A New “Silver-Bullet” to treat caries in children – Nano Silver Fluoride: A randomised clinical trial

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A B S T R A C T

Background: Untreated dental caries in children remains a public health challenge in poor communities.
Objectives: This prospective controlled clinical trial investigated the effectiveness of a new anti-caries agent, Nano Silver Fluoride (NSF), applied once a year to arrest caries in children.
Methods: One hundred thirty decayed primary teeth were randomly divided into two groups: NSF as the experimental agent and water as the control group. Teeth were clinically diagnosed and treated by one masked examiner and followed up at seven days and five and 12 months by another calibrated examiner who was blinded to the type of treatment. The criteria of the ICDAS II were followed to determine the activity of lesion and the diagnosis of caries. The Pearson’s chi-square test was used to compare the groups during different follow-up exams.
Results: At seven days, 81% of teeth in the NSF group exhibited arrested caries, whereas in controls, no teeth had arrested decay (p < 0.001) [PF, prevented fraction = 81%]. After five months, the NSF group had 72.7% with arrested decay, and the control group had 27.4% (p < 0.001) [PF = 62.5%]. At 12 months, 66.7% of the lesions treated with NSF were still arrested, while the control group had 34.7% remaining arrested (p = 0.003) [PF = 50%]. The number needed to treat (NNT) at five months was two, and at 12 months, the number was three.
Clinical Significance: The NSF formulation is effective to arrest active dentine caries and not stain teeth.
Conclusions: NSF was demonstrated to be effective in arresting caries in children in poor communities.
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1. Introduction

Dental caries is one of the most common chronic childhood diseases,¹,² and in underprivileged parts of the world, many decayed teeth are left untreated.³ Innovative approaches are needed to increase access to dental care for those at need. The development of anti-caries agents capable of reducing caries rates in underprivileged populations has represented a challenge for dental researchers and clinicians. A variety of chemotherapeutic agents have been tested for preventing and arresting caries, including antibiotics, metal ions and various types of fluoride agents.⁴,⁵

A recent review⁶ on the use of SDF revealed that this caries agent, when applied once a year, has the ability to prevent new caries and to arrest existing caries with a high rate of success. However, adverse effects have been reported, such as staining carious tissue black due to the oxidation process of ionic silver contained in its formulation and reversible slightly painful lesions in oral mucosa caused by accidental contact of SDF solution, which may disappear within 48 h.⁵ Nano Silver Fluoride⁶ (NSF), a new experimental formulation containing silver nanoparticles, chitosan and fluoride combines preventive and antimicrobial properties and was developed to be an effective anti-caries agent without staining the porous dental tissues black, as does silver diamine fluoride and amalgam.

This new substance is safe to be used in humans and has excellent antimicrobial properties against Mutans streptococci and Lactobacilli, the primary pathogens responsible for the development of dental caries. NSF is available as a yellow solution that proved to be stable for three years. This substance is eco-friendly and low in cost.⁷

Thus, this prospective controlled clinical trial investigated the effectiveness of a new anti-caries agent for preventing and arresting caries in children. The null hypothesis tested was that there were no differences in the effectiveness of Nano silver fluoride solution and water in arresting dentine caries.

Fig. 1 – Transmission electron microscopy of the NFS.
The present paper reports the final 12-month results of this study.

2. Materials and methods

2.1. Preparation of Nano Silver Fluoride*

To prepare the colloidal silver, 1.0 g of chitosan was dissolved in 200 mL of acetic acid 2% (V/V). The solution was stirred overnight and subsequently filtered under vacuum. Next, an aliquot of 60 mL of chitosan solution was placed in an ice bath while being stirred, and 4.0 mL was added to a solution of silver nitrate \( \times 0.012 \text{ mol L}^{-1} \), 30 min before the addition of sodium borohydride. The relationship between AgNO\(_3\) and NaBH\(_4\) was maintained at a 1:6 mass and added dropwise. The reduction of Ag\(^+\) was initiated immediately, as the solution changed from colourless to light yellow and ended up reddish. The silver nanoparticles had an average size of 3.2 ± 1.2 nm and a spherical shape (Fig. 1). Fluoride (NaF) was added only at the end of the experiment, which improved the stability of the solution. The concentrations of each component, as expressed in micrograms per millilitre, were as follows: Chitosan [28,585 μg/mL], Ag\(^+\) [375.3 μg/mL] and Sodium fluoride [5033.8 μg/mL].

