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Comparison of the Effect of Two Experimental Varnishes Containing Nano- Hydroxyapatite with MI Varnish and Sodium Fluoride Varnish on Remineralization of Enamel White Spot Lesions: An In Vitro Study

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Research Article

Keywords: Enamel remineralization, Nano-hydroxyapatite, White spot lesion, Dental varnish, Surface microhardness, CPP-ACP, Fluoride

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11119319/v1

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Additional Declarations: No competing interests reported.

Abstract

Introduction:

White Spot Lesions (WSLs), as the earliest clinical manifestation of enamel caries, result from subsurface mineral loss in the presence of dental biofilm and, if left untreated, may progress to cavitated lesions. The use of biomimetic remineralizing agents such as nano-hydroxyapatite (nano-HA) as an alternative or adjunct to fluoride has gained attention in recent years. This study aimed to compare two experimental varnishes containing nano-hydroxyapatite with MI varnish and sodium fluoride varnish on the remineralization of white spot lesions.

Materials and Methods

In this in-vitro study, fifty-five extracted human premolar teeth were divided into five groups (n = 11): experimental varnish containing nano-hydroxyapatite and fluoride, nano-hydroxyapatite varnish, MI varnish containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), 5% sodium fluoride varnish, and a control group. After inducing white spot lesions using a demineralizing solution, the specimens underwent a pH-cycling protocol for 10 days. Surface Microhardness (SMH) was measured using the Vickers microhardness test before demineralization, after demineralization, and after remineralization. One-way Analysis of Variance (ANOVA) with Tukey's post-hoc test and Repeated-measures ANOVA were used to compare SMH values ($p < 0.05$ in all tests).

Results

The results showed that after pH cycling, the highest SMH recovery was observed in the group receiving the varnish containing nano-hydroxyapatite with fluoride, whereas the group receiving sodium fluoride varnish and the control group exhibited the least remineralization ($p < 0.05$). Moreover, no statistically significant difference was observed between the nano-hydroxyapatite-containing groups and the MI varnish group.

Conclusion

Varnishes based on nano-hydroxyapatite, particularly in combination with fluoride, demonstrated greater remineralization potential compared to sodium fluoride varnish in initial enamel lesions and may serve as an effective approach for the non-invasive management of white spot lesions. Clinical studies are recommended to confirm the efficacy of these formulations.

Introduction

Dental caries is a prevalent biofilm-mediated disease driven by an imbalance between demineralization and remineralization at the tooth surface (1, 2). The earliest detectable manifestation of enamel caries is the White Spot Lesion (WSL), which represents subsurface mineral loss while the outer enamel surface remains intact (3). WSLs are particularly common after orthodontic treatment due to plaque retention around appliances, leading to esthetic and structural issues (4). Since WSLs are reversible pre-cavitation, they are a primary target for minimally invasive prevention (5). WSL formation arises from biofilm-enamel interactions, where acidogenic bacteria lower local pH below ~ 5.5, causing calcium and phosphate ion loss from the enamel crystal lattice (6). Consequently, a porous subsurface region forms, enabling remineralization upon the return of the oral environment to supersaturation (7). Saliva buffers the pH and supplies calcium and phosphate ions for reprecipitation into partially dissolved crystals upon neutralization (8). Fluoride-based agents are the cornerstone of prevention, enhancing remineralization and inhibiting demineralization (9). Fluoride forms fluorapatite-like crystals, which are less acid-soluble than hydroxyapatite, increasing enamel resistance. However, fluoride-induced remineralization occurs mainly on the surface, potentially leaving the lesion's internal structure vulnerable to progression (10). Furthermore, concerns about excessive fluoride exposure (especially in children) have prompted exploration of alternative agents that directly supply calcium and phosphate ions to enamel (11). Biomimetic calcium-phosphate systems such as CPP-ACP have been introduced; these complexes stabilize amorphous ions, facilitating penetration into porous lesions and enhancing mineral redeposition (12). Nano-hydroxyapatite (nano-HA) offers unique advantages due to its structural and compositional similarity to natural enamel crystals (13). Nano-HA particles act as nucleation centers, integrating into enamel and restoring mineral density biomimetically (14). Moreover, nano-HA forms a protective mineral layer on enamel and occludes interprismatic spaces in initial lesions (15). Its combination with fluoride may synergistically increase ion bioavailability and form fluoridated apatite, reducing enamel solubility (16). Despite several studies on nano-HA in dentifrices and pastes, its efficacy in varnish formulations—recognized as clinically relevant delivery systems for prolonged contact and sustained ion release—is limited (17, 18). Therefore, this in-vitro study aimed to compare the remineralization potential of two experimental nano-HA varnishes (with/without fluoride) against MI varnish and 5% NaF varnish on artificial WSLs in human enamel. Efficacy was assessed via Vickers surface microhardness changes following a pH-cycling regimen.

