

# A Comparative Evaluation of Effect of Three Different Remineralizing Dentifrices on Surface Microhardness of Artificially Demineralized Human Enamel: An In-Vitro Study

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## **Abstract:**

**Objectives:** Aim of this in vitro study was to evaluate and compare the effect of three different remineralizing dentifrices on surface microhardness of artificially demineralized human enamel.

**Materials and Methods:** Artificial white spot lesions were created in enamel blocks obtained from thirty sound human molars. Specimens were then randomly divided into three groups ( $n = 10$ ) based on the test dentifrice used, Group 1: Colgate Sensitive Plus toothpaste, Group 2: Aclaim toothpaste and Group 3: SHY-XT toothpaste. Samples were subjected to Vickers microhardness testing.

**Statistical Analysis:** One-way ANOVA and post hoc Tukey's test were applied for intergroup comparisons. A  $p$ -value  $< 0.05$  was considered statistically significant.

**Results:** All three dentifrices showed significant remineralization ( $p < 0.001$ ). SHY-XT exhibited the highest post-treatment hardness, followed by Aclaim and Colgate Sensitive Plus.

**Conclusion:** SHY-XT was most effective due to its combined fluoride and nHAp content. Biomimetic agents like Aclaim also demonstrated promising results.

**Keywords:** Remineralization, white spot lesions, nano-hydroxyapatite, fluoride, dentifrices, surface microhardness, in-vitro.

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Date of Submission: 05-07-2025

Date of Acceptance: 16-07-2025

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## **I. Introduction**

White Spot Lesions (WSLs), the earliest visible signs of enamel demineralization, appear as chalky white, opaque areas and are marked by subsurface mineral loss while the surface remains intact. If untreated, WSLs can progress to cavitated lesions, causing both aesthetic and functional issues (1,2,3).

Dentifrices containing various fluoride compounds are known to be effective in the remineralization of early WSLs. However, it is difficult for fluoride to result in oriented and ordered mineral crystals on the surface of enamel under physiological conditions due to the lack of ability to guide the formation of mineral crystals. The ordered orientation is essential for the mechanical properties of enamel. (3)

Dentifrices containing biomimetic agents like Pro-Argin™ and Nano-hydroxyapatite (nHAp) have emerged as effective alternatives or adjuncts to fluoride in managing WSLs. Pro-Argin™ promotes remineralization by delivering calcium and phosphate ions deep into the lesion body (4). Hence it significantly increases the surface microhardness and reduces the lesion porosity in WSLs, comparable to the effects seen with fluoride-based interventions. (5,6). While, nHAp mimics natural enamel, facilitating mineral deposition and reducing lesion depth while improving surface microhardness (7). Hence, it is proven to be more effective than fluoride in improving the reducing the lesion depth (8,9). Additionally, nHAp avoids risks like fluorosis, making it a safer long-term option (10).

Recently introduced, nHAp dentifrice containing fluoride has shown synergistic remineralization effects. It forms a fluorapatite layer more acid-resistant than hydroxyapatite while strengthening both subsurface & surface enamel (8,9). Studies have shown that such a combination boosts the long-term enamel protection by reducing the lesion depth thereby producing significantly higher surface microhardness recovery than those dentifrices containing either constituent alone. (8,9)

In research, Surface microhardness (SMH) indentations provide a relatively simple, nondestructive and rapid method in demineralization and remineralization studies

Hence, this in-vitro study evaluates the remineralization potential of these three novel dentifrices on surface microhardness of demineralized enamel with WSLs.

## **II. Material And Methods**

A total of 30 extracted sound human molars, free from caries, restorations, cracks, or structural defects, were collected and stored in 0.1% thymol solution at room temperature to prevent microbial growth until use [11]. The crown portion of each decoronated molar was then sectioned mesiodistally and buccolingually, resulting in three equal sized enamel blocks per tooth [12]. Each enamel block was embedded in self-cure acrylic resin, exposing the outer enamel surface for testing [13].

The exposed enamel surface of each block was demarcated into three equal occlusogingival sections [14]. The cervical third (baseline normal section) of each tooth will be coated with a nail varnish leaving behind two thirds of the tooth, which will be subjected to pH cycling. After pH cycling the middle third (baseline demineralized Section) will be coated with nail varnish while the occlusal third will be subjected to the treatment using the respective three dentifrices.

The specimens were randomly divided into three groups (n = 30 per group) based on the remineralizing dentifrice used:

Group 1: Colgate Sensitive Plus toothpaste (containing Pro-Argin Technology) [15]

Group 2: Aclaim toothpaste (containing nano-hydroxyapatite) [16]

Group 3: SHY-XT toothpaste (containing potassium nitrate, nano-hydroxyapatite, and fluoride) [17]

### **Artificial Demineralization Process**

To create white spot lesions (WSLs), the enamel blocks (excluding the control section) were immersed in a demineralizing solution at 37°C for 10 days [18]. The demineralizing solution contained: 0.1 M lactic acid, 4.0 mM calcium chloride, and 8.0 mM potassium phosphate. The pH was adjusted to 4.5 to mimic cariogenic conditions [19]. After 10 days, the middle third was sealed with nail varnish, preserving it as the "demineralized section" for comparison.

### **Remineralization Protocol**

The lower third of each enamel block was subjected to a 14-day remineralization regimen simulating clinical conditions [20]:

Each block was brushed with the assigned dentifrice using a soft-bristled toothbrush [20].

Brushing simulation was performed for 5 minutes every 12 hours (morning and evening) [21].

Between treatments, samples were stored in artificial saliva (composition: 1.5 mM CaCl<sub>2</sub>, 0.9 mM KH<sub>2</sub>PO<sub>4</sub>, 130 mM KCl, and 20 mM HEPES buffer, pH 7.0), maintained at 37°C and replenished daily [22].

### Microhardness Testing

Following the remineralization period, the nail varnish coated on cervical and middle third section of each block was removed and then all samples were subjected to Vickers microhardness testing for each section