2.2. Clinical trial

This study was approved by the International Review Board (IRB) of the University of Pernambuco (Protocol No. 119/12) in accordance with the World Medical Association Declaration of Helsinki. Written information explaining the purpose of the study was sent to the parents who sent back signed written consent. Verbal consent was obtained from the children prior to treatment. Patients requiring extractions were scheduled for dental appointments. This study was conducted between 2012 and 2013 in a poor community of the city of Gravatá, in Pernambuco, northeastern Brazil, where the public water supply is not fluoridated. The school meals are rich on sugary products as they are more affordable for the education authorities and also for the regular families.

All children were provided with a toothbrush, fluoridated toothpaste (1000 ppm F) and oral hygiene and healthy diet instructions before their dental examinations. Children were excluded from the trial if they presented syndromes or were undergoing medical treatment for chronic or acute diseases, to avoid bias for reduced salivary flow. The sample size was determined using the PC-SIZE program, version 1.01(c), 1990 (Gerard E. Dallal, Andover, MA, USA). Based on the pilot study, the rate of success for NSF was 73.1%, whereas the rate of success was 27.4% for controls, indicating that 54 primary teeth in each group would comprise the sample to generate statistically significant data, considering a type 1 error of 5% and a CI of 99.9%. In addition, to make up for possible losses, the sample size was extrapolated by 20% to enhance precision. Thus, 65 primary teeth were intended to act as the experimental group, Nano Silver Fluoride (NSF), and 65 teeth were intended to act as the control group, water.

The investigation comprised primary teeth with active caries lesions at the dentine level. The cavities had an average shallow depth and no pulpal exposure or fistula, corresponding to the International Caries Detection and Assessment System, code 5 (ICDAS II) for occlusal and smooth surfaces. At baseline, the children did not present caries in their permanent teeth. A single calibrated investigator selected and treated the subjects.

This study design was a randomised, controlled, double-blind trial. The method of randomisation was performed to maintain a similar distribution of the number of teeth in each group, as blocks of four teeth were included in different combinations in two sealed envelopes for each type of intervention. The teeth were clinically treated by one examiner. The follow up exams were performed by another calibrated examiner who was blind to the type of treatment. The children and guardians were also blind to the type of treatment.

For caries treatment in both techniques, no effort was made to remove the caries or unsupported enamel. For both techniques, were used cotton rolls to isolate the teeth from saliva.

The NSF (33,989.8 μg/mL) solution was left in contact with the tooth surface for 2 min. Each tooth received two drops of NSF with a micro brush, equivalent to a dose of 10 mg of the solution. For the control group, only one drop was given. Both treatments were performed only once in 12 months.

The teeth were assessed clinically using visual and tactile inspection by a trained blind examiner after a week, five months and 12 months. The ICDAS II criteria were used to classify active caries lesions in both groups. Active caries was recorded with a blunt probe that, when applied with light force, easily penetrated the dentine, whereas arrested caries was recorded if the dentine could not be penetrated.

For data analysis, descriptive statistical techniques and the chi-squared and Fisher's exact tests for categorical variables were used. Normal distribution of quantitative data was checked by the Kolmogorov-Smirnov test and the Mann-Whitney test was applied to compare the quantitative variables between groups. Statistical tests were performed with a margin of error of 5.0%. Data were stored on EXCEL spreadsheet and statistics were calculated using the Statistical Package for the Social Sciences (SPSS) version 17 (IBM, Chicago, IL, USA). This statistical analysis is in agreement with the recommendations for oral and dental research.9

On separate occasions, 10% of the sample was randomly selected to be re-examined for intra-examiner reproducibility. Intra-examiner reproducibility for caries diagnosis was calculated by Cohen's kappa test. The kappa for intra-examiner agreement was 0.90 for caries and 1 for arrested caries.

