Assessment of the antimicrobial effect of fluoride varnish containing nanosilver and conventional fluoride varnish on oral streptococci: an in-vitro study

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Background/aim
Oral streptococci are commonly found in the human oral cavity and are considered the foremost cariogenic pathogens in tooth decay. The aim of this study was to assess the antimicrobial effect of fluoride varnish containing nanosilver particles with different concentrations and conventional fluoride varnish on oral streptococci.

Materials and methods
Streptococcus mutans (ATCC 25175) and *Streptococcus salivarius* (ATCC 13419) were grown in nutrient agar medium with 5% blood agar in the bacteriological laboratory. Then both strains were further cultured on Mueller–Hinton agar media separately; three different concentrations of nanosilver fluoride (Nsf) varnish (432, 216, and 108 μg/ml) were prepared and applied on the Mueller–Hinton plates. Conventional fluoride varnish was included as the control sample. The plates were further incubated anaerobically for 24 h at 37°C in the incubator. The antimicrobial effect of different varnishes was assessed by measuring the diameter of inhabitation zones in millimeters by a ruler. Statistical analysis was performed using analysis of variance test, followed by Tukey's post-hoc test.

Results
The Nsf varnish showed antimicrobial effect against *S. mutans*, where the mean value of inhibition zone size (mm) was increased by increasing the concentration of Nsf. For *S. salivarius*, the mean value of inhibition zone (mm) was gradually decreased by increasing concentration of Nsf.
Conventional fluoride varnish showed no inhibition zones in both bacterial strains.

Conclusion
Nsf varnish had better antibacterial effect than conventional fluoride varnish against both *S. mutans* and *S. salivarius*.

Keywords:
antimicrobial, dental caries, fluoride varnish, nanosilver, oral streptococci

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Introduction

Dental caries is an epidemic, microbiological disease of the teeth that ends in localized dissolution and damage of the calcified structure of the teeth [1]. This disease is considered one of the most prevalent dental biofilm-related diseases. Although dental biofilms can be composed of numerous bacterial species, oral streptococci are considered as the imperative etiologic factor in dental caries. To decrease the prevalence of caries, an improved understanding of the role of these microorganisms in dental diseases is needed [2].

The prevention of dental caries in children is regarded as a priority for dental services and considered more cost-effective than its treatment. Several methods for caries prevention have been developed; among these methods are dietary control, proper oral hygiene measures, and topical protection of the tooth surface as topical fluoride application [3].

Besides the conventional materials for caries prevention, new innovative approaches are needed to increase access to dental care for those at need. A new material (nanosilver) can be used to inhibit the cariogenic bacteria. Silver has a significant antimicrobial activity and is effective against streptococci of the human oral cavity and periodontal pathogens especially when applied in nanometer sizes [4,5].

The biocidal effect of nanosilver particles with its broad spectrum of activity including bacterial, fungal, and viral agents makes it an excellent choice for multiple roles in the medical field. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. Moreover, it can inhibit bacterial adherence to surfaces and biofilm formation, so it can be applied as a useful antibacterial additive to fluoride varnishes or pits and fissure sealants or even glass ionomers and composites [6,7]. Therefore, the aim of the present in-vitro study was to assess the antimicrobial effect of fluoride varnish containing nanosilver and conventional fluoride varnish on two types of oral streptococci (*Streptococcus mutans* and *Streptococcus salivarius*).

Materials and methods

Materials

(1) Fluor protector 1 mm ampoules produced by IvoclarVivadent (Amherst, New York, USA) were bought from local market and used as the control group.
(2) Nanosilver fluoride varnish was prepared in three different concentrations (432, 216, and 108 μg/ml) by the aid of Naqaa Nanotechnology Network (Giza, Egypt).
(3) Freeze-dried *S. mutans* (ATCC 25175) and *S. salivarius* (ATCC 13419) were brought from Al-Magd for laboratory and medical supplies (Helmiet Al-Zitoon, Cairo Egypt).
(4) Nutrient agar media (HiMedia Laboratories, Mumbai, Maharashtra, India) were purchased from local market and prepared according to the manufacturer's instructions.
(5) Blood agar media (HiMedia Laboratories) were purchased from local market and prepared according to the manufacturer's instructions.
(6) Mueller–Hinton agar media (HiMedia Laboratories) were purchased from local market and prepared according to the manufacturer's instructions.

