ORIGINAL RESEARCH

Evaluation of Remineralization Efficacy of Nanoparticle-based Materials on White Spot Lesions in Children: A Comparative Clinical Study

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Abstract

Background: Despite the introduction of several anticaries products, dental caries continues to be a global problem. In recent years, there has been a rise in interest in noninvasive treatment for noncavitated caries lesions by employing remineralization concepts. Each remineralizing agent has its own drawbacks. Therefore, it is desirable to seek new agents that offer the advantages of earlier counterparts with lower detrimental reactions.

Aim: The purpose of this research is to evaluate the remineralization efficacy of nanoparticle-based materials on white spot lesion (WSL) in children.

Materials and methods: A total of 45 children between the age-group of 4 and 8 years with WSLs were selected and randomly divided into three groups. At baseline, the teeth with WSLs were confirmed and identified using International Caries Detection and Assessment System II (ICDAS II) criteria, and the dimensions of the lesions were measured using photographic methods. Then, they were randomly placed into three groups of 15 samples each—group I nanosilver fluoride (NSF), group II nanohydroxyapatite (nano-HAP) serum, and group III MI varnish. Following that, the varnish was applied, and follow-up was done in the 2nd, 4th, 12th, and 24th week.

Results: By the 4th week, all three groups had a statistically significant difference ($p < 0.05$). Baseline measurements for groups I, II, and III showed that their respective mean WSL dimensions were 4.9 ± 0.66, 4.27 ± 0.69, and 5.44 ± 2.95. The dimensions of each group were reduced by the 24th week to 1.22 ± 0.46, 0.93 ± 0.41, and 2.19 ± 1.40, respectively. Overall, group II (nano-HAP serum) showed a statistically significant decrease in the dimension of the lesion at the end of the 24th week, followed by groups I and III.

Conclusion: The remineralization of enamel was induced by all three agents. Nano-HAP serum is more successful than MI Varnish and NSF.

Keywords: MI varnish, Nanohydroxyapatite serum, Nanosilver fluoride, Remineralization, White spot lesion.

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Introduction

Dental caries in children is still a major health problem in underdeveloped and developing countries, despite improvements in the dental care system.[1] Children with a poor socioeconomic background have a higher prevalence of early childhood caries (ECC) and pulp involvement. Most children acquire the germs, most often *Streptococcus mutans*, from their mothers or caretakers through salivary contact spanning from 6 to 30 months of age. This period of time is known as the distinct "window of infectivity."[2]

The demineralization process is dynamic, and if diagnosed early enough, it can be reversed. If episodes of demineralization occur more frequently than periods of remineralization, caries develops. A white patch emerges as the enamel continues to lose minerals as demineralization progresses. Visual changes in the tooth surface attributable to disease progression begin at the subclinical stage (white spots) and progress to cavitation.[3,4] The treatment of extensive carious involvement becomes unaffordable for people in low socioeconomic communities. Hence, preventive concepts are always better than undertaking extensive traditional restorative procedures.[2]

Preventing ECC in children has become a challenging task for researchers for years. Various remineralizing agents have been used to treat early enamel caries lesions, including fluorides and nonfluoride remineralizing agents, such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), bioactive glass, xylitol, α-tricalcium phosphate, β-tricalcium phosphate, dicalcium phosphate dehydrate, theobromine, arginine, and oligopeptides.[5]

Now, the emerging trend in dentistry is nanotechnology-based materials, which have numerous benefits like remineralizing potential, antimicrobial efficacy, and cariostatic effect as well. Because of their capacity to break the cell wall, silver nanoparticles (AgNPs) have shown a high antibacterial effect against both gram-positive and gram-negative bacteria.[2,6] Nanosilver also has significant antiviral and antifungal potency. Dental varnishes using

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nanosilver have been suggested by Tirupathi et al. According to clinical investigations, nanosilver-containing fluoride varnish preparations were more effective than the controls at halting the advancement of caries in primary teeth.[7]

Recently, nanohydroxyapatite (nano-HAP) serum (PrevDent®nHAp™, Riga, Latvia) and nanosilver fluoride (NSF) (Nano™ Research Elements) have gained attention as remineralizing potentials in a few of the *in vitro* studies,[1,4,8] There are very few clinical studies on the remineralization potential of these materials to date. Hence, using the current concepts of nanotechnology-based materials, this clinical trial was carried out to assess the remineralization efficacy of three different nanoparticle-based varnishes on white spot lesions (WSL) in children.

Materials and Methods

The present research was an experimental, single-blinded, randomized clinical trial conducted among children aged 4–8 years (both male and female) reporting to the Department of Pedodontics and Preventive Dentistry, Sri Siddhartha Dental College and Hospital, Tumakuru, Karnataka, India. Ethical approval was obtained from the Institutional Ethics Committee. Consolidated Standards of Reporting Trials (CONSORT) guidelines were followed.

