

**COMPARATIVE EVALUATION OF THE EFFECT OF CPP-ACP, BIOACTIVE GLASS,
AND NANO-HYDROXYAPATITE ON DENTIN MICROHARDNESS AND SURFACE
ROUGHNESS OF NON-CARIOUS LESIONS: AN IN VITRO STUDY**

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ABSTRACT

Aim: To evaluate and compare the effect of three different remineralizing agents on dentin microhardness and surface roughness of non-carious lesions. **Materials and Methods:** Forty human mandibular molar teeth extracted for periodontal or orthodontic reasons were collected, cleaned, disinfected, decoronated, and stored in distilled water. Enamel from buccal and lingual surfaces was removed to expose dentin (0.5 mm depth), and specimens were polished for standardization. Each tooth was sectioned, mounted in acrylic resin blocks, and prepared for microhardness and surface roughness analysis. Baseline dentin microhardness was measured using a Shimadzu Vickers microhardness tester, and baseline surface roughness was assessed using a profilometer. Specimens were randomly divided into four groups (n=10): CPP-ACP [GC Tooth Mousse plus], bioactive glass [Elsenz], nano-hydroxyapatite [Perfora], and artificial saliva (control). Demineralization was induced using 37% phosphoric acid for 15 seconds. Post-demineralization measurements were recorded, followed by application of the respective remineralizing agents for 5 minutes daily for 15 days. Control specimens were stored in artificial saliva at 37°C. Final dentin microhardness and surface roughness were then evaluated. **Results:** All groups showed reduced dentin microhardness and increased surface roughness after demineralization. Following remineralization, all experimental groups showed significant improvement compared with the control group ($p < 0.001$). Bioactive glass demonstrated the highest microhardness and lowest surface roughness, followed by nano-hydroxyapatite and CPP-ACP. **Conclusion:** All three remineralizing agents improved dentin microhardness and reduced surface roughness, with bioactive glass showing the highest remineralization potential.

KEYWORDS: Dentin microhardness, surface roughness, bioactive glass, nano-hydroxyapatite, CPP- ACP, remineralization.

INTRODUCTION

A non-cariou lesion is the gradual loss of tooth structure characterized by the formation of a smooth, polished surface, irrespective of its etiology. It includes attrition, abrasion, erosion, and abfraction which can be described as 'wasting disease' or as regressive alterations of the teeth that is not associated with microorganisms.^[1] Worldwide prevalence of non-cariou lesions is increasing due to improved life expectancy. As age increases, prolonged exposure to multifactorial etiological factors leads to progressive loss of cervical tooth structure, making NCCLs more common in middle-aged and older individuals. These lesions can adversely affect the long-term survival of the teeth.^[2] Clinically, these lesions vary from shallow grooves to broad scooped-out lesions to large notched or wedge-shaped lesions. Clinical appearance of non-cariou lesions can vary depending on the type and severity of the etiological factors involved.^[3] Out of all possible etiological factors for non-cariou lesion, occlusal stress forces have received maximum attention over the years. Without proper diagnosis and treatment, it can cause continuous loss of tooth structure, dentin hypersensitivity or even tooth loss.^[4]

The successful management of these lesions is challenging. Due to the significance of conservative dentistry, researchers have long been in search of enamel and dentin remineralizing agents. Various remineralizing agents can prevent or minimize the progression of these lesions. Remineralization of tooth structure typically occurs due to an increase in the concentration of calcium, phosphate, and fluoride ions, along with other salivary buffering agents, which together facilitate the deposition of minerals into demineralized enamel and dentin, thereby restoring structural integrity and resistance to acid attack.^[5]

Bioactive materials such as sodium calcium phosphosilicate (bioactive glass) have shown promising results in enhancing mineral deposition. Elsenz paste is a bioactive glass-based remineralizing and desensitizing agent designed to enhance enamel and dentin mineral recovery. The formulation contains calcium sodium phosphosilicate, which releases calcium, phosphate, and sodium ions upon contact with saliva or an aqueous environment. This ion release promotes the formation of a hydroxycarbonate apatite layer on the tooth surface, closely resembling the natural mineral phase of enamel and dentin. The newly formed apatite layer facilitates occlusion of exposed dentinal tubules, thereby reducing dentin hypersensitivity and enhancing surface integrity.

