

ANTIMICROBIAL EFFICACY OF NANO SILVER FLUORIDE, FLUORIDATED SELF-ASSEMBLING PEPTIDE AND SODIUM FLUORIDE VARNISHES ON ORAL *STREPTOCOCCUS MUTANS*

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ABSTRACT

Aim: This study was designed to evaluate the antimicrobial efficacy of Nano Silver Fluoride, Fluoridated Self-assembling peptide, and Sodium Fluoride Varnishes on oral *Streptococcus mutans* bacteria.

Materials and Methods: The Antimicrobial efficacy was evaluated using agar-disk diffusion method. Three Muller-Hinton agar plates cultured with *Streptococcus mutans* (ATCC1402) were prepared. Each plate received three remineralizing agents (Group 1: Nano Silver Fluoride, Group 2: Fluoridated Self-assembling peptide; and Group 3: Sodium Fluoride varnish; as a positive control group) on three filter paper discs. Then the plates were incubated anaerobically for 24 h at 37°C. The diameter of the formed inhibition zones was measured in millimeters by a ruler to assess the antimicrobial effect. Statistical analysis was done using Kruskal–Wallis test and Mann–Whitney *post hoc* Test. The level of significance was set at $P \leq 0.05$.

Results: Both Fluoridated Self-assembling peptide and Nano Silver Fluoride had antimicrobial effects against *Streptococcus mutans*. However, the median value of inhibition zone size (mm) was greater in Fluoridated Self-assembling peptide 21 (18-22) as compared to Nano Silver Fluoride 9 (8-9), with a statistically significant difference (P value = 0.046). Enamelast Sodium Fluoride varnish showed no inhibition zones.

Conclusions: Fluoridated Self-assembling peptide had a better antimicrobial effect compared to Nano Silver Fluoride and conventional Sodium Fluoride varnish against *Streptococcus mutans*.

KEYWORDS: Antimicrobial, *Streptococcus mutans*, Nano Silver Fluoride, Fluoridated Self-assembling peptide.

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INTRODUCTION

Dental caries is considered one of the most prevalent chronic dental diseases affecting children.⁽¹⁾ It is a multifactorial disease caused by bacterial fermentation of food particles accumulated on the tooth surface and acid production, causing destruction and demineralization of the dental hard tissue.⁽²⁾

Cariogenic bacteria, especially *Streptococcus mutans* (*Strep. mutans*) and *Lactobacillus*, play an important role in the pathogenesis of dental caries. They are characterized by their ability to grow in an acidic medium and their rapid metabolism of sugars into organic acids, including lactic acid. It is believed that *Strep. mutans* is the main factor responsible for the initiation of enamel caries, while *Lactobacillus* is responsible for dentin caries development.⁽²⁾

A proper knowledge of the role of the microorganism in dental caries is important to decrease the disease prevalence and develop techniques for caries prevention. Dental caries prevention in children is considered a priority and can be done by several methods as dietary control, adequate oral hygiene measures, and professionally applied agents as topically applied fluoride.⁽³⁾

Fluoride is the cornerstone of the non-invasive management of early non cavitated carious lesions with the highest level of supporting evidence.⁽⁴⁾ One of the theories suggesting the fluoride mode of action on dental caries relates it to its antimicrobial effect on oral microbial flora either by direct inhibition of bacterial cellular enzymes or increasing the permeability of the bacterial cell membrane by the formation of hydrogen fluoride.⁽⁵⁾

However, many researchers tend to ignore the antimicrobial role of fluoride since it is effective only in high concentration and as they found no differences in the *Strep. mutans* count in persons living in fluoridated and in non-fluoridated areas.⁽⁵⁾ Moreover, its antimicrobial effect is only evident

for a short time and could be neglected for topical fluorides applied once every several months.⁽⁶⁾

One of the methods to enhance efficacy of topical fluoride varnish is by the addition of some metals as silver e.g. Silver Diamine Fluoride (SDF). Silver Diamine Fluoride (SDF) is an ammonia solution containing fluoride and silver ions.⁽⁷⁾ Although, The addition of silver ions produces a synergistic antimicrobial effect with the fluoride, silver precipitation produces dark black staining of the carious lesion, so its clinical use in the aesthetic region is discouraged.^(8,9)