Sample Size Calculation

$$n = z^2pq / e^2$$

At baseline, WSL in groups I, II, and III had mean dimensions of 4.11 ± 1.8, 4.58 ± 2.46, and 4.69 ± 2.63, respectively. By the 24th week, these dimensions decreased to 2.52 ± 1.42, 3.25 ± 2.22, and 1.21 ± 0.78, respectively, with 90, 95, and 100% reduction observed in the respective groups as noted in previous literature. The sample size was estimated to be 15 in each group, resulting in a total sample size of 45, with a power of 80%, an accepted error of 0.1%, and a significant level of α = 0.05.

Sample Size Selection

A sample of 45 children was selected according to the inclusion criteria—healthy and cooperative children with WSLs and ECC between 4 and 8 years of age; teeth with intact enamel surfaces; teeth without fluorosis, cracks, hypoplasia, and children who are not allergic to milk products. Exclusion criteria included uncooperative children, special care children, children with severe ECC, teeth with developmental anomalies, deep dentinal caries that are asymptomatic or symptomatic, and teeth that have already been restored or fractured.

Methodology

Patient Preparation

Following the screening of the children, a case sheet was used to record the patient's medical history. This sheet asked for information, such as the patient's name, age, sex, class, International Caries Detection and Assessment System II (ICDAS II) score, number of filled teeth with decay, and the index of that decayed surface, as well as information about diet and a provisional diagnosis. Parental consent was obtained after describing the study's protocol to them.

Randomization

Teeth with WSLs in children were chosen by the professionally trained investigator after the teeth were air-dried for 5 seconds.

Fig. 1: Nanoparticle-based varnishes

A simple random sampling technique was employed, and the participants were randomly allocated into three different groups using computer-generated random numbers. This randomization method was carried out by an independent observer. Following that, the second investigator applied three different nanoparticle-based varnishes (Fig. 1) over the WSLs in children. The baseline and follow-up evaluations were also determined by the same trained investigator. The participants were allocated as follows:

- Group I (n = 15): Nanosilver fluoride.
- Group II (n = 15): Nanohydroxyapatite.
- Group III (n = 15): MI varnish.

Blinding

Over the duration of the study, the professionally trained investigator was blinded to the randomization process and to the clinical procedures that were performed. However, the investigator only evaluated the WSL dimension.

Procedure for Selection of the Case

In this study, teeth with WSLs were identified visually and confirmed with the air-drying method by the trained examiner. Scoring was done according to the ICDAS II criteria, and the cases were randomly selected for the NSF, nano-HAP, and MI varnish procedures. Before performing procedures in all the groups, the salivary pH was checked using salivary pH strips. After the application of the materials to the different groups, follow-up was conducted in the 2nd, 4th, 12th, and 24th weeks to evaluate the same. The CONSORT flowchart depicts this process in Figure 2.

Procedure for Group I (Nanosilver Fluoride)

In this group, the subjects' teeth were wiped with a cotton pellet, especially the surfaces with WSLs. Subsequently, the surface was cleaned and dried using a three-way air syringe, and the chosen tooth received two drops of NSF (33,989.8 µg/mL) (Nano™ Research Elements) utilizing the tip of a microbrush applicator (Fig. 3), which is comparable to 10 mg of the solution. The solution was left in contact with the tooth surface for 2 minutes, and instructions were given to the children and their parents not to rinse their mouths and not to eat for 30 minutes after the procedure. Patients were then called for follow-up.

Procedure for Group II (Nanohydroxyapatite)

In this group, the subjects' teeth were wiped with a cotton pellet, especially the surfaces with WSLs, and then the surface

was cleaned and dried using a three-way air syringe. The nano-HAP serum (PrevDent International BV®, Netherlands) was applied with its sponge for three minutes according to the manufacturer's instructions (Fig. 4), and patients were called for follow-up.

Procedure for Group III (MI Varnish)

In this group, the subjects' teeth were wiped with a cotton pellet, especially the surfaces with WSLs, and then the surface was cleaned and dried using a three-way air syringe. In accordance with the manufacturer's recommendations, MI varnish was applied to the WSLs (Fig. 5). Patients were given post-varnish guidelines, including a 4-hour prohibition on eating anything hard, hot, or sticky, and patients were called for follow-up.

Identification of White Spot Lesions

The teeth with WSLs were observed on the labial/buccal surfaces toward the cervical margin of the selected participants after prolonged air drying for 5 seconds and were analyzed using ICDAS II criteria code 2 [first visual change in enamel—opacity or discoloration (white or brown) is visible at the entrance to the pit or fissure seen after prolonged air drying].[9]

Assessment of White Spot Lesion Dimensions (At Baseline)

Standardization of Photographs

Preoperative and postoperative intraoral photographs were taken with a Canon EOS 3000D camera. A distance of 80 cm was maintained as a standard measurement between the camera and the patient while using a tripod and maintaining the camera's maximum focal length of 5.6 in a dimly illuminated room. The orthopantomogram machine's cephalostat was used to support the patients' heads while they were made to bite down on a custom-built occlusal plate to standardize pictures (Fig. 6).

