver nanoparticles (AgNPs) are one of the most widespread nanomaterials in consumer products, mainly due to their antimicrobial properties. Promising results have been reported regarding their effectiveness against *S. mutans* which means they can be potentially used for caries arrestment and oral care. Any formulation designed to meet this application must be non-toxic, effective as a bacteriostatic/bactericidal agent and capable of preventing biofilm formation.

Regarding toxicity, the hen's egg test (HET), which is well known as a basic test for embryotoxicity, measures the acute effects induced by a test substance on the small blood vessels and proteins of the chorioallantoicmembrane (CAM). It is a fast, non-animal which has been accepted as a valid in vitro assay for the prediction of the irritation potential of irritating substances.

Here, we describe results of this cytotoxicity tests together with biofilm inhibition experiments performed with AgNPs/chitosan formulations aiming their application for caries arrestment. A clinical trial was also carried out with promising results.

Results and Discussion

Silver nanoparticles were synthesized by reduction with sodium borohydride in chitosan solution and NaF was also added in the formulation. The features of each formulation tested are summarized in the following Table.

Table 1. Size and shape of the silver nanoparticles in the formulations tested in this study.

Spherical		Triangular		Elliptical	
%	Size (nm)	%	Size (nm)	%	Size (nm)
100.0	8.7 ± 3.1	–	–	–	–
97.5	15.0 ± 7.9	2.5	22.2 ± 9.5	–	–
77.3	31.8 ± 10.4	15.9	27.1 ± 10.1	6.8	33.2 ± 7.8 13.6 ± 3.6
75.2	43.2 ± 14.3	23.3	38.2 ± 14.8	1.5	38.4 ± 11.6 17.9 ± 7.3

Biofilm inhibition was studied using the fluorescence protocol Filoche *et al.* Biofilms subjected the four formulations tested presented 0% cell viability, while the positive control presented 36.5% bacterial viability.

HET-CAM tests were performed according to the methodology described by Steiling *et al.*. The test was carried out using sterile deionized water to hydrate the membrane in all samples. Membranes were observed for 5 min for signs of vasoconstriction, hemorrhage, and coagulation. Different sizes and morphologies of AgNPs showed no irritating potential.

The clinical trial was in accordance with the World Medical Association Declaration of Helsinki and registered at www.clinicaltrials.gov under the protocol NCT01950546. After seven days of follow-up, 81% of decayed teeth in the AgNP group showed hard arrested dentine, which was not observed in the control group. After five months, the AgNP group had 72.7% of the teeth showing arrested cavities, and the control group had 27.4%. At 12 months, 66.7% of the lesions in teeth treated were still arrested, while the control group showed 34.7%.

Conclusions

The formulations are as effective silver diamine fluoride (SDF) 30% as an anti-caries agent was when applied once a year, with the advantage of not staining the dental tissue black. Unlike SDF, the NSF solution had no metallic taste.

The total silver amount is approximately 600 times lower than SDF.

Aknowledgements

CAPES, CNPq, FACEPE.

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