Recent advances focus on nanotechnology-based systems, including nano-hydroxyapatite particles, which offer improved penetration and controlled ion release.^[9] Perfora toothpaste is a fluoride-free, mineral-based oral care formulation designed to promote enamel protection and overall oral health. The toothpaste primarily contains nano-hydroxyapatite and calcium-based compounds that

mimic the natural mineral composition of enamel. Upon application, these bioavailable minerals interact with the tooth surface, facilitating remineralization of early enamel defects and contributing to the repair of microstructural irregularities.^[10]

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is a biomimetic remineralizing agent consisting of a milk-derived casein phosphopeptide complexed with amorphous calcium phosphate. The phosphorylated peptide chains in CPP-ACP stabilize calcium and phosphate ions within an amorphous nanocomplex, serving as a reservoir of bioavailable minerals.^[11] GC Tooth Mousse Plus is the formulation of CPP-ACP with incorporated fluoride to a level of 900 ppm, where fluoride gives additive effect in reducing caries.^[12]

The microhardness of enamel and dentin indicates the mineral content of their surface. Several studies have measured microhardness as an indicator for the degree of mineralization of enamel and dentin. Surface roughness of dentin is a key parameter that influences plaque retention, bacterial adhesion, dentin permeability, hypersensitivity, and the bonding effectiveness of restorative materials. Various remineralizing agents not only facilitate mineral deposition but also induce notable changes in the surface roughness and topography of dental hard tissues, which can influence bacterial adhesion and overall clinical outcomes.^[15,16] Thus, the present study aimed to evaluate and compare the effectiveness of nano-hydroxyapatite, bioactive glass, and CPP-ACP-based remineralizing agents on dentin surface microhardness and surface roughness in non-cariou lesions.

MATERIALS AND METHOD

Forty human mandibular first molars extracted for periodontal or orthodontic reasons were collected. The extracted teeth were stored in 0.1% thymol solution at room temperature. Soft tissue residues were removed using a scalpel, and the teeth were cleaned with a prophylaxis brush and low-speed handpiece under running water. The teeth were then decoronated using a diamond disc and stored in distilled water. The enamel from the middle and coronal thirds of the buccal and lingual surfaces was removed using a diamond bur (3 mm × 5 mm) to expose dentin to a depth of 0.5 mm. For standardization of specimens, the exposed dentin surfaces were polished using 600-grit abrasive discs. Each crown was bisected into buccal and lingual halves. The specimens were mounted in auto-polymerizing acrylic resin to form blocks measuring 3 cm in length and 2 cm in width. The samples were divided into **four groups** based on the remineralizing agents used, Group 1—CPP-ACP, Group 2—Bioactive glass, Group 3—Nano-hydroxyapatite, and Group 4—Artificial saliva (control). Each group was further subdivided into **Subgroup A** for evaluation of microhardness on the buccal surface and **Subgroup B** for evaluation of surface roughness on the lingual

surface. The baseline dentin microhardness of each mounted specimen was measured using a digital Vickers microhardness tester (Shimadzu HMV-G31DT, India), and all specimens were standardized as far as possible with respect to baseline microhardness values. Baseline surface roughness was also recorded using a surface profilometer. To induce demineralization, all specimens were etched with 37% phosphoric acid (Scotchbond etchant, 3M) for 15 seconds, rinsed with saline for 1 minute, and stored in a buffering solution with a pH of 7.4 at room temperature. Post-demineralization dentin microhardness and surface roughness values were recorded. Specimens in the experimental groups were treated with CPP-ACP, bioactive glass, or nano-hydroxyapatite particles according to the manufacturers'

instructions for 5 minutes daily for a period of 15 days and were stored in artificial saliva between applications. The control group specimens were stored in artificial saliva at 37 °C for the same duration. After 15 days, final dentin microhardness was measured using the Vickers microhardness tester, and final surface roughness was evaluated using surface profilometry.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics version 22. Descriptive statistics included mean and standard deviation. Inferential statistics included the Kruskal–Wallis test for intergroup comparison and Mann–Whitney U test for pairwise comparison. Statistical significance was set at $p < 0.05$.

RESULTS

Table 1.