Fig. 3: Application of NSF

Fig. 4: Application of nano-HAP serum

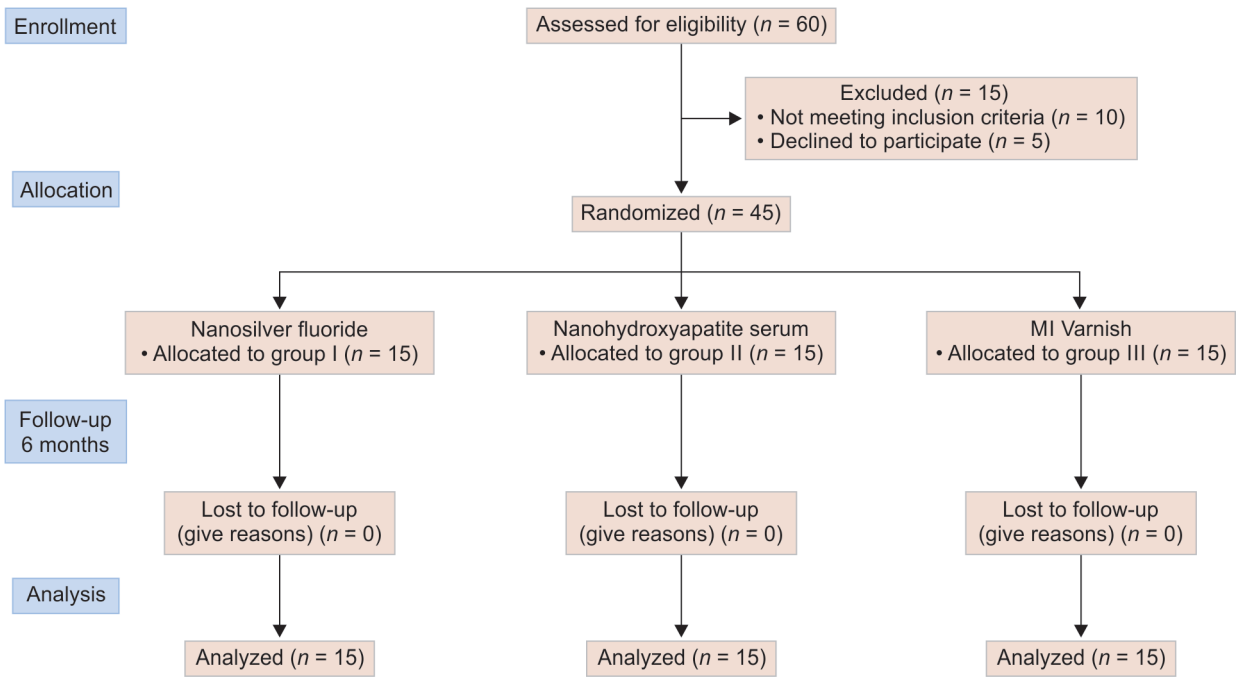

Fig. 2: Consolidated Standards of Reporting Trials flowchart

Measurement of White Spot Lesion Dimension

The maximum lesion dimension was calculated using software (Adobe Photoshop 7.0) after the standard photographs were uploaded to the laptop. After launching Adobe Photoshop 7.0 on the laptop, the images that would be studied were shown. The polygonal lasso tool was then selected from the toolbar, and it was used to select the region of the WSLs (Fig. 7). The pixels were then measured using the following formula:

$$\text{WSL} = \frac{\text{Number of pixels occupied by the lesion}}{\text{Number of pixels in the labial surface}} \times 100$$

Following this criterion, the scoring was given on frequent follow-up periods. The acquired findings were analyzed using statistical analysis.

Fig. 5: Application of MI varnish

Fig. 6: Standardization of photographs using cephalostat

Statistical Analysis

The statistical software Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, Illinois) was used to analyze the data, and the level of significance was set at ($p < 0.05$). Descriptive statistics were used to evaluate the mean and standard deviation (SD) of the relevant groups. The Shapiro–Wilk test was used to determine whether the data were normally distributed. Since the data followed a normal distribution, a parametric test was employed to analyze the data. Inferential statistics to find out the difference between and within the groups were conducted using one-way analysis of variance (ANOVA) test and repeated measures of ANOVA followed by Tukey's honestly significant difference (HSD) *post hoc* test.

Results

A sample of 15 children with WSLs was comprised in each group of our study. The dimension of the lesion was measured at the baseline, 2nd week, 4th week, 12th week, and 24th week (regular intervals of follow-up). There was no statistically significant difference found in the baseline of all three groups ($p = 0.06$). For all three groups, the lesion's dimension remained unchanged until the 2nd week. By the 4th week, a significant difference was found in all three groups ($p = 0.03$) (Table 1).

At baseline, the mean WSL dimensions for groups I, II, and III were 4.96 ± 0.66, 4.27 ± 0.69, and 5.44 ± 2.95, respectively. The dimensions of each group decreased; by the 24th week, they were 1.22 ± 0.46, 0.93 ± 0.41, and 2.19 ± 1.40, respectively. There was a significant decrease in the dimension of the lesion in group II, followed by groups I and III (Table 1 and Fig. 8).