This leads to the development of Nano-silver fluoride (NSF) as a trial to overcome the aesthetic adverse effect of SDF using Nano- sized silver particles (AgNPs). Unlike silver ion solutions, the intrinsic optoelectronic properties of AgNPs cause considerably less tooth discoloration. In addition, the nano size of silver particles increases contact area with microbial cells and results in easier penetration of the bacterial cell wall, producing broad-spectrum antibacterial activity in low concentrations.⁽¹⁰⁾

Recently, the biomimetic approach for the non-invasive management of non-cavitated carious lesions has shown promising results. One of the biomimetic re-mineralizing agents is Self-assembling peptide (P11-4).⁽¹¹⁾ P11-4 is a rationally designed small molecule that has the ability to self-assemble into a three-dimensional fibrillar scaffold in response to characteristic environmental stimuli.⁽¹²⁾

The proposed mechanism of action of P11-4 is that monomers or small aggregates diffuse into the subsurface lesion and self-assemble into fibers, forming a three-dimensional matrix that mimics enamel matrix proteins. This three-dimensional matrix has a high affinity for Ca^{2+} ions and acts as a nucleator for de novo hydroxyapatite formation, resulting in remineralization of the lesion. This suggests that the P11-4 matrix enhances natural salivary remineralization of more pronounced lesions but not the cavitated ones.⁽¹³⁻¹⁵⁾

Despite the recent development of different remineralizing materials and techniques. There is insufficient evidence about the antimicrobial efficacy of the recently introduced remineralizing agents. Therefore, this study was performed with the null hypothesis that there is no significant difference between the antimicrobial effects of (Nano Silver Fluoride, Sodium Fluoride varnish, and Fluoridated Self-assembling peptide).

MATERIALS AND METHODS

Sample size estimation:

The calculated least sample size for this study was three samples for each group (Total 9 samples). Sample size was calculated using the G*power version 3.1.9.2, Germany sample size calculator at 5% level of significance and 95% power of the study. The outcome of interest is inhibition zone as a measurement of antimicrobial activity of silver nanoparticles with Effect size = 6.46; based on Mean H_0 =8 mm Bharkhavy et al.(2022)⁽¹⁶⁾, Mean H_1 =16.4 mm, SD=1.3 Elsayed et al.(2021)⁽³⁾, α error prob = 0.05, β error prob =0.05 and power (1- β error prob) = 0.95.

Study design:

This study is a laboratory study. Three plates of *Strep. mutans* microorganisms were prepared. Each plate received three different materials: Nano Silver Fluoride varnish (Group 1), Curodont™ Repair Fluoride plus; Fluoridated self-assembling peptide (Group 2) and, Enamelast Sodium Fluoride varnish (Group 3) on filter paper discs.

Materials:

1. Nano silver fluoride (NSF). (Nano Gate Company, Cairo)
2. Curodont™ Repair Fluoride plus, Fluoridated Self-assembling peptide (F-SAP). (Credentis AG, Switzerland)

3. Enamelast Sodium Fluoride varnish (NaF). (Ultradent Company, USA).
4. Freeze-dried *Streptococcus mutans* (ATCC1402). (Microbiology department, faculty of medicine, Mansoura University).
5. Nutrient agar media. (From the local market)
6. Blood agar media. (From the local market)
7. Mueller-Hinton agar media (From the local market).

Methods:

Nano Silver Fluoride preparation and characterization:

Silver nanoparticle solution was prepared by the chemical reduction of silver nitrate ($AgNO_3$) with sodium borohydride and with the addition of chitosan as a stabilizing agent as described by dos Santos et al. 2014⁽¹⁷⁾. 1.0 g of chitosan was dissolved in 200 mL of 2% acetic acid (V/V); the solution was stirred overnight and filtered under vacuum. Next, 4.0 ml of silver nitrate (0.012 mol L^{-1}) was added to 60 ml of chitosan solution 30 minutes before the addition of sodium borohydride. Sodium fluoride (NaF) was added at the end and improved the stability of the solution. The prepared solution has Ag^+ [376.5 mg/mL] and Sodium fluoride [5028.3mg/mL]. The size and shape of silver nanoparticles were confirmed using transmission electron microscopy (TEM) and UV-VIS spectroscopy.

Microbiological test:

This study was performed using an agar disk-diffusion test. The steps of the test were employed as suggested by Balouiri et.al.⁽¹⁸⁾ A suspension of *Strep. mutans* (ATCC1402) 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. Then three Mueller-Hinton agar plates were prepared. Each plate received 0.5 McFarland

suspensions of *Strep. mutans*, and then it was spread on the Mueller-Hinton surface by a sterile swab applicator. Each plate containing *Strep. mutans* culture received 50 μ l of the three remineralizing agents on a filter paper disc with the aid of the micropipette device. The plates were further incubated anaerobically for 24h at 37°C in the incubator. The antimicrobial properties of materials were assessed by measuring the diameter of the formed bacterial inhibition zones in millimeters using a ruler (from the edge of the zone from one end to the next edge).