This clinical trial was registered at www.clinicaltrials.gov under the protocol NCT01950546 and adhered to the CONSORT guidelines.

3. Results

The sample comprised 60 school children, mean age 6.31 ± 0.60 years; of these, 26 (44.1%) were male, and 33 (55.9%) female (p > 0.05). From the total, 73% of the treatment
were in posterior teeth and 23% were in anterior teeth, 64.6% of the carious lesions involved only one carious surface and 35.4% two or more surfaces. The mean dmft (decayed, missing and filled teeth) at baseline was 4.76 ± 2.65 with no statistically significant difference between the two groups (p > 0.05).

One hundred thirty decayed primary teeth were randomly divided into two groups: 63 teeth for the NSF group and 67 for the control group. The number of teeth in each group was not similar because each child had more than one tooth included in the study, and the protocol required the same type of treatment for the same mouth, resulting in no statistically significant differences (p > 0.05).

After one week from the examination, there was no loss of participants or teeth due to exfoliation or extraction. At 12 months, there were 12 losses in the NSF group and 18 in the control group. Those losses were predictable; therefore, was applied a correction factor of 1.2 to the sample size to make up for possible losses (Diagram 1).

After seven days of follow-up, 81% of decayed teeth in the NSF group showed hard arrested dentine, which was not observed in the control group (p < 0.001). After five months, the NSF group had 72.7% of the teeth showing arrested cavities, and the control group had 27.4% (p < 0.001). At 12 months, 66.7% of the lesions in teeth treated with NSF were still arrested, while the control group showed 34.7% (p = 0.003). The preventative fraction showed that the use of NSF decreased by 81%, 62.5% and 50% the risk of caries remaining active in the intervals of seven days, five months, and 12 months, respectively, when compared to the control group. The number need to treat (NNT) at five

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Diagram 1 – Number of teeth that were randomly assigned, received the intended treatment and were analysed.
months was two; at 12 months, the number was three (Table 1).

The effectiveness of the NSF was not associated with the number of decayed surfaces, at 5 months (p = 0.257) and after 12 months (p = 0.545), however, it was more effective in anterior than in posterior teeth, at 5 months (p = 0.012) and at 12 months (p = 0.010). The same applied to the control group that did not show statistically significant results for number of involved surfaces at 5 months (p = 0.147) and at 12 months (p = 0.232). For the control group there was not association between number of decayed surfaces and type of teeth at 5 months (p = 0.319), however, at 12 months results were

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>NNT</th>
<th>PF or RRR</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days</td>
<td></td>
<td>1.23</td>
<td>81%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Success</td>
<td>NSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>51 (81.0)</td>
<td>0 (0.0)</td>
<td>1.23</td>
<td>81%</td>
</tr>
<tr>
<td></td>
<td>12 (19.0)</td>
<td>67 (100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Months</td>
<td></td>
<td>2.22</td>
<td>62.5%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Success</td>
<td>NSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>40 (72.7)</td>
<td>17 (27.4)</td>
<td>2.22</td>
<td>62.5%</td>
</tr>
<tr>
<td></td>
<td>15 (27.3)</td>
<td>45 (72.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Months</td>
<td></td>
<td>3.12</td>
<td>50%</td>
<td>0.003</td>
</tr>
<tr>
<td>Success</td>
<td>NSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>34 (66.7)</td>
<td>17 (34.7)</td>
<td>3.12</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>17 (33.3)</td>
<td>32 (65.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square test.