Methods and Materials

This in-vitro study used fifty-five freshly extracted human premolars, obtained for orthodontic purposes. Teeth with intact buccal surfaces, caries-free, crack-free, and restoration-free, were selected. After extraction, specimens were cleaned and stored in 1% chloramine at room temperature to prevent microbial growth and preserve enamel structure (19). Buccal surfaces were polished with SiC abrasive papers (320, 600, 1200 grit) under water irrigation to standardize surfaces. A 2×4 mm window was demarcated using acid-resistant nail varnish, exposing only the test area. Roots were sectioned at the CEJ using a diamond disc under coolant. Baseline SMH was measured using a Vickers tester with a 200

g load applied for 10 seconds. Three indentations were made within the window, and the mean was recorded as baseline SMH (20).

To create artificial white spot lesions, specimens were immersed in a demineralizing solution containing 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid (pH 4.4) at room temperature for 72 hours (21). Surface microhardness was then re-measured to confirm lesion formation. Demineralized specimens were randomly allocated to five groups (n = 11) according to the remineralizing agent: Group 1: experimental varnish with 7% nano-HA and fluoride; Group 2: experimental varnish with 7% nano-HA; Group 3: MI varnish (GC Corporation, USA) containing CPP-ACP with 900 ppm fluoride; Group 4: 5% sodium fluoride varnish; Group 5: control varnish without active agent. All varnishes were diluted 1:3 with artificial saliva prior to application. Specimens underwent a 10-day pH-cycling regimen to simulate demineralization-remineralization cycles. Each daily cycle included two 3-hour demineralization periods separated by a 2-hour remineralization period. Overnight, specimens were kept in a remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl, pH 7.0) (22).

Therapeutic agents were applied thrice daily: before the first demineralization phase, and before and after the second demineralization phase. The varnish was placed on the exposed enamel using a microbrush and left for 30 minutes. Specimens were then rinsed with distilled water and transferred to the next cycle phase. Upon completing the pH-cycling protocol, final surface microhardness was measured under baseline conditions. Percentage SMH recovery was calculated per specimen using pre-demineralization, post-demineralization, and post-remineralization values. Statistical analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA). Data were reported as mean ± standard deviation. Normality was assessed using the Kolmogorov–Smirnov test. Between-group SMH comparisons used one-way ANOVA with Tukey's post-hoc test, while within-group SMH changes across stages were evaluated using repeated-measures ANOVA. Significance was set at $p < 0.05$ for all tests.

This study was an in vitro investigation using extracted human premolar teeth and did not involve human participants. The teeth were extracted for orthodontic reasons and were anonymized before use; no identifiable human data were collected. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the institutional guidelines of Alborz University of Medical Sciences, and also the study protocol was approved by the Ethics Committee of Alborz University of Medical Sciences, Karaj, Iran (approval code: IR.ABZUMS.REC.1404.000.)

Results

A total of fifty-five extracted human premolar teeth were examined and randomly allocated to five experimental groups (n = 11). The Kolmogorov–Smirnov test confirmed normal distribution of data in all groups at each experimental stage ($p > 0.05$ for all measurements), supporting the use of parametric statistical tests.

Baseline and post-demineralization microhardness:

As shown in Table 1, baseline SMH values were comparable among all groups (p = 0.97), confirming initial enamel uniformity. After 72 hours of demineralization, SMH significantly decreased in all groups, with no statistically significant differences between groups (p = 0.28), indicating uniform artificial white-spot lesion formation.