Group	Time point	Maximum	Minimum	Mean \pm SD
Group 1	Baseline	56.83	55.37	56.10 \pm 0.73
	After acid etching	36.11	35.18	35.65 \pm 0.47
	Final	49.85	48.92	49.39 \pm 0.47
Group 2	Baseline	57.85	57.25	57.55 \pm 0.30
	After acid etching	36.76	36.38	36.57 \pm 0.19
	Final	50.50	50.12	50.31 \pm 0.19
Group 3	Baseline	58.93	58.23	58.58 \pm 0.35
	After acid etching	37.45	37.00	37.23 \pm 0.22
	Final	49.95	49.50	49.73 \pm 0.22
Group 4	Baseline	59.12	55.68	57.40 \pm 1.72
	After acid etching	37.57	35.38	36.48 \pm 1.10
	Final	38.23	36.19	37.21 \pm 1.02

Table 1 presents the measures of central tendency and dispersion for dentin surface microhardness among the study groups following intervention. Intergroup comparison using the Kruskal–Wallis test revealed a statistically significant difference among the four groups ($p < 0.001$). The bioactive glass group showed the highest mean microhardness value, followed by the nano-hydroxyapatite and CPP-ACP groups, whereas the artificial saliva group demonstrated the lowest mean value. Pairwise comparison using the Mann–Whitney U

test revealed significant differences between the CPP-ACP group and the bioactive glass group ($p < 0.001$), and between the CPP-ACP group and the artificial saliva group ($p < 0.001$). However, no statistically significant difference was observed between the CPP-ACP and nano-hydroxyapatite groups ($p = 0.105$). Overall, all three remineralizing agents significantly improved dentin microhardness following demineralization, with bioactive glass showing the greatest remineralization potential.

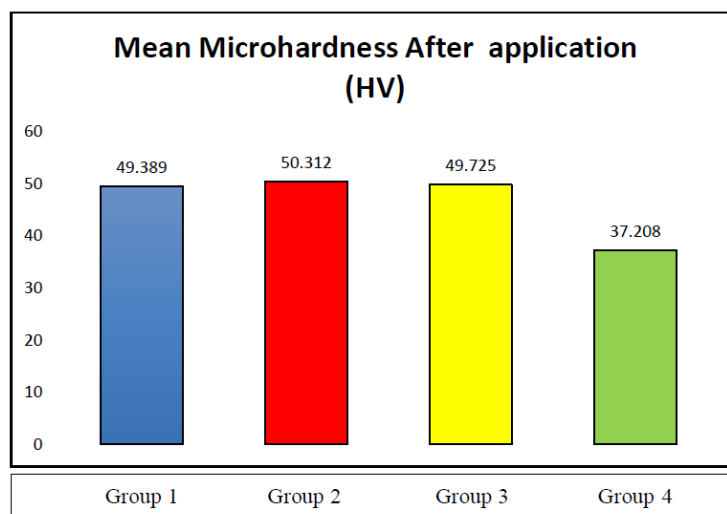
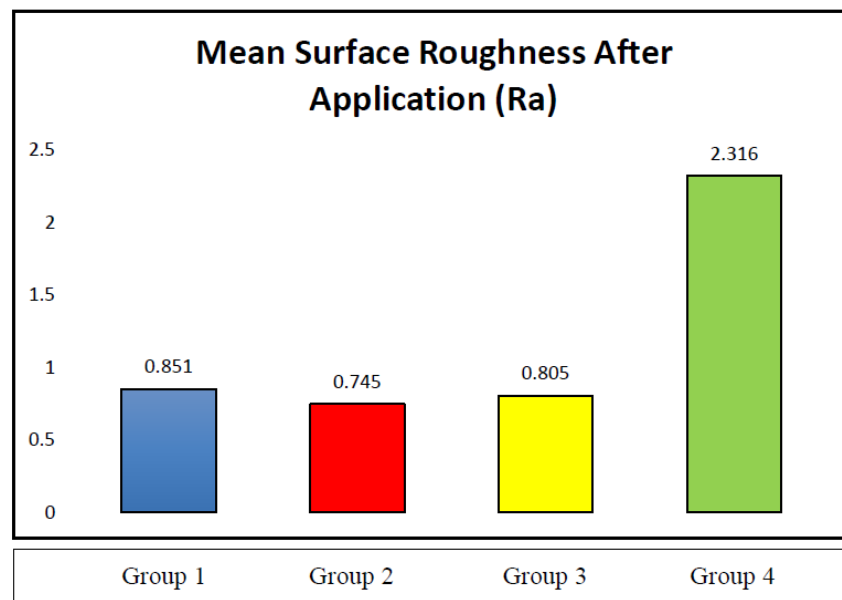


Table 2.