Table 2 – Final logistic regression model for the evaluation of the effectiveness, according to the type of tooth and the number of surfaces involved.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 months</th>
<th>Total</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Success</td>
<td>Fail</td>
<td></td>
</tr>
<tr>
<td>NSF</td>
<td>Number surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>27 (79.4)</td>
<td>7 (20.6)</td>
<td>34</td>
</tr>
<tr>
<td>Multiple (Two or more) Type</td>
<td>13 (61.9)</td>
<td>8 (38.1)</td>
<td>21</td>
</tr>
<tr>
<td>Anterior</td>
<td>17 (94.4)</td>
<td>1 (5.60)</td>
<td>18</td>
</tr>
<tr>
<td>Posterior</td>
<td>23 (62.1)</td>
<td>14 (37.9)</td>
<td>37</td>
</tr>
<tr>
<td>Control</td>
<td>Number surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>13 (35.1)</td>
<td>24 (64.9)</td>
<td>37</td>
</tr>
<tr>
<td>Multiple (Two or more) Type</td>
<td>4 (16.0)</td>
<td>21 (84.0)</td>
<td>25</td>
</tr>
<tr>
<td>Anterior</td>
<td>5 (26.0)</td>
<td>8 (74.0)</td>
<td>13</td>
</tr>
<tr>
<td>Posterior</td>
<td>12 (24.4)</td>
<td>37 (75.6)</td>
<td>49</td>
</tr>
<tr>
<td>Treatment</td>
<td>12 months</td>
<td>Total</td>
<td>p value*</td>
</tr>
<tr>
<td></td>
<td>Success</td>
<td>Fail</td>
<td></td>
</tr>
<tr>
<td>NSF</td>
<td>Number surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>22 (70.9)</td>
<td>9 (29.1)</td>
<td>31</td>
</tr>
<tr>
<td>Multiple (Two or more) Type</td>
<td>12 (60.0)</td>
<td>8 (40.0)</td>
<td>20</td>
</tr>
<tr>
<td>Anterior</td>
<td>14 (93.3)</td>
<td>1 (6.70)</td>
<td>15</td>
</tr>
<tr>
<td>Posterior</td>
<td>20 (55.5)</td>
<td>16 (44.5)</td>
<td>36</td>
</tr>
<tr>
<td>Control</td>
<td>Number surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>11 (44.0)</td>
<td>14 (66.0)</td>
<td>25</td>
</tr>
<tr>
<td>Multiple (Two or more) Type</td>
<td>6 (25.0)</td>
<td>18 (75.0)</td>
<td>24</td>
</tr>
<tr>
<td>Anterior</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>6</td>
</tr>
<tr>
<td>Posterior</td>
<td>12 (27.9)</td>
<td>31 (72.1)</td>
<td>43</td>
</tr>
</tbody>
</table>

* Fisher’ exact test.
initiation and development of carious lesions, respectively. Furthermore, the small size of the NSF nanoparticles (3.2 ± 1.2 nm) and their spherical shapes potentiate the antimicrobial effect by increasing the contact surface14–16 (Fig. 1). There are several theories explaining the mechanism of action of fluoride, which invoke its actions towards demineralisation,17 enhancement of remineralisation18 and inhibition of bacterial activity in the plaque.19 The nanoparticles of silver are a promising component to combat residual bacteria in tooth cavity and invading bacteria at the margins of the restorations. Thus, Zhang et al. (2013)20 suggest the use of silver nanoparticles in adhesives, composites, cements and sealants to inhibit biofilms and caries.

Targino et al. (2014)7 evaluated the antimicrobial and cytotoxic activity of Nano Silver Fluoride against Streptococcus mutans in comparison to chlorhexidine and silver diamine fluoride. It was found that the NSF is a bacteriostatic and bactericidal compound and the MIC and MBC values for the ATCC (25,175) strains were 33.54 ± 14.52 µg/mL and 50.32 µg/mL, respectively. The difference between the MIC values (p = 0.032) and the MBC (p = 0.035) of the tested substances were assessed for statistical significance. The NSF was not toxic at any concentration tested for any type of erythrocyte and is more biocompatible than SDF.