Statistical analysis:

The statistical analysis was done using SPSS computer program (version 22) using Kruskal-Wallis test between the three studied groups and Mann-Whitney test between every two groups. The P-value was adjusted to ≤ 0.05 . The data was expressed as median (minimum-maximum) as the data was non-parametric (Shapiro-Wilk to ≤ 0.05), as shown in Table 1.

RESULTS

The results of this study showed the ability of Nano Silver Fluoride and Fluoridated Self-assembling peptide (Curodont™ Repair fluoride plus) to produce an inhibitory effect against *Strep. mutans* bacteria. However, the median value of inhibition zones was greater in Fluoridated Self-assembling peptide 21 (18-22) as compared to Nano Silver Fluoride 9 (8-9), with a statistically significant difference ($p=0.046$), while Enamelast Sodium Fluoride varnish produces no inhibitory effect against *Strep. mutans* bacteria, as shown in (Table 1, Figure 1).

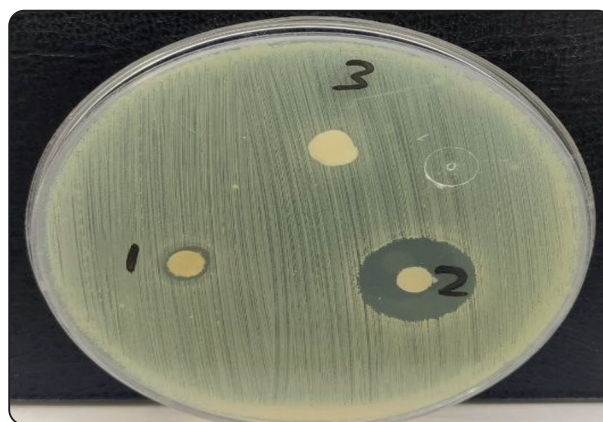


Fig. (1) Inhibition zones of *Streptococcus mutans* Produced by three materials: (1: Nano Silver Fluoride, 2: Curodont™ Repair fluoride plus and 3: Enamelast Sodium Fluoride varnish).

TABLE (1) Comparison between Nano Silver Fluoride, Fluoridated Self-assembling Peptide, and Sodium Fluoride varnishes regarding inhibition zones of oral *Streptococcus mutans* (mm).

Groups	Median	Minimum	Maximum	Kruskal-Wallis test (P-value)	Mann-Whitney test
Group 1 (NSF)	9	8	9	= 0.023*	P1 = 0.046*
Group 2 (F-SAP)	21	18	22		P2 = 0.034*
Group 3 (NaF)	0	0	0		P3 = 0.037*

P1: NSF VS F-SAP P2: NSF VS NaF P3: F-SAP VS NaF

***Statistically significant.**

DISCUSSION

Even though there is a great improvement in dental caries management techniques and materials, dental caries remains the most common chronic disease affecting children and adults.⁽¹⁹⁾ Dental caries is a multifactorial disease caused by the combination of several factors such as susceptible host, cariogenic microorganisms, and tooth surface.⁽²⁰⁾

Streptococcus mutans bacteria is considered the primary etiological factor of dental caries, it has the ability to adhere to the dental pellicle, produce lactic acid from the fermentation of simple carbohydrates, and form an acidic medium around the tooth that eventually results in tooth demineralization.⁽²¹⁾ For this reason, *Strep. Mutans* was selected as the test microorganism in this study as controlling the growth of this microorganism is considered an effective method of caries prevention.

Fluoride therapy has been the gold standard for caries prevention.⁽²²⁾ The ability of fluoride to control caries is related to its topical effect on the tooth surface and its antimicrobial effect against cariogenic bacteria.⁽³⁾ However, fluoride as an antimicrobial agent against *Strep. mutans* is only effective at a specific concentration, and further increases in the concentration to increase its effectiveness can lead to toxicity.⁽²¹⁾

Recently, new approaches have been developed to increase the antimicrobial and remineralization abilities of sodium fluoride. One of these new approaches depends on the addition of nanosilver ions to sodium fluoride. The nanosize of silver particles increases contact area with microbial cells and results in easier penetration of the bacterial cell wall, producing broad spectrum antibacterial activity in low concentrations.⁽¹⁰⁾ Several studies have demonstrated the strong antimicrobial effect of nano silver fluoride against *Strep. Mutans* bacteria.⁽²³⁻²⁶⁾

Recently, biomimetic approaches for enamel regeneration have attracted researchers' attention. One of these approaches is self-assembling peptide

(P11-4). Many studies have demonstrated the remineralizing ability of p11-4⁽²⁷⁻³⁰⁾ but there is a little available evidence on their antimicrobial efficacy against *Strep. Mutans* bacteria.

