RESEARCH ARTICLE

Nano-silver Fluoride at Higher Concentration for Caries Arrest in Primary Molars: A Randomized Controlled Trial

Manuela Arnaud[1], Paulo CM Junior[2], Maria GS Lima[3], Amitis VC e Silva[4], Joas T Araujo[5], Andre Gallembeck[6], Arnaldo de França Caldas Júnior[7], Aronita Rosenblatt[8]

Abstract

Aim and objective: To compare the arresting caries effectiveness of two different silver nanoparticle (AgNP) concentrations of nano-silver fluoride (NSF), namely 400 and 600 ppm. The hypothesis is that in posterior primary teeth with occlusal and approximal active dentin carious lesions, NSF 600's effectiveness will be higher than that of the NSF 400 solution over a 6-month follow-up period.

Materials and methods: This was a double-blind randomized clinical trial (RCT) conducted in the city of Recife, Brazil. A total of 337 children aged 5–7 years who attended the University of Pernambuco Dental School's clinics were examined. A single-blinded investigator conducted the examinations and treatment of the children. After baseline examination and recording of the dmft index, children were allocated to one of two study groups (NSF 600—intervention and NSF 400—positive control). In both groups, each tooth received two drops of NSF and treatments were performed only once in 6 months. The follow-up examinations were visual and tactile, performed in 30, 90, and 180 days to determine the activity of caries. The carious lesions that were not arrested in 30 days were recorded and referred for restorative treatment.

Results: The NSF 600 showed higher rate of success in arresting caries (72.7%, $p = 0.025$) compared with NSF 400 (56.5%).

Conclusion: The higher rate of success of NSF 600 can be explained by the higher concentration of AgNPs.

Clinical significance: Nano-silver fluoride has emerged as an excellent alternative to silver diamine fluoride (SDF), as it adds a high antibacterial effect to better esthetic results. Treatment is simple, non-invasive, and incurs low cost. It is ideal for use in community-based programs to increase the access to dental care without staining teeth black like other silver products.

Keywords: Dental caries, Primary teeth, Randomized controlled trial, Silver nanoparticles.

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Introduction

The American Academy of Pediatric Dentistry (AAPD) recognizes that dental caries continues to be a prevalent and severe disease in children.[1] In many developing countries, caries remain untreated due to under-developed oral healthcare systems.[2]

Untreated carious lesions may advance to pain, spreading of the infection, and malnutrition due to feeding difficulty.[3] Restorative care for dental caries in the developed world often requires the use of sedation and general anesthesia; it involves high costs, it may be a health risk, and it may not be the best way of preventing the recurrence of lesions. As a result, caries treatment currently focuses on preventing and arresting the disease processes.[4]

Silver diamine fluoride (SDF), a material with easy application and low cost, has been used to arrest and prevent caries with a high rate of success since 1969.[5] It is widely tested and it seems to provide good caries protection, with a once yearly application.[6] However, its use promotes the staining of the dental carious tissue black due to the oxidation process of ionic silver contained in its formulation.[6–9]

Silver nanoparticle (AgNP) anticaries agents are not irritating and have an effective bactericidal effect against *S. mutans* dynamic biofilm. The properties of these new products suggest their potential as an effective cariogenic biofilm inhibitor, with esthetic and antimicrobial advantages over traditional silver fluoride products.[10]

Nano-silver fluoride (NSF) is an AgNP product comprising chitosan and fluoride that arrests and prevents carious lesions and does not cause toxicity. Nano-silver fluoride has already been tested clinically, and it proved to be effective in arresting caries in the enamel and dentin without staining the teeth.[11] Treatment with NSF is simple, non-invasive, and of low cost. This new anticaries agent is ideal for use in disadvantaged communities in developing countries,[11] as it can help in increasing the access to dental care, without stigmatizing individuals through the development of black teeth, as SDF does.[12]

This study aimed to compare the arresting caries power of two different concentrations of NSF AgNPs, namely NSF 400 ppm and NSF 600 ppm. The hypothesis was that in posterior primary teeth with occlusal and proximal active dentin carious lesions, NSF 600's

[1–5]Department of Pediatric Dentistry, University of Pernambuco, Recife, Pernambuco, Brazil

[6]Department of Chemistry, Federal University of Pernambuco, Recife, Pernambuco, Brazil

[7]Department of Clínica Integrada, Faculdade de Odontologia de Pernambuco - Universidade de Pernambuco, Camaragibe, Brazil

[8]Department of Postgraduation/Pediatric Dentistry, University of Pernambuco, Camaragibe, Brazil

Corresponding Author: Manuela Arnaud, Department of Pediatric Dentistry, University of Pernambuco, Recife, Pernambuco, Brazil, Phone: +5581981031983, e-mail: manuelaarnaud83@gmail.com

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effectiveness would be higher than that of the NSF 400 solution over a 6-month follow-up period.