Study design

This study was an observational case-controlled study. A total of 16 plates of *S. mutans* microorganisms (n=16) and 16 plates of *S. salivarius* microorganisms (n=16) were prepared, where four plates of *S. mutans* received the fluoride varnish as a control group and the other 12 plates received the three different concentrations of nanosilver fluoride (Nsf) varnish separately as the study group (four plates received 432 μg/ml concentration, four plates received 216 μg/ml concentration, and the last four plates received 108 μg/ml concentration). The same steps were repeated for *S. salivarius* microorganisms.

Methods

Silver nanoparticles synthesis

The synthesis of silver nanoparticles in an aqueous solution was successfully carried out via the chemical reduction of silver nitrate with sodium borohydride and chiston biopolymer as a stabilizing agent. For the synthesis, $AgNO_3$ (1 ml, 0.11 M) and chitosan (28.7 ml, 2.5 mg/ml), which had been previously dissolved in a 1% acetic acid solution, were mixed under magnetic stirring until homogeneous. Next, the mixture was transferred to an ice-cold bath, and freshly prepared $NaBH_4$ (0.3 ml, 0.8 M) was then added drop by drop while stirring vigorously. The flask was then removed from the ice bath, and the fluoride (10.147 ppm of fluorine) was incorporated. The stirring was maintained overnight. The silver nanoparticles had an average size of 1.2–5.2 nm and a spherical shape, which was checked by scanning electron microscope [8].

Bacteria preparation

This study was carried out by agar diffusion test. The steps of the disc diffusion method were employed as suggested by Kumar *et al.* [9].

A suspension of *S. mutans* (ATCC 25175) and *S. salivarius* (ATCC 13419) was grown on nutrient agar medium with 5% blood agar, and then the plates were incubated at 37°C for 24 h anaerobically in the incubator.

A total of 32 plates of Mueller–Hinton agar were further prepared previously and divided into two main groups: the first group (16 plates) received the *S. mutans* and the other group (16 plates) received *S. salivarius* bacteria. McFarland standards was used as a reference to adjust the turbidity of the bacterial suspensions. Each Mueller–Hinton plate received 0.5 McFarland suspensions of *S. mutans*, and then it was spread on the Mueller–Hinton surface by sterile swab applicator. The same step was applied on *S. salivarius* as well.

Each plate containing *S. mutans* culture received 50 μl of Nsf varnish (432 μg/ml) by the aid of the micropipette device. This step was repeated for the rest of the concentrations (216 and 108 μg/ml) and the conventional fluoride varnish, respectively. The same steps were applied on *S. salivarius* as well. The plates were further incubated anaerobically for 24 h at 37°C in the incubator. The antimicrobial property of materials was assessed, at which it appeared to from circular zones of bacterial inhibition (halo) around each hole. The diameter of these zones of bacterial inhibition was measured in millimeters using a ruler (from the edge of the zone from one end to the next edge).

Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS 19; SPSS, Chicago, Illinois, USA). As the quantitative data showed a parametric distribution, one-way analysis of variance (ANOVA) test, followed by Tukey's post-hoc test was used for comparison between groups. The level of significance was set at *P* value less than 0.05 [10,11].

Results

Streptococcus mutans (inhibition zones)

The mean value of inhibition zone (mm) gradually increased by increasing the concentration of Nsf. The highest mean value (16.38±1.25) was recorded in Nsf at 432 μg/ml, whereas the least value in Nsf (13.25±1.5) was recorded in Nsf 108 μg/ml. Conventional fluoride varnish control recorded an inhibition zone of 0 mm (Fig. 1). ANOVA test revealed that the difference was highly statistically significant ($P<0.0001$) (Table 1). Tukey's post-hoc test revealed that the concentration of Nsf 432 μg/ml was statistically significant in comparison with the other two studied concentrations (Nsf 108 and 216 μg/ml) ($P=0.01$), whereas there was no significant difference between the concentration of Nsf 216 μg/ml and the other two studied concentrations.