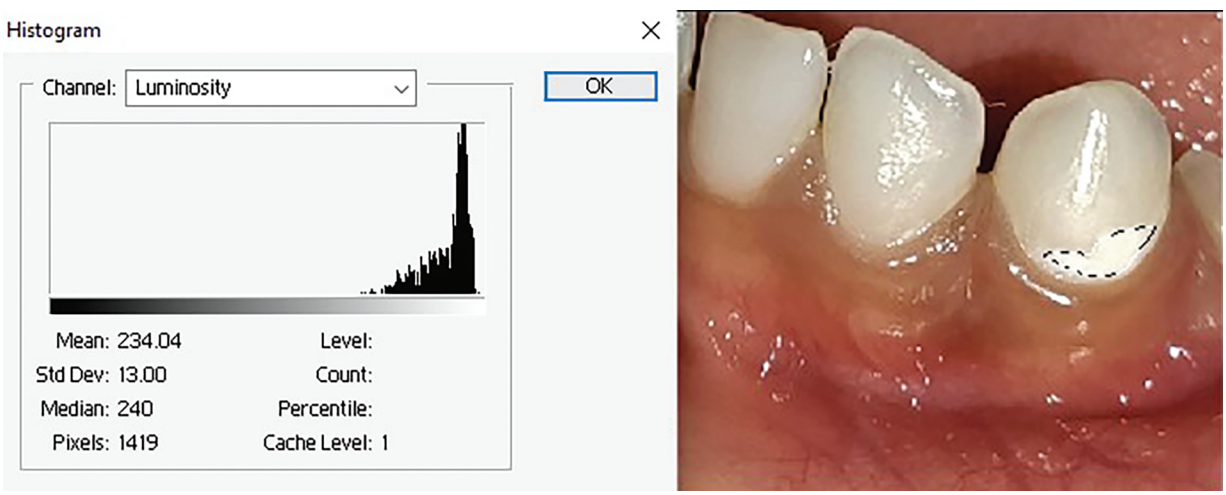

Fig. 7: Preoperative measurement of WSL dimension

One-way ANOVA analysis was used to compare the effectiveness of remineralization for WSLs in children with ECC among the three groups—NSF, nano-HAP serum, and MI varnish. The research showed that changes in WSL dimension were equivalent among the three groups in the 2nd week. By the 4th week, groups II and I exhibited significantly less WSL dimension ($p < 0.0001$) than the other group, at a greater level. In comparison to the other two groups, group II also had a higher degree of WSL dimension reduction at the 12-week mark, and this difference was significant.

According to a *post hoc* Tukey HSD test, the decline in the lesion's dimension started in the 2nd week and continued through week 24 of the assessment. The dimension of WSLs for groups I, II, and III was examined using repeated measures ANOVA to assess changes from baseline to the 2nd, 4th, 12, and 24 weeks. Between-group analysis was carried out using one-way ANOVA to compare groups I vs II, which showed statistically insignificant results between the groups ($p = 0.6$). However, when comparing groups I

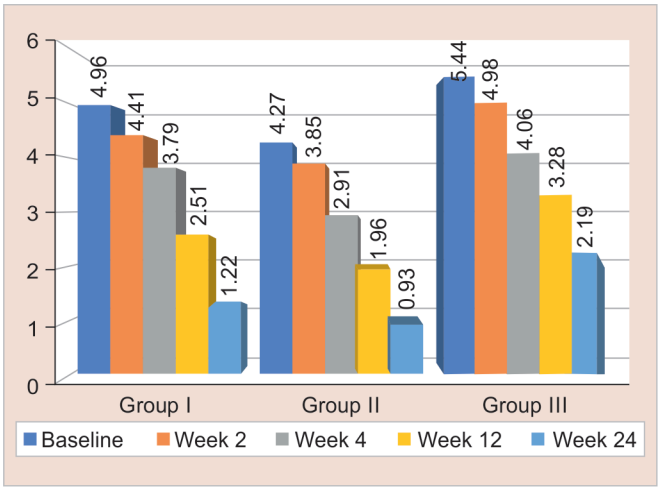

Fig. 8: Within group analysis

Table 1: Descriptive data within group analysis

Groups	*Baseline*	*2nd week*	*4th week*	*12th week*	*24th week*	*p-value ANOVA*	*Post hoc*	*p-value*
Group I	4.96 ± 0.66	4.41 ± 0.66	3.79 ± 0.61	2.51 ± 0.67	1.22 ± 0.46	0.0001*	B vs W2	0.15
							B vs W4	0.0001*
							B vs W12	0.0001*
							B vs W24	0.0001*
							W2 vs W4	0.03*
							W2 vs W12	0.0001*
							W2 vs W24	0.0001*
							W4 vs W12	0.0001*
							W4 vs W24	0.0001*
							W12 vs W24	0.0001*
Group II	4.27 ± 0.69	3.85 ± 0.59	2.91 ± 0.92	1.96 ± 0.71	0.93 ± 0.41	0.0001*	B vs W2	0.35
							B vs W4	0.0001*
							B vs W12	0.0001*
							B vs W24	0.0001*
							W2 vs W4	0.002*
							W2 vs W12	0.0001*
							W2 vs W24	0.0001*
							W4 vs W12	0.001*
							W4 vs W24	0.0001*
							W12 vs W24	0.0006*
Group III	5.44 ± 2.95	4.98 ± 1.78	4.06 ± 1.77	3.28 ± 1.60	2.19 ± 1.40	0.0001*	B vs W2	0.92
							B vs W4	0.18
							B vs W12	0.004*
							B vs W24	0.0001*
							W2 vs W4	0.63
							W2 vs W12	0.04*
							W2 vs W24	0.0001*
							W4 vs W12	0.70
							W4 vs W24	0.02*
							W12 vs W24	0.40