This encouraged us to perform this study to compare the antimicrobial efficacy of two of the recent remineralizing agents; Nanosilver fluoride and Fluoridated Self-assembling peptide (Curodont™ Repair fluoride plus) to the conventional sodium fluoride varnish against *Strep. Mutans* bacteria using agar disk diffusion method. Agar disk diffusion, also known as Kirby-Bauer method, is considered the gold standard for susceptibility testing because of its simplicity, its ability to test different microorganisms and different materials, its ease of result interpretation, and its high levels of reproducibility.^(18,31)

In the present study, Fluoridated Self-assembling peptide (Curodont™ Repair Fluoride Plus) revealed a larger zone of inhibition 21(18-22) against *Strep. Mutans* compared to Nano silver fluoride 9(8-9) and Enamelast sodium fluoride varnish. Enamelast sodium fluoride varnish did not reveal any zones of inhibition, indicating no antimicrobial activity against *Strep. mutans*. So, the null hypothesis was rejected due to the presence of statistically significant differences in the antimicrobial efficacy of different agents tested (P = 0.023).

In this study, fluoridated self-assembling peptide (Curodont™ Repair Fluoride Plus) showed the highest antimicrobial efficacy against *Strep. mutans*. Curodont™ Repair fluoride plus is a relatively new material, so a direct comparison of its antimicrobial efficacy with other studies could not be done because of a shortage of literature.

However, according to our knowledge, only one study has used a different material (Curodont™ Protect; Credentis, Switzerland) with the same remineralization technique (self-assembling -based remineralization).⁽²¹⁾ Curodont™ Protect differs from Curodont™ Repair Fluoride Plus in the form and mode of action. Curodont™ Protect is a

remineralizing tooth gel containing the biomimetic *Oligo-Peptide 10³* technology for in-office and home use that is used for protection from caries and erosion. While Curodont™ Repair Fluoride Plus is a liquid containing revolutionary *Monomer-Peptide 10⁴* technology for in office only use that is used for Regenerative treatment of initial carious lesions.

Gayas et al.⁽²¹⁾ evaluated the antimicrobial efficacy of curodont protect on *Strep.mutans* using a time-kill assay over a period of 24 h and concluded that The self-assembling peptide P11-4-based tooth remineralization agent (curodont protect) showed an inhibitory effect on *Strep. mutans* and its application can inhibit the formation of the cariogenic dominant biofilm.

In this study, NSF also showed an antimicrobial effect against *Strep. mutans* 9(8-9) compared to conventional sodium fluoride varnish. This result was in agreement with Elsayed et al.⁽³⁾ who found that NSF varnish showed an antimicrobial effect against *Strep.mutans* while conventional sodium fluoride varnish showed no inhibition zone, while Haghgoo et al.⁽³²⁾ revealed that the minimum nanosilver concentration needed for inhibition of the growth of *Strep. mutans* was more than 0.5% and less than 1%.

Moreover, in the present study, Sodium fluoride varnish (Enamelast) showed no inhibition zone against *Strep. mutans*. Matar et al.⁽³³⁾ assumed that this low antimicrobial performance could be related to its weak fluoride release caused by its hydrophobic resinous content. This is consistent with Al Dehailan et al.⁽¹⁹⁾, who related the fluoride varnishes mechanism of action to their composition.

Limitation of the study:

This in vitro study design is not able to reproduce the complex intraoral conditions present during tooth demineralization. Moreover, in this study, the antimicrobial effect of the tested agents was determined only on *Strep. mutans* due to its main role in the initiation of dental caries. However, because of the polymicrobial etiology of dental caries, further studies are needed on other cariogenic bacteria.

CONCLUSION

Curodont™ Repair Fluoride Plus showed a better antimicrobial effect as compared to Nano Silver Fluoride and Enamelast Sodium Fluoride varnish against *Strep. mutans*.

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