Table 1
Surface microhardness (mean ± SD) before and after demineralisation induction in the five study groups. One-way ANOVA showed no significant differences between groups at either time point (p > 0.05), indicating initial enamel uniformity and consistent lesion formation.

Group	Before Demineralisation	After Demineralisation
Nano-HA + Fluoride varnish	507.4 ± 19.5	228.8 ± 32.3
7% Nano-HA varnish	510.0 ± 41.3	207.5 ± 18.7
MI varnish	517.1 ± 42.8	216.0 ± 21.3
5% Sodium fluoride varnish	508.7 ± 38.6	219.0 ± 20.3
Control	514.3 ± 47.2	232.8 ± 46.1
p-value (between groups)	*0.97*	*0.28*

Post-treatment microhardness and temporal changes:

Following the 10-day pH-cycling protocol, significant differences in SMH recovery were observed among the groups (p < 0.001). The highest mean SMH was recorded in the nano-HA + fluoride group (465.2 ± 51.6), followed by MI varnish (434.2 ± 29.8) and nano-HA alone (432.1 ± 40.2). The sodium fluoride group showed lower recovery (327.8 ± 87.0), while the control group exhibited the least remineralisation (232.9 ± 29.1). Repeated-measures ANOVA (Table 2) showed significant within-group changes over time (p < 0.001) in all groups, and the between-group comparison also revealed significant differences in the rate of SMH recovery across conditions (p < 0.001). The temporal pattern of microhardness recovery (Fig. 1) demonstrates progressive mineral gain in treatment groups, with the most pronounced recovery observed in the nano-HA + fluoride group.

Table 2
Longitudinal changes in surface microhardness (mean ± SD) before demineralisation, after demineralisation, and after pH-cycling in the five study groups. Repeated-measures ANOVA demonstrated significant within-group changes over time ($p < 0.001$ for all groups), and the between-group comparison over time was also significant ($p < 0.001$).

Group	Baseline	Post-Demineralisation	Post-pH-Cycling	*p* (within-group)
Nano-HA + Fluoride varnish	507.4 ± 19.5	228.8 ± 32.3	465.2 ± 51.6	< 0.001
7% Nano-HA varnish	510.0 ± 41.3	207.5 ± 18.7	432.1 ± 40.2	< 0.001
MI varnish	517.1 ± 42.8	216.0 ± 21.3	434.2 ± 29.8	< 0.001
5% Sodium fluoride varnish	508.7 ± 38.6	219.0 ± 20.3	327.8 ± 87.0	< 0.001
Control	514.3 ± 47.2	232.8 ± 46.1	232.9 ± 29.1	< 0.001
p (between-groups)	*0.97*	*0.28*	*< 0.001*	

Pairwise comparisons:

Post-hoc analysis (Table 3) revealed no statistically significant differences between the nano-HA + fluoride group and either the nano-HA alone or MI varnish groups ($p > 0.05$). However, the nano-HA + fluoride group showed significantly higher SMH compared to the sodium fluoride and control groups ($p < 0.001$). All nano-HA-containing groups demonstrated superior remineralisation compared to the control ($p < 0.001$).

Table 3
Pairwise comparison of post-pH-cycling microhardness between groups (Tukey's post-hoc test). Statistically significant differences are indicated by $p < 0.05$.

Group Comparison	Mean Difference	p-value
Nano-HA + Fluoride vs. 7% Nano-HA	**33.0**	**0.57**
Nano-HA + Fluoride vs. MI varnish	**31.0**	**0.63**
Nano-HA + Fluoride vs. 5% NaF	**137.5**	**< 0.001**
Nano-HA + Fluoride vs. Control	**232.3**	**< 0.001**
5% NaF vs. MI varnish	**106.4**	**< 0.001**
5% NaF vs. 7% Nano-HA	**104.3**	**< 0.001**
5% NaF vs. Control	**94.9**	**< 0.001**
7% Nano-HA vs. Control	**199.2**	**< 0.001**

Discussion

This in-vitro study compared the remineralization potential of two experimental nano-HA varnishes against MI varnish and NaF varnish on artificial WSLs. Based on post-treatment SMH values following pH-cycling, the nano-HA + fluoride varnish showed the highest efficacy, followed by MI varnish and nano-HA alone. NaF varnish showed lower recovery, whereas the control group showed the least. These findings suggest that nano-HA formulations may enhance enamel mineral recovery beyond that achieved by fluoride alone.