*p <0.05 is considered statistically significant

and III, statistically significant results were found by the 24th week ($p = 0.01$). Similarly, in the comparison between groups II and III, statistically significant results were found by the 2nd week ($p = 0.02$) (Table 2 and Fig. 9).

Comparison of baseline, 2, 4, 12, and 24 weeks of follow-up in terms of changes in the WSL dimension of group I (NSF) was carried out. Within-group comparisons revealed that the mean rank at baseline, 2, 4, 12, and 24 weeks were 4.96 ± 0.66, 4.41 ± 0.66, 3.79 ± 0.61, 2.51 ± 0.67, and 1.22 ± 0.46, accordingly with a *p*-value of 0.0001 found to be significant. Therefore, it can be said that the WSLs effectively shrank from baseline to the 24-week follow-up period after applying NSF varnish (Table 1 and Fig. 8).

Comparison between baseline and regular intervals in follow-up was carried out in terms of changes in the WSL dimension in group II (nano-HAP serum). Within-group comparisons revealed that the mean rank at baseline, 2, 4, 12, and 24 weeks were 4.27 ± 0.69, 3.85 ± 0.59, 2.91 ± 0.92, 1.96 ± 0.71, and 0.93 ± 0.41 accordingly, with a *p*-value of 0.0001 found to be significant. Therefore, it can be stated that between baseline and 24 weeks of follow-up, nano-HAP serum showed considerably higher changes in decreasing the dimension of the WSL (Table 1 and Fig. 8).

Comparison between baseline, 2, 4, 12, and 24 weeks of follow-up in terms of changes in the WSL dimension of group III (MI varnish) was carried out. Within-group comparisons revealed that the mean rank at baseline and regular intervals were 5.44 ± 2.95, 4.98 ± 1.78, 4.06 ± 1.77, 3.28 ± 1.60, and 2.19 ± 1.40 accordingly, with a *p*-value of 0.0001 found to be significant. As a result, it is also possible to draw the conclusion that MI varnish steadily reduced the dimension of the WSL from baseline to the 24-week follow-up (Table 1 and Fig. 8).

Therefore, group II experienced a substantial decrease in WSL dimension compared to the other two groups at the 4th, 12th, and 24th weeks.

Discussion

Dental caries should indeed be prevented rather than treated. ECC, which is characterized by rapid and progressive carious lesions in newborns and younger children, presents a challenge to pedodontists because it typically necessitates general anesthesia for treatment.[10]

The most mineralized and hardened tissue in the human body is enamel. It is designed to withstand chemical, abrasive, and mechanical injury. Long-term demineralization causes excessive mineral loss, which results in the loss of enamel structure and cavitation, both of which are hallmarks of dental caries.[11] The equilibrium of remineralization and demineralization is crucial for preventing dental caries. Remineralization is a unique, noninvasive way of treating early carious lesions. However, in individuals with elevated caries activity, saliva alone is unable to remineralize incipient caries lesions because of the low phosphorus and calcium ion concentration gradient between saliva and decalcified areas.[12]

Fluoride is one of the most potent remineralizing agents for cavity prevention. However, certain challenges have been raised regarding fluorosis and overall fluoride intake.[1] It therefore sparked the development of materials based on nanotechnology, and in recent years, several researchers have experimented with using nanoparticles in restorative and preventive dentistry. One such example is nano-HAP, a unique class of nanoparticle used in dentistry. Due to its resemblance to the bone and mineral structure of teeth, biocompatibility, and bioactivity, nano-HAP was perceived to be promising. The particles resemble dental apatite in terms of appearance and crystal structure.[8]

It has been demonstrated that the 20 nm-sized HAP, known as nano-HAP, is the most bioactive and biocompatible type of synthetic apatite and has remarkable potential to biomimetically rebuild the enamel destroyed by acid. A greater surface area and a strong affinity for the enamel surface are characteristics of nano-HAP. It is interesting to observe that a layer of nano-HAP that develops on the enamel surface has high acid resistance and can shield the underlying enamel from demineralization in the future.[5,8] In our research, nano-hydroxyapatite (nano-HAP) serum started reversing the WSLs by the 2nd week when compared to other materials. These findings were in agreement with the research done by Ali Nozari et al., where nano-HAP serum showed good results in remineralizing the initial caries. We utilized nano-HAP (PrevDent International BV, Netherlands) in the form of a serum that included mentha piperita oil, linalool, limonene, xylitol, nano-HAP, and