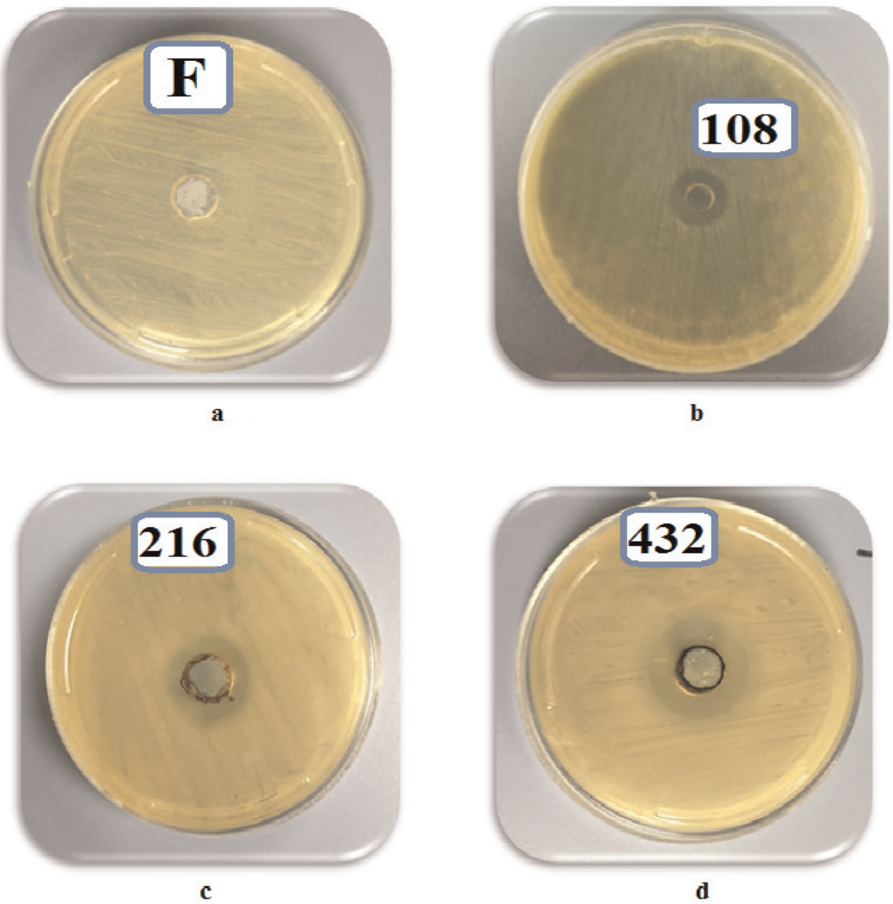
Streptococcus salivarius (inhibition zones)

The mean value of inhibition zone (mm) gradually decreased by increasing concentration of Nsf. The highest mean value (18±8.12) was recorded in Nsf 108 μg/ml, whereas the least value in Nsf (16.25 ±5.56) was recorded in Nsf 432 μg/ml. Conventional fluoride varnish recorded an inhibition zone of 0 mm (Fig. 2). ANOVA test revealed that the difference was statistically significant ($P<0.002$) (Table 2). However, Tukey's post-hoc test revealed no significant difference among the three different concentrations of Nsf (432, 216, and 108 μg/ml).

Discussion

Dental caries is a major oral health problem in most industrialized countries, affecting 60–90% of school children and the vast majority of adults. Considering the vivid importance of oral streptococci in caries etiology, prevention of the growth of these microorganisms can be effective in prevention of dental caries [4]. One of the most well-known topical fluoride products is the fluoride varnish, which is widely used by dentists all over the world. The mechanisms of