Fluoride is a recognized remineralizing agent, forming fluorapatite-like crystals with lower acid solubility (9). However, fluoride-induced remineralization occurs mainly on the enamel surface, limiting ion penetration into deeper subsurface regions (10). This surface-level effect impedes complete WSL restoration, sparking interest in biomimetic calcium-phosphate agents for deeper ion delivery (17). In the present study, nano-HA varnishes showed significantly greater SMH recovery than NaF varnish, consistent with Huang et al., who reported enhanced remineralization of initial lesions with nano-HA (13). The structural similarity of nano-HA to natural enamel crystals may enable them to act as nucleation sites for mineral deposition, facilitating enamel lattice repair through a biomimetic mechanism (14). Furthermore, nano-HA increases the supersaturation of calcium and phosphate ions around the lesion, promoting mineral redeposition in subsurface areas (15).

The superior remineralization in the nano-HA with fluoride group may reflect a synergistic interaction: nano-HA supplies bioavailable calcium and phosphate, while fluoride stabilizes newly formed mineral phases via fluoridated hydroxyapatite, enhancing acid resistance. This synergy was previously reported by Hannig and Hannig (16). MI varnish (CPP-ACP) also showed considerable efficacy, as CPP-ACP

stabilizes calcium and phosphate ions in a metastable state, facilitating penetration into lesions (12). These findings align with prior studies reporting comparable effects between CPP-ACP and fluoride agents in vitro (23). However, the lack of significant difference between nano-HA and MI varnish in this study may relate to variations in lesion characteristics, application protocols, or evaluation methods across studies (24). The pH-cycling model used is a reliable in-vitro simulation of intraoral cycles, but it cannot fully replicate the oral cavity's biological complexity (salivary flow, biofilm, diet). Therefore, clinical extrapolation requires caution. Within these limitations, nano-HA varnishes—especially with fluoride—showed higher remineralization than NaF varnish in artificial WSLs, suggesting they may serve as an alternative or adjunct in managing initial enamel lesions.

Conclusion

Within the limitations of this in-vitro study, varnish formulations containing nano-hydroxyapatite (nano-HA) demonstrated a higher remineralization potential compared to 5% sodium fluoride varnish in artificially induced White Spot Lesions (WSLs) in enamel. This was confirmed by a significant increase in the recovery of enamel surface microhardness (SMH) following a standard pH-cycling protocol. The combination of nano-hydroxyapatite with fluoride exhibited the highest remineralization efficacy among the tested agents, which may indicate a synergistic interaction in enhancing mineral deposition and structural restoration of enamel.

MI varnish containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) also showed considerable remineralization capability; however, its effect was not statistically significantly different from that of the varnish containing nano-hydroxyapatite alone. In contrast, sodium fluoride varnish, under the in-vitro conditions employed in this study, exhibited lower remineralization performance compared to the other experimental groups.

The findings of this study suggest that varnishes based on nano-hydroxyapatite — particularly in combination with fluoride — may serve as an alternative or adjunctive preventive strategy in the management of initial enamel caries lesions. Further in-vivo studies are recommended to evaluate the long-term efficacy of these formulations under the dynamic conditions of the oral environment.

Abbreviations

1. **MI Varnish** - Casein Phosphopeptide-Amorphous Calcium Phosphate Varnish

2. **WSL** - White Spot Lesion

3. **nano-HA** - Nano Hydroxyapatite

4. **PH-cycling** - pH cycling

5. **SEM-EDX** - Scanning Electron Microscopy-Energy Dispersive X-ray

6. **SPSS** - Statistical Package for the Social Sciences

7. **ANOVA** - Analysis of Variance

8. **p-value** - Probability value used in statistical hypothesis testing

9. **Vickers's microhardness** - A method to measure the hardness of materials, specifically enamel in this study

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations and the ethical principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Alborz University of Medical Sciences, Karaj, Iran (approval code: IR.ABZUMS.REC.1404.000). Informed consent was obtained from all patients (or their legal guardians) for the use of their extracted teeth for research purposes.

Consent for publication

"Not applicable."

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that there is no conflict of interest in this study.