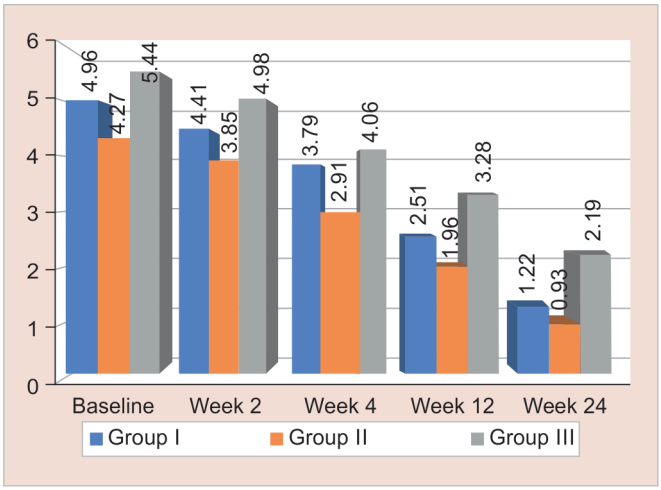

Fig. 9: Between the group analysis

Table 2: Between group analysis

	Groups	*Baseline*	*2nd week*	*4th week*	*12th week*	*24th week*
	Group I	4.96 ± 0.66	4.41 ± 0.66	3.79 ± 0.61	2.51 ± 0.67	1.22 ± 0.46
	Group II	4.27 ± 0.69	3.85 ± 0.59	2.91 ± 0.92	1.96 ± 0.71	0.93 ± 0.41
	Group III	5.44 ± 2.95	4.98 ± 1.78	4.06 ± 1.77	3.28 ± 1.60	2.19 ± 1.40
	p-value	0.06	0.02*	0.03*	0.007*	0.0007*
Post hoc	I vs II	0.24	0.28	0.10	0.27	0.63
	I vs III	0.44	0.44	0.86	0.19	0.01*
	II vs III	0.06	0.02*	0.03*	0.005*	0.0008*

*p <0.05 is considered statistically significant

potassium chloride. For 10 days, we used the nano-HAP serum on a daily basis. Another study that produced findings similar to ours suggested daily usage of 10% nano-HAP because of its efficacy, which was comparable to that of 1000 ppm fluoride. Fluorosis is the major fluoride disadvantage in children; hence, nano-HAP serum might be suggested as an alternative. Additionally, Tschoppe et al. suggested nano-HAP as an alternative to fluoride, which is compatible with our observations.[1,13]

Based on clinical trials, it was found that using fluoride varnish reduced cavities in deciduous teeth by 30% over the course of 6 months to 3 years. Radha et al.'s findings from a comparable study showed that the active WSLs turned inactive over the course of 24 weeks. As a result, this study was also designed to last for 6 months.[3]

In this current research, we evaluated the effectiveness of NSF as a remineralization solution. At the 4th week, NSF showed better results ($p = 0.001$), and over the period of 24 weeks, NSF efficiently decreased the dimension of the lesion. These results were consistent with the previous studies.[1,4,6]

A novel colloid based on AgNPs, chitosan, and fluoride called NSF was created as a caries-arresting substance that also has remineralizing and antimicrobial capabilities. It has been characterized as a yellowish solution that was created as an experimental solution and was shown to be stable for 3 years. Moreover, the material is inexpensive and environmentally safe.[14] AgNPs are widely used due to their broad-spectrum antibacterial properties. There are two possible explanations for how nanosilver particles function. The first is the interaction of AgNPs with the bacterial cell membrane. AgNPs can cause cell death by creating "pits" in the bacterial cytoplasmic membrane *via* reactive oxygen species. Second, once AgNPs penetrate the membrane, a flood of silver ions is released inside the bacterial cells. These silver ions interact with DNA replication, cell inclusions, enzymes, and respiratory chain processes, potentially inhibiting cell division and leading to cell death.[15] Additionally, the NSF nanoparticles' modest size (3.2 ± 1.2 nm) and their spherical forms amplify the antibacterial activity by enhancing the contact surface.[2]

Chitosan was added to the AgNPs as it acts as a carrier and stabilizes the solution. Fluoride acts as an anticariogenic agent in NSF. Fluoride was added to diminish the growth and adherence of biofilms, as well as the development of acid and inhibit demineralization. There are many hypotheses describing how fluoride works. NSF has the benefit of not producing tooth discoloration. According to Zhang et al., NSF did not produce oxides when in contact with oxygen in the medium and did not impart a metallic taste as does sodium fluoride treatment.[6,16] Since NSF applications are affordable, most communities can afford them. The treatment process is simple and doesn't demand extensive dental equipment or a clinical setting.