Group	Time point	Maximum	Minimum	Mean \pm SD
Group 1	Baseline	0.72	0.64	0.68 \pm 0.04
	After acid etching	2.12	1.88	2.00 \pm 0.12
	Final	0.89	0.81	0.85 \pm 0.04
Group 2	Baseline	0.82	0.75	0.79 \pm 0.03
	After acid etching	2.52	2.28	2.40 \pm 0.12
	Final	0.78	0.72	0.75 \pm 0.03
Group 3	Baseline	0.92	0.85	0.89 \pm 0.03
	After acid etching	2.75	2.15	2.45 \pm 0.30
	Final	0.84	0.78	0.81 \pm 0.03
Group 4	Baseline	0.94	0.78	0.86 \pm 0.08
	After acid etching	2.72	2.10	2.41 \pm 0.31
	Final	2.50	2.13	2.32 \pm 0.19

Table 2 presents the measures of central tendency and dispersion for dentin surface roughness among the study groups following intervention. Intergroup comparison using the Kruskal–Wallis test revealed a statistically significant difference among the four groups ($p < 0.001$). The bioactive glass group demonstrated the lowest mean surface roughness value, followed by the nano-hydroxyapatite and CPP-ACP groups, whereas the artificial saliva group exhibited the highest surface roughness value. Pairwise analysis using the Mann–

Whitney U test showed statistically significant differences between CPP-ACP and bioactive glass groups ($p < 0.001$), CPP-ACP and nano-hydroxyapatite groups ($p = 0.015$), and between all remineralizing agent groups and the artificial saliva group ($p < 0.001$). Overall, all three remineralizing agents significantly reduced dentin surface roughness following demineralization, with bioactive glass demonstrating the greatest improvement in surface characteristics.



DISCUSSION

Non-carious cervical lesions (NCCLs) consist of irreversible loss of mineralized tissue unrelated to carious pathology. Generally, NCCLs are located in the cervical third of the tooth at the level of cemento-enamel junction and tend to extend from the latter toward the tooth root. The lesions may form as a smooth surface, rounded (saucerlike) depression into the cervical area of a tooth, or a V-shaped indentation.^[17] Regressive alterations may vary in etiology, extent, and clinical presentation among individuals and may be associated with physiologic or pathologic processes. There can be sensitivity, no sensitivity, or limited sensitivity, with

even potential for loss of pulp vitality. The cemento-enamel junction proves to be more prone to loss of substance because the thickness of enamel is greatly reduced and, consequently, the enamel–dentin bond is much weaker. As age increases, prolonged exposure to multifactorial etiological factors leads to progressive loss of cervical tooth structure, making NCCLs more common in middle-aged and older individuals.^[18]

Non-carious cervical lesions require a multifactorial treatment approach based on severity, etiology, and patient-related factors. Management strategies range from preventive measures to restorative interventions.

Remineralizing pastes have been advocated as a conservative treatment modality due to their ability to replenish lost mineral content and improve the physicochemical properties of demineralized tooth structure. These agents act primarily by delivering bioavailable calcium and phosphate ions, with or without fluoride, facilitating re-precipitation of minerals into partially demineralized enamel and dentin.^[19] In NCCLs, where dentin exposure is common, remineralizing pastes may promote tubule occlusion, increase surface microhardness, and reduce surface porosity, thereby enhancing resistance to further acid dissolution and mechanical wear.^[20]