Materials and Methods

This study was a double-blind randomized clinical trial (RCT) conducted in the city of Recife, Brazil. It was conducted in accordance with the Declaration of Helsinki and Brazil's Health National Council (resolution 466/2012), and approved by the University of Pernambuco's ethics committee (no. 08170412.0.0000.5207) and the Health Secretary of the State of Pernambuco.

Recife is the capital and largest city of the state of Pernambuco. With 3,995,949 inhabitants, non-fluoridated water, and the highest country proportions of dental decay, the city is located in the Northeast region of Brazil. The children enrolled in this RCT attended the University of Pernambuco Pediatric dentistry's clinics and its institutional Oral Health Promotion program.

Initially, 337 children aged 5–7 years were examined. Of these, 74 children with at least one active dentin occlusal or proximal carious lesion in the primary molars (ICDAS 5), without signs or symptoms of pain or pulp infection, were included in the study. The teeth with both occlusal and proximal caries (occlusoproximal) were classified as proximal carious lesions; still, the same tooth with two or more separated cavities (occlusal and proximal), was also counted as proximal for data analysis at tooth and surface levels.

All children presented informed consent signed by the parents/tutors before the implementation of the study. A single trained investigator examined, selected, and treated the subjects. A different trained investigator worked on allocation concealment and randomization of the subjects.

The sample calculation was based on a previous pilot study with both solutions tested in this RCT (NSF 400 ppm and NSF 600 ppm) with a 6-month follow-up period. With the success rates for the solutions (NSF 600, 87.5%; NSF 400, 53.5%), a power of 90%, and a significance level of 1%, the calculation indicated that 52 teeth should be included per group. With an additional 20% of possible losses, the final sample calculation indicated 63 teeth per group.

After baseline examination and recording of the decayed, missing, filled teeth (dmft) index, the children received computer-generated random numbers inside opaque sealed envelopes that indicated the study group they were allocated to. Each child could only have one type of solution applied to the teeth to avoid interactions between solutions and study bias. Therefore, the randomization process was based on the subjects with an allocation ratio of 1:1.

All participants were provided with toothbrushes and toothpaste (1,500 ppm of fluoride). Before clinical examinations and solution application, the children were asked to brush their teeth to remove food debris. For caries treatment, no effort was made to remove the carious tissue or unsupported enamel. Cotton rolls were used to isolate the teeth from saliva. The NSF solutions were left in contact with the tooth surface for 2 minutes in both the intervention (NSF 600) and control (NSF 400) groups. Each tooth received two drops of NSF with a micro brush, equivalent to a dose of 10 mg of the solution. Both treatments were performed only once in 6 months.

Nano-silver fluoride application protocol:

- Children brush teeth to remove food debris.
- Children positioned seated on a regular chair.
- Isolate the decayed tooth with cotton rolls.
- Remove excess saliva by a gauze or cotton pellet, if needed, but no air drying.
- Apply the solution on the carious tooth surfaces with a micro brush and rub for 10 seconds.
- Wait 2 minutes to remove cotton rolls and conclude the application protocol.
- Inform the parent or tutor that the child should not eat, drink, or rinse mouth in the next 30 minutes.

The dmft indexes and caries diagnostics were recorded based on the World Health Organization[13] recommendations at 30, 90, and 180 days after NSF applications, with natural light and a ball-end probe. The exams were visual and tactile. If a wall or floor of the lesion was soft and easily penetrated by the probe using light force, then it was diagnosed as active. Arrested caries showed smooth, hard surfaces. At the child level, the decayed, missing, and filled tooth (dmft) index was adopted for recording dental caries experience. At tooth surface level, caries activity (arrested/active) and surfaces involved (occlusal and proximal) were evaluated.

For the statistical analysis of the categorical data, descriptive statistics and Pearson's Chi-square test were used. The continuous variables (dmft and age) were analyzed through means and standard deviation (SD). Dmft was also categorized (low and high) and analyzed through Pearson's Chi-square test. The missing data were related to the sample lost, described in sample flowchart (Flowchart 1) and were not included in the statistical analysis. The data normality assumption was checked through Kolmogorov–Smirnov test. Data were analyzed using the software SPSS 23.0 for Windows (SPSS Inc., Chicago, USA). The level of statistical significance was set at 0.05.