Figure 1



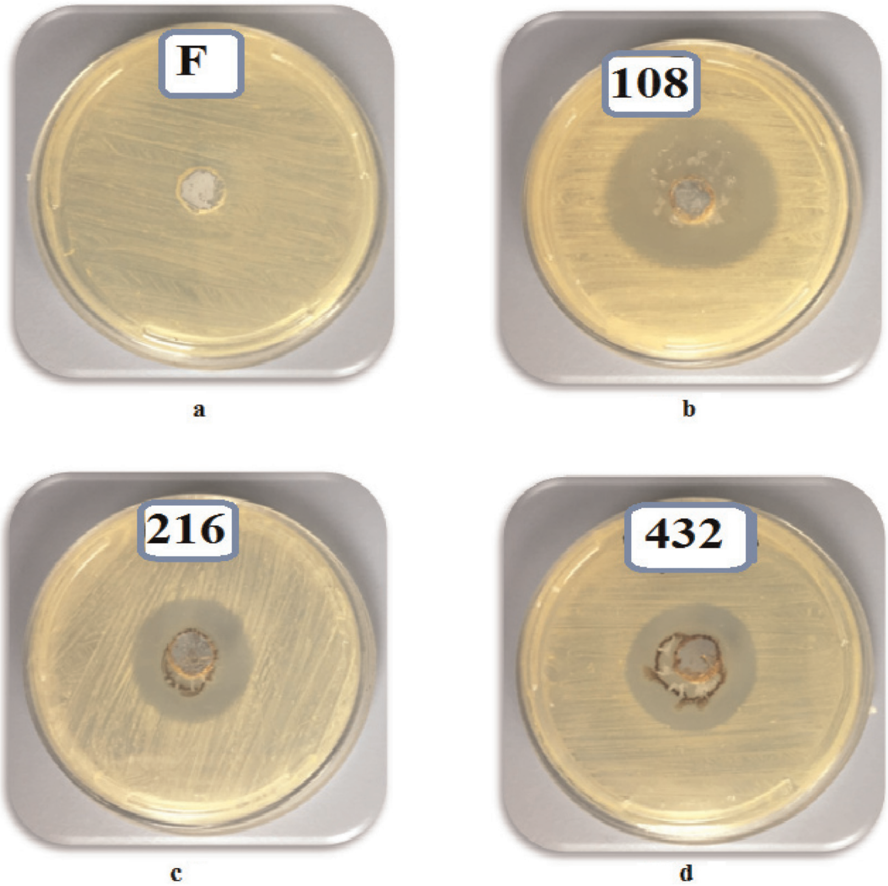
Mueller–Hinton agar media with *Streptococcus mutans* showing the zones of inhibition around the applied materials: (a) fluoride varnish, (b) nanosilver fluoride varnish 108 μg/ml, (c) nanosilver fluoride varnish 216 μg/ml, and (d) nanosilver fluoride varnish 432 μg/ml.

Table 1 Comparison between conventional fluoride varnish (control group) and different concentrations of nanosilver fluoride (study groups) regarding inhibition zone of *Streptococcus mutans* (mm)

	Mean±SD	Minimum	Maximum	*P*
Streptococcus mutans				
F (control group)	0±0.00^{a}	00.00	0.00	
Nsf 108 (μg/ml)	13.25±1.50^{b}	12.00	15.00	<0.0001
Nsf 216 (μg/ml)	14.75±1.50^{c,b}	13.00	16.00	
Nsf 432 (μg/ml)	16.375±1.25^{c}	15.00	18.00	

Different letters a, b, care significant at *P* value less than 0.05, using analysis of variance test.

Figure 2



Mueller–Hinton agar media with *Streptococcus salivarius* showing the zones of inhibition around the applied materials: (a) fluoride varnish, (b) nanosilver fluoride varnish 108 μg/ml, (c) nanosilver fluoride varnish 216 μg/ml, and (d) nanosilver fluoride varnish 432 μg/ml.

Table 2 Comparison between conventional fluoride varnish (control group) and different concentrations of nanosilver fluoride (study groups) regarding inhibition zone of *Streptococcus salivarius* (mm)

	Mean±SD	Minimum	Maximum	*P*
Streptococcus salivarius				
F (control group)	0±0.00^{a}	00.00	00.00	
Nsf 108 (μg/ml)	18±8.12^{b}	11.00	26.00	<0.002
Nsf 216 (μg/ml)	16.5±5.92^{b}	11.00	23.00	
Nsf 432 (μg/ml)	16.25±5.56^{b}	11.00	22.00	

Different letters a, b are significant at *P* value less than 0.05, using analysis of variance test.

antibacterial effect of fluoride are well known, and the ability of fluoride to control caries is primarily owing to its topical effect on tooth surfaces. Unfortunately, this traditional approach is not fully effective because it has been demonstrated that fluoride ion affects the intact or relatively intact enamel. Fluoride is not entirely effective on the teeth with destroyed enamel and severely defunct dentin filled with bacteria [12].