At week of 12th (Fig. 10), the dimension of the lesion began to shrink when MI varnish was applied ($p = 0.004$), and over the course of 24 weeks, it gradually diminished the dimension of the lesion. This supports the findings of the study by Girish Babu et al., which found no discernible difference between the remineralizing potential of varnish containing CPP-ACP, and fluoride and varnish containing primarily fluoride. The technique with CPP-ACP reduces dental enamel demineralization and facilitates enamel remineralization. Additionally, it prevents plaque buildup.[17]

In order to promote remineralization, CPP-ACP is extremely soluble in water and offers large quantities of free calcium and phosphate ions that are numerous times higher than those typically present in saliva.[18] CPP-ACP has recently been combined with fluoride and made available as a varnish. However, the remineralization potential of varnish containing CPP-ACP with fluoride showed up in our investigation, was not statistically significant. This could be caused by the varnish's physical properties, which were used. Since the varnish with CPP-ACP and fluoride was thicker, the surface may not have been adequately moisturized.[4,17]

Prior to the procedures in our study, participants' salivary pH was measured using a salivary pH kit. The mean value was 6.83 ± 0.49, which is in the normal range of salivary pH (6.2–7.6). Thus, it shows that there is no correlation between salivary pH and caries activity, which is in accordance with the study conducted by Jayaraj and Ganesan and Kaveh et al.[19,20]

Dental caries is diagnosed and evaluated using the ICDAS II, a diagnostic grading system. According to these factors, code 02 was used as the inclusion criterion in the current investigation. In contrast to other scoring systems, this one can be used to identify and evaluate lesions on coronal surfaces, root surfaces, enamel caries, dentinal caries, and noncavitated lesions. When Kuhnisch et al.[21] compared the diagnostic results of the World Health Organization (WHO)

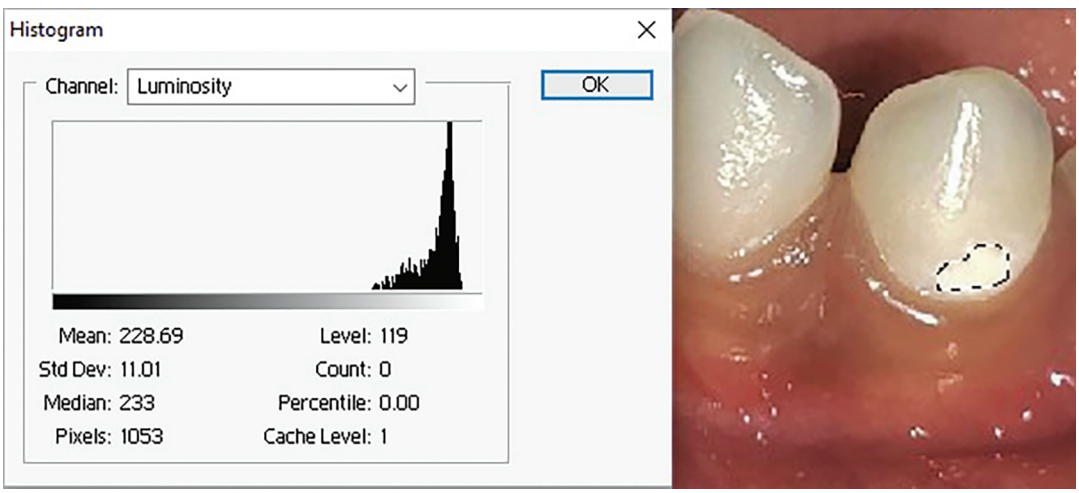

Fig. 10: Postoperative measurement of WSL dimension (12th week)

criteria, the ICDAS II criteria, and laser fluorescence measurements, they discovered that ICDAS II had a greater diagnostic potential than the conventional WHO criteria and was also effective at identifying cavities that were not cavitated. The study also highlighted DIAGNOdent limited utility in field investigations when employing the ICDAS approach for recording caries.[9]

Conclusion

Based on the data from the study, the following conclusion was drawn.

All test groups demonstrated a substantial capacity for remineralization. Among the three groups, nano-HAP serum showed the greatest remineralizing efficacy, followed by NSF and MI varnish, respectively. In order to address reversible carious lesions (WSLs), the therapeutic application of these substances will provide a novel, patient-friendly, noninvasive approach. However, additional *in vivo* research is necessary to conclude the superiority.