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 via Cohen's kappa test, which indicated 0.90 for caries and 1 for arrested caries.

Results

At baseline, 337 children were screened. Of these, 72 that matched the inclusion criteria were included and randomly allocated to the study groups. The experimental group (NSF 600) comprised 36 children, and 36 children were allocated to the NSF 400 group. By this time, there was a sample loss of four children in the NSF 400 group; one child moved to another town, and three children dropped out of the study.

The flowchart (Flowchart 1) shows the number of children enrolled and the number of teeth in each study group.

After 6 months of follow-up, the final sample comprised 68 children with 173 decayed primary molars treated with NSF solutions, with 29 (42.6%) boys and 39 (57.4%) girls ($p = 0.038$), who had a mean age of 6.06 years (SD = 0.844 years) and mean dmft of 5.69 (SD = 3.17). Of these, 88 teeth (50.9%) received the NSF 600 application, and 85 (49.1%) received NSF 400 ($p = 0.025$).

At surface level, more than half of the dental surfaces included in this study were occlusal (56.6%), while 43.4% were proximal (Table 1). The difference in the caries arrest rates according to the type of surfaces was statistically significant ($p = 0.035$), showing a higher number of arrested caries in the occlusal lesions (40.5%) than proximal ones (24.3%), while using the fractions of a total number of teeth analyzed (Table 2).

Flowchart 1: Recruitment, enrolment, randomization, withdrawal, and completion of subjects

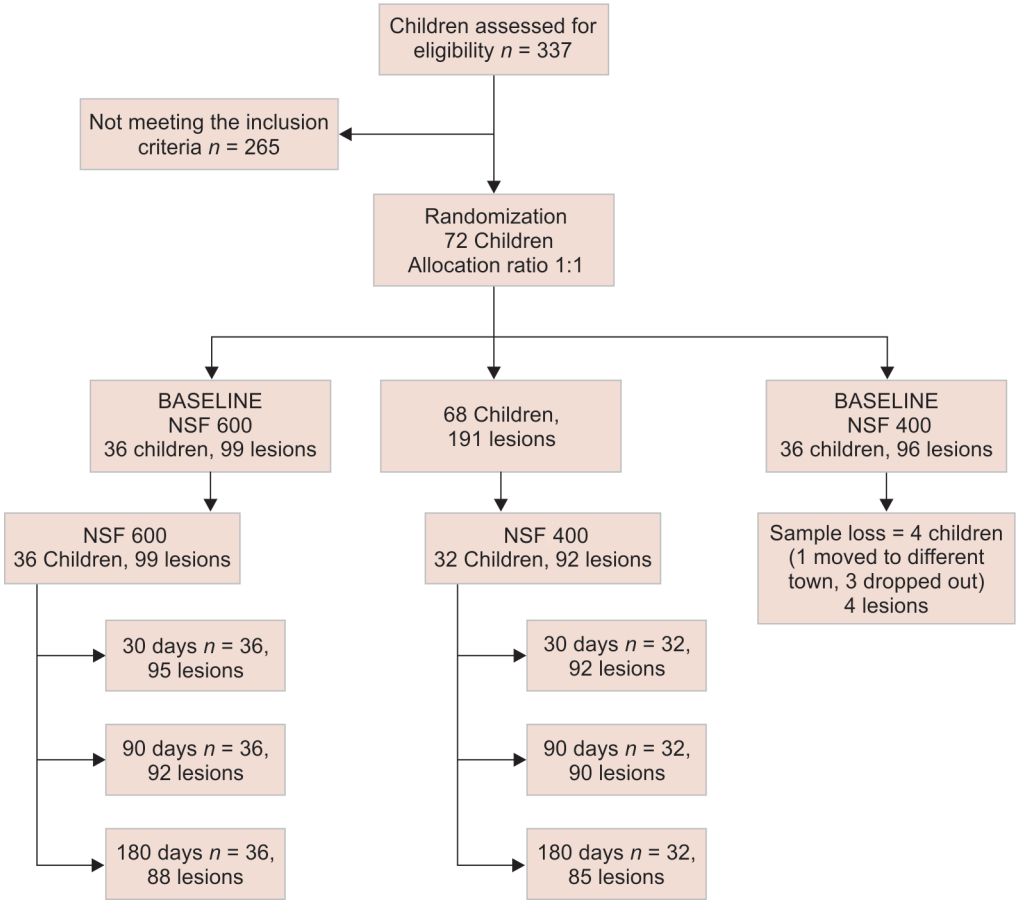

Table 1: Descriptive statistics with final sample characteristics—a 6-month follow-up