Funding

This research was financially supported by Alborz university of medical sciences.

Authors' contributions

SYR: Investigation, Methodology, Data curation, Validation, Performing the experiments, measurements, and assisted in manuscript writing. SKT: Supervision, Conceptualization, Project administration. FS:

Supervision, Scientific guidance. MQ: Formal analysis, Statistical data analysis. MBK: Writing - original draft, Writing - review & editing, Manuscript preparation. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the staff of the Dental Research Center and the Faculty of Dentistry at Alborz University of Medical Sciences for their support.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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Figures

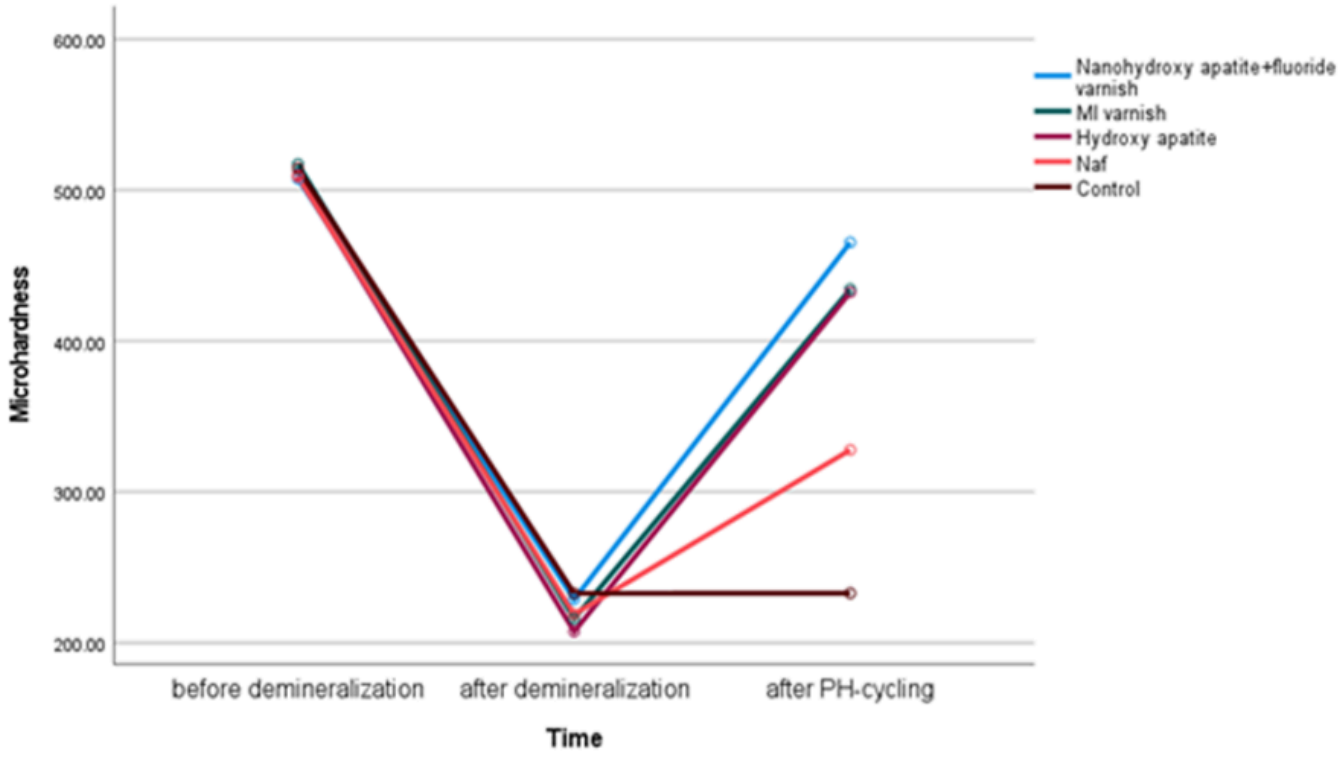


Figure 1

Linear graph showing the trend of surface microhardness changes before demineralisation, after demineralisation, and after pH-cycling in the five study groups. The nano-HA + fluoride group exhibited the steepest recovery, approaching baseline values, whereas the sodium fluoride and control groups showed markedly less mineral gain.