Because of the growing interest in the use of SDF, a preliminary study on the pharmacokinetics of silver fluoride has been reported by Vasquez et al. (2012)21. Results suggest that serum concentrations of fluoride and silver after topical application of SDF should pose little toxicity risk when used in adults21 and inhibit the demineralisation and preserve the collagen from degradation in demineralised dentine.22 However, the same has not been clarified for nano silver fluoride yet.

The effectiveness of this new anti-caries agent (66.7%) was similar to that of silver diamine fluoride (SDF) 30% (66.9%)23 when applied once a year, and the agent also had the advantage of not staining the dental tissue black (Fig. 2). Nano-silver compounds do not form oxides when contacting oxygen in the medium. Also, unlike SDF, the NSF solution had no metallic taste.20 In the same way as is true in SDF treatment,10,11 NSF application is inexpensive and thus can be afforded by most communities. The treatment procedure is simple and requires no full dental equipment or a clinical setting. Because this process requires non-invasive procedures, the risk of cross-infection is significantly reduced.

As far as dietary controls are concerned, this important item was not considered because it remains a very delicate issue to be addressed and evaluated in underprivileged communities, which often contain extreme poverty, hunger and malnutrition. In such communities, school meals consist mainly of high calorific, cariogenic foods. However, in each follow-up examination, all children received a toothbrush, fluoridated toothpaste, and dietary and oral hygiene instructions. These interventions may explain the positive results for caries arrest in the control group, considering that the public water supply is not fluoridated.

According to the accuracy of ICDAS II system used in this study, a systematic review24 compared this system with a histological classification system and concluded that is possible to assess the activity of primary coronal caries

Fig. 2 – Teeth treated with NSF after 12 months.

Statistically significant (p = 0.015) for type of teeth, which could be explained by the physiological exfoliation of the primary anterior teeth that might have generated a confounding variable (Table 2).

At no time did the carious lesions of the NSF group turn black, which is a chemically desired effect for not stigmatising poor children (Fig. 2). Upon attempts to remove dentine treated with NSF, the tissue turned hard and took on a crumbly consistency, while the control exhibited a blackened arrested caries.

4. Discussion

The null hypothesis of this study was not supported by the present report. It was found that the annual application of the NSF solution was more effective in hardening and arresting dentine caries in primary teeth than the placebo. The prevented fraction in this context indicates caries arrest in the experimental group compared to the control group (higher is better) and a decreased risk of caries was actively maintained at seven days and five and 12 months (Table 1).

The NSF-prevented fraction of caries arrest in the primary teeth was similar to the SDF (Silver Diamine Fluoride)-prevented fraction reported in the clinical trial conducted by Llodra et al. (2005)10 (>55%) but lower than those reported by Chu et al. (2002)11 (>96%). This can be explained by differing designs and application intervals. However, both substances demonstrated effectiveness in arresting caries.

The NNT indicates the number of teeth needs to be treated to prevent the development of 1 additional dental caries (lower is better). Therefore, it provides a measure of the efficacy of the treatment. The NNT can also be used to extrapolate the effects for individuals. The NNT for NSF in five and 12 months were substantially low, at two and three, respectively.

The effectiveness of NSF in arresting caries, as found in this work, can be explained by the synergism of the components of its formulation (chitosan silver nanoparticles and fluoride). Various studies show that chitosan and silver nanoparticles have antimicrobial activity against mutans streptococci and lactobacilli,12,13 the main pathogens responsible for the
lesions accurately by using the combined knowledge obtained from the visual appearance and tactile sensations during probing.

More studies are required to investigate alternative protocols. Furthermore, the applications of NSF for treating tooth sensitivity and root caries need to be evaluated.

5. Conclusions

NSF was demonstrated to be effective in arresting caries in children in poor communities.

Acknowledgments

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