		*NSF** 600 N (%)*	*NSF** 400 N (%)*
Number of children		36/68 (52.9%)	32/68 (47.1%)
Number of teeth		88/173 (50.9%)	85/173 (49.1%)
Mean age		6.06 (SD = 0.860)	6.06 (SD = 0.840)
Gender	Female	21/39 (54%)	18/39 (46%)
	Male	15/29 (51.7%)	14/29 (48.3%)
Mean dmft***		5.7 (SD = 3.30)	5.6 (SD = 3.07)
Tooth carious surfaces*	Occlusal	43/98 (43.9%)	55/98 (56.1%)
	Proximal	45/75 (60%)	30/75 (40%)

*The teeth with both occlusal and proximal caries (occlusoproximal) were classified as proximal carious lesion; still, the same tooth with two or more separated cavities (occlusal and proximal), was also counted as proximal for data analysis at tooth and surface levels
**NSF, nano-silver fluoride
***dmft, decayed, missing, filled teeth

Regarding the caries arrest rates at tooth level, 64.7% (112/173) of the teeth showed arrested carious lesions. The difference in the arrested caries rates between the groups was significant ($p = 0.025$). The NSF 600 group showed 72.7% (64/88) arrested caries, while the rate in the NSF 400 group was 56.5% (48/85; Table 2).

At the child level analysis, a similar trend was observed in caries arrest after 6 months of follow-up. Higher NSF concentration showed higher success (53 vs 38%), and it was seen more on the occlusal surfaces than proximal (63 vs 40%). Higher arrest rates were observed with low dmft group defined lower or equal to three (63 vs 39%). All these findings were not statistically significant possibly due to the small sample size, as shown in Table 3.

Discussion

Nano-silver fluoride 400 is an AgNP, chitosan, and fluoride solution produced by the Chemistry Department of the Federal University of Pernambuco in association with the Northeast Technology Center (CETENE-MCT-PE; patent register number BR10201501008) in 2012. During the production process, several AgNP concentrations were tested, but only the NSF with 400 ppm reached the expected stability, and it later proved to be stable for 3 years.[11]

The problem of antibiotic resistance has encouraged the development of new antibacterial strategies. In the last 5 years, many authors[14–16] have illustrated that the effectiveness of AgNPs

Table 2: Final caries arrest rate and independent variables at tooth level—a 6-month follow-up

		Caries arrest rate after 6 months of follow-up		
Independent variables		*Success***	*Failure****	*p value*
NSF* concentration	600	64/88 (72.7%)	24/88 (27.3%)	0.025[1]
	400	48/85 (56.5%)	37/85 (43.5%)	
Type of involved surfaces	Occlusal	70/98 (71.4%)	28/98 (28.6%)	0.035[1]
	Proximal	42/75 (56%)	33/75 (44%)	

[1]Pearson's Chi-square test
*NSF, nano-silver fluoride
**Success was defined based on caries activity (arrested) when visual and tactile examination showed smooth and hard surfaces after a previous active lesion treatment with the solutions
***Failure was defined based on caries activity (active) if a wall or floor of the lesion was soft and easily penetrated by the probe using light force through examination

Table 3: Caries arrest rates and independent variables at child level—a 6-month follow-up

		Caries arrest rate after 6 months of follow-up			
Independent variables		*Success***	*Failure****	*Both*****	*p value*
NSF** concentration	600	19/36 (52.7%)	6/36 (16.6%)	11/36 (30.5%)	0.220[1]
	400	12/32 (37.5%)	11/32 (34.4%)	9/32 (28.1%)	
Type of involved surfaces	Occlusal	17/27 (63%)	6/27 (22.2%)	4/27 (14.8%)	0.086[1]
	Proximal	6/15 (40%)	5/15 (33.3%)	4/15 (26.7%)	
	Occlusal and proximal surfaces involved	8/26 (30.8%)	6/26 (23.1%)	12/26 (46.1%)	
Dmft	Low (≤3)	12/19 (63.2%)	3/19 (15.8%)	4/19 (21%)	0.191[1]
	High (>3)	19/49 (38.8%)	14/49 (28.6%)	16/49 (32.6%)	