In the past few years, new approaches have been considered to control dental caries such as topical application of antibacterial compounds on the surface of those teeth that are at risk of or affected by dental caries. The silver ions exhibited high bactericidal activity against oral streptococci [6]. Several studies have demonstrated that silver nanoparticle-containing materials achieved strong antibacterial effects against microorganisms biofilms and the oral pathogenic species of streptococci [13–15].

Fluor protector fluoride varnish 1 mm ampoules by IvoclarVivadent was selected as a control group owing to its properties, as it spreads easily and readily flows into complex surface structures, dries quickly, and shows excellent adhesion to teeth [16].

Prepared Nsf varnish was selected as an intervention group to be investigated in this study in inhibiting the bacterial growth. It was tested in many studies regarding its effect on inhibition of oral streptococci owing to the high content of fluoride in addition to nanosilver particles, which has very strong bactericidal effect [6].

In this study, the null hypothesis was rejected because the results showed that there was a statistically significant difference in the mean inhibition zone diameters between conventional fluoride varnish fluor protector and all three concentrations of Nsf varnish (432, 216, and 108 μg/ml) against both *S. mutans* and *S. salivarius.* These results were supported by studies conducted by Haghgoo *et al.* [4] and Shah *et al.* [17]. However, it was in contrast with another study conducted by Fujun *et al.* [13], which concluded that there was no statistically significant difference between nano silver-containing varnish and conventional fluoride varnish.

The significant difference between conventional fluoride varnish fluor protector and all three concentrations of Nsf varnish (432, 216, 108 μg/ml) against both *S. mutans* and *S. salivarius* may be owing to the ability of the nanosilver particles to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell [14,18,19].

Regarding *S. mutans*, there was a direct correlation between the inhibition zones diameter and the concentrations of nanosilver particles in the Nsf varnish. These results were in accordance with the results of studies conducted by Haghgoo [4] *et al.* and Shah *et al.* [17]. On the contrary, *S. salivarius* showed an inverse relationship between the inhibition zones diameter and the concentrations of nanosilver particles in the Nsf varnish. These results were not supported before by any other studies, as there were no sufficient studies conducted on the antibacterial effect of nanosilver particles on *S. salivarius*. Only one study conducted by Haghgoo *et al.* [4] concluded that the *S. salivarius* was more susceptible to Nsf varnish than that of *S. mutans*.

For *S. salivarius*, the varnish containing 0.1% (1000 μg/ml) nanosilver prompted bacterial reduction, and varnishes containing higher concentrations showed a significant bacterial reduction. Therefore, these results may be owing to using different concentrations of Nsf varnish (432, 216, and 108 μg/ml) in this study. Moreover, in this study, conventional fluoride varnish fluor protector showed no antibacterial effect on both *S. mutans* and *S. salivarius*, as there were no inhibition zones generated around the applied fluoride varnish on Mueller–Hinton agar media. These results might be owing to insufficient fluoride concentration in this product (1000 ppm), as some studies showed that the reduction of oral streptococci in saliva requires from 250 to 12 300 ppm of fluoride continuously [16].

Other studies stated that the other possible reason might be owing to some bacteria have evolved different abilities to withstand certain levels of fluoride. Fluoride-resistant strains of several oral bacterial species, including *S. mutans*, *S. salivarius*, and *Streptococcus sanguinis*, have been created in laboratories. Generally, a fluoride-resistant strain is able to grow in an environment containing 400–1000 ppm of fluoride, depending on the strain. This level of fluoride was at least three times higher than that which fluoride-sensitive strains could withstand [20,21].

Conclusion

Within the limitations of this study, the following could be concluded:

(1) The three concentrations of Nsf varnish had better antibacterial effect than conventional fluoride varnish against both *S. mutans* and *S. salivarius*.
(2) The antibacterial effect of Nsf varnish against *S. mutans* organisms gradually increases with the increase of the nanosilver particle concentration.
(3) The increase in concentration of nanosilver particles in the Nsf varnish did not affect the growth of *S. salivarius* organisms.

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Conflicts of interest
There are no conflicts of interest.

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