The present study evaluated the effect of three different remineralizing agents on dentin microhardness and surface roughness, which are critical determinants of the structural integrity and functional longevity of dentin affected by non-carious lesions. Dentin microhardness is a direct indicator of mineral content and resistance to demineralization, while surface roughness influences plaque accumulation, bacterial adhesion, and the overall success of restorative procedures. In the present *in vitro* study, changes in dentin microhardness and surface roughness were used to evaluate the remineralization potential of different agents on non-carious cervical lesions. At baseline, dentin microhardness values among the four study groups were comparable, ranging from 56.10 to 58.58 HV, indicating uniformity of the samples prior to experimental intervention. Following acid etching, a marked reduction in dentin microhardness was observed across all groups, with mean values decreasing to 35.65–37.23 HV. Similarly, baseline surface roughness values were low and comparable among groups, ranging from 0.68 to 0.89 μm . After etching, surface roughness values increased considerably in all groups, with mean Ra values rising to 2.00–2.45 μm . Following application of the remineralizing agents, a statistically significant increase in dentin microhardness and a corresponding reduction in surface roughness were observed in all experimental groups when compared with the artificial saliva group ($p < 0.001$). Among the treated groups, Group 2 demonstrated the highest mean microhardness value (50.31 ± 0.19 HV), followed by Group 3 (49.73 ± 0.22 HV) and Group 1 (49.39 ± 0.47 HV), whereas the artificial saliva group showed minimal recovery (37.21 ± 1.02 HV). Post-application Ra values were significantly reduced in Groups 1, 2, and 3, whereas the artificial saliva group showed persistently high surface roughness. The reduction in surface roughness in the experimental groups can be attributed to mineral precipitation within surface irregularities and dentinal tubules, resulting in surface smoothing.

The bioactive glass-containing toothpaste (Elsenz) demonstrated the highest recovery in microhardness and the greatest reduction in surface roughness, suggesting superior mineral deposition and surface smoothing. The nano-hydroxyapatite-containing toothpaste (Perfora) and the CPP-ACP-containing toothpaste (GC Tooth

Mousse) also showed significant improvements, reflecting their ability to promote remineralization and partial occlusion of dentinal tubules. In contrast, the artificial saliva group exhibited minimal improvement in microhardness and persistently higher surface roughness values, highlighting its limited remineralization capacity. Overall, the inverse relationship observed between dentin microhardness and surface roughness indicates effective remineralization, with bioactive glass showing the most pronounced effect, followed by nano-hydroxyapatite and CPP-ACP formulations.

The differences observed among the remineralizing agents can be attributed to variations in their composition and mechanism of action. Bioactive glass (BAG) is a ceramic material composed of amorphous sodium–calcium–phosphosilicate, which exhibits high reactivity in aqueous environments. Upon contact with saliva, bioactive glass releases calcium, phosphate, and sodium ions, leading to the formation of a hydroxycarbonate apatite layer on the tooth surface, chemically similar to natural tooth mineral.^[21] Several studies have demonstrated its effectiveness in reducing dentin hypersensitivity through dentinal tubule occlusion and promoting remineralization. These findings are consistent with studies by Geeta R D *et al.* (2020) and Mohadese Asadi *et al.* (2024), who reported significantly greater remineralization potential and improvement in surface microhardness with bioactive glass and nano-hydroxyapatite compared with CPP-ACP formulations. Similar results were also reported by Kinza Manzoor *et al.* (2024), who demonstrated superior dentin remineralization and tubule occlusion with bioactive glass-based formulations when compared with CPP-ACP on artificially demineralized dentin surfaces, thereby supporting the findings of the present study.

Nano-hydroxyapatite (nHA) is the primary mineral constituent of teeth and bone, making it highly biocompatible and an effective source of calcium and phosphate ions. Its nanoscale size provides larger surface area, higher solubility, increased surface energy, and superior bioactivity, enabling effective interaction with enamel and dentin.^[21] When applied in toothpastes or gels, nHA particles penetrate enamel porosities and form a protective mineral coating, restoring microhardness and reducing surface roughness.

CPP-ACP is a well-established biomimetic agent used for tooth remineralization. It stabilizes calcium and phosphate ions in a bioavailable nanocomposite form and delivers these ions into demineralized zones of enamel and dentin, creating a concentration gradient that promotes remineralization while inhibiting further demineralization.^[22]

From a clinical perspective, the use of bioactive and nanomaterial-based remineralizing agents, such as BAG, nHAp, and CPP-ACP, offers a promising and minimally invasive approach to managing non-carious dentin

lesions. These agents enhance dentin microhardness and reduce surface roughness, thereby strengthening the tooth structure and restoring its functional integrity. Incorporation of such biomimetic remineralization strategies supports conservative treatment protocols, avoids unnecessary tooth removal, and improves long-term tooth resilience.

CONCLUSIONS

Within the limitations of this *in vitro* study, the results demonstrated that all three remineralizing agents improved dentin microhardness and reduced surface roughness following demineralization. Among the tested agents, bioactive glass exhibited the highest remineralization potential, followed by nano-hydroxyapatite and CPP-ACP.

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