[1]Pearson's Chi-square test
*$p < 0.05$, statistical significance
**NSF: nano-silver fluoride
**Success was defined based on caries activity (arrested) when visual and tactile examination showed smooth and hard surfaces after a previous active lesion treatment with the solutions
***Failure was defined based on caries activity (active) if a wall or floor of the lesion was soft and easily penetrated by the probe using light force through examination
****Children with success and failure cavities

is size and dose-dependent; in these studies, the MIC and MBC were tested with different AgNP concentrations. These results are from previous studies,[14–19] which reported that bacterial growth is directly affected by higher AgNP concentrations.

Thus, in 2015, based on the high-quality evidence, and after several improvements on the process of the innovative product, which comprises the expertise of increasing the concentration of silver nanoparticles without enlarging the size of the silver particle, CETENE-MCTI, produced the NSF 600 ppm solution.

Silver diamine fluoride was tested previously in different concentrations (12, 30, and 38%) and application protocols to establish an effective and safe path against caries. Based on a similar purpose, the present randomized controlled trial tested two different concentrations of NSF, namely 400 and 600 ppm.

The NSF caries arrest effectiveness in anterior and posterior teeth was reported[11] in a previous study with water as a negative control. NSF 400 showed 72.7% caries arrest, with a preventive fraction (PF) of 62.5% at the 5-month evaluation ($p < 0.001$) and a higher effectiveness in carious lesions of the anterior teeth (94.4%) than posterior teeth (62.1%, $p = 0.012$).

Since the children enrolled in the study presented high caries risk,[20] this RCT was conducted with a positive control group with fewer risks than a placebo group, and all children were treated with the solutions for ethical reasons,[21] as also reported by other authors dealing with children at risk.[8,9] Critics of placebo-controlled trials that include an untreated control group cite Article 11.3 of the Declaration of Helsinki as a report that every patient including those of the control group should be assured of the best proven diagnostic and therapeutic methods and no patient should suffer from unnecessary pain.[22]

This RCT tested the effectiveness of two different concentrations of NSF in posterior primary teeth with occlusal and proximal dentin-level carious lesions. The higher rate of success in arresting caries with NSF 600 in this clinical trial (72.7%, $p = 0.025$) compared with NSF 400 (56.5%) can be explained by the higher concentration of AgNPs, which increased its antibacterial effect.[17–19]

The lack of statistical significance between NSF 400 and NSF 600 at the child level was due to the categorization of the children (children with only success in caries arrestment; children with failures and children with both teeth with success and with failure). Therefore, predicting the model of treatment effectiveness at the subject level would not be precise, just as it occurred with other reported clinical investigations.[23]

This RCT indicated an effective rate of success in arresting caries in occlusal decayed surfaces. However, there was a significant difference in the caries arrest rates between occlusal and proximal cavities ($p = 0.035$). It is also important to emphasize that occlusal surface cavities comprised the majority of the decayed teeth included in this study. This was a study limitation.

Other study limitations include the randomization process performed on the subject level while the analysis was mainly on the teeth level due to previous sample calculation based on teeth

level. Thus, the analysis did not consider the correlation of the teeth within each subject.

More studies with the equally distributed type of surfaces need to be conducted to clarify this matter.

So far, the studies with the SDF and NSF comprised anterior and posterior cavities, which is directly reflected in the higher caries arrest success rates and better clinical parameters. All previous studies used different application intervals and designs and demonstrated a substantial beneficial effect on caries arrest.

This clinical trial showed relevant data related to caries arrest in posterior teeth, supporting the paradigm shift in the caries approach and indicating that the use of simple caries arrest treatment in deprived communities may be of significant importance for caries control. Therapy with NSF is safe, effective, and easy to accomplish, with no staining of the tooth tissues.

Conclusion

- The arresting effectiveness of NSF 600 at tooth level is higher than that of NSF 400 for a 6-month evaluation with a single application.
- The caries arrest effectiveness dependent on the type of dental surfaces.
- More clinical studies should be conducted to clarify the effect of different arresting caries products in dental cavities on multiple tooth surfaces and at the subject level.

Clinical Significance

Nano-silver fluoride has emerged as an excellent alternative to SDF, as it adds a high antibacterial effect to better esthetic results. Treatment is simple, non-invasive, and low cost. It is ideal for use in community-based programs to increase the access to dental care without staining teeth black like other silver products.

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Manufacturer Name

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