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References

1. Nozari A, Ajami S, Rafiei A, et al. Impact of nano-hydroxyapatite, nano silver fluoride and sodium fluoride varnish on primary teeth enamel remineralization: an in vitro study. J Clin Diagn Res 2017;11(9):ZC97–ZC100. DOI: 10.7860/JCDR/2017/30108.10694
2. Nagireddy VR, Reddy D, Kondamadugu S, et al. Nanosilver fluoride—a paradigm shift for arrest in dental caries in primary teeth of schoolchildren: a randomized controlled clinical trial. Int J Clin Pediatr Dent 2019;12(6):484–490. DOI: 10.5005/jp-journals-10005-1703
3. Radha S, Kayalvizhi G, Adimoulame S, et al. Comparative evaluation of the remineralizing efficacy of fluoride varnish and its combination varnishes on white spot lesions in children with ECC: a randomized clinical trial. Int J Clin Pediatr Dent 2020;13(4):311–317. DOI: 10.5005/jp-journals-10005-1673
4. Silva AV, Teixeira JD, Melo PC, et al. Remineralizing potential of nano-silver-fluoride for tooth enamel: an optical coherence tomography analysis. Pesqui Bras Odontopediatria Clín Integr 2019;19(1):1–13. DOI: 10.4034/PBOCI.2019.191.50
5. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. Int J Clin Pediatr Dent 2019;12(2):139–144. DOI: 10.5005/jp-journals-10005-1603
6. Silva A, Teixeira JA, de Oliveira Mota CCB, et al. In vitro morphological, optical and microbiological evaluation of nanosilver fluoride in the remineralization of deciduous teeth enamel. Nanotechnol Rev 2018;7(6):509–520. DOI: 10.1515/ntrev-2018-0083
7. Tirupathi S, Svsg N, Rajasekhar S, et al. Comparative cariostatic efficacy of a novel Nano-silver fluoride varnish with 38% silver diamine fluoride varnish a double-blind randomized clinical trial. J Clin Exp Dent 2019;11(2):e105–e112. DOI: 10.4317/jced.54995
8. Memarpour M, Shafiei F, Rafiee A, et al. Effect of hydroxyapatite nanoparticles on enamel remineralization and estimation of fissure sealant bond strength to remineralized tooth surfaces: an in vitro study. BMC Oral Health 2019;19(1):1–4. DOI: 10.1186/s12903-019-0785-6
9. Gugnani N, Pandit IK, Srivastava N, et al. International caries detection and assessment system (ICDAS): a new concept. Int J Clin Pediatr Dent 2011;4(2):93–100. DOI: 10.5005/jp-journals-10005-1089
10. Kooshki F, Pajoohan S, Kamareh S. Effects of treatment with three types of varnish remineralizing agents on the microhardness of demineralized enamel surface. J Clin Exp Dent 2019;11(7):e630–e635. DOI: 10.4317/jced.55611
11. Sharma A, Rao A, Shenoy R, et al. Comparative evaluation of nanohydroxyapatite and casein phosphopeptide–amorphous calcium phosphate on the remineralization potential of early enamel lesions: an in vitro study. J Orofac Sci 2017;9(1):28–33. DOI: 10.4103/0975-8844.207941
12. Konagala RK, Mandava J, Anwarullah A, et al. Synergistic effect of arginine on remineralization potential of fluoride varnish and nanohydroxyapatite on artificial caries lesions: an in vitro study. J Contemp Dent Pract 2020;21(9):1048–1053.
13. Tschoppe P, Zandim DL, Martus P, et al. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. J Dent 2011;39(6):430–437. DOI: 10.1016/j.jdent.2011.03.008
14. Akyildiz M, Sönmez IS. Comparison of remineralising potential of nano silver fluoride, silver diamine fluoride and sodium fluoride varnish on artificial caries: an in vitro study. Oral Health Prev Dent 2019;17(5):469–477. DOI: 10.3290/j.ohpd.a42739
15. Al-Nerabieah Z, Abo-Arrag E, Comisi JC, et al. Effectiveness of a novel nano-silver fluoride with green tea extract compared with silver diamine fluoride: a randomized, controlled, non-inferiority trial. Int J Dent Oral Sci 2020;7(6):753–761. DOI: 10.19070/2377-8075-20000148
16. Zhang Q, Zou J, Yang R, et al. Remineralization effects of casein phosphopeptide amorphous calcium phosphate crème on artificial early enamel lesions of primary teeth. Int J Paediatr Dent 2013;21(5):374–381. DOI: 10.1111/j.1365-263X.2011.01135.x
17. Girish Babu KL, Subramaniam P, Teleti S. Remineralization potential of varnish containing casein phosphopeptides-amorphous calcium phosphate with fluoride and varnish containing only fluoride: a comparative study. Saudi J Oral Sci 2018;5(1):35–40. DOI: 10.4103/sjos.SJOralSci_44_17
18. Vinod D, Gopalakrishnan A, Subramani SM, et al. A comparative evaluation of remineralizing potential of three commercially available remineralizing agents: an in vitro study. Int J Clin Pediatr Dent 2020;13(1):61–65. DOI: 10.5005/jp-journals-10005-1715
19. Jayaraj D, Ganesan S. Salivary pH and buffering capacity as risk markers for early childhood caries: a clinical study. Int J Clin Pediatr Dent 2015;8(3):167–171. DOI: 10.5005/jp-journals-10005-1307
20. Kaveh S, Karamifar A, Kousha S, et al. Comparative efficacy of MI paste plus and fluoride varnish remineralizing agents for preventing white spot lesions in patients on fixed orthodontic treatment: a clinical trial. Middle East J Rehabil Health Stud 2022;9(1):e118635. DOI: 10.5812/mejrh.118635
21. Kühnisch J, Berger S, Goddon I, et al. Occlusal caries detection in permanent molars according to WHO basic methods, ICDAS II and laser fluorescence measurements. Community Dent Oral Epidemiol 2008;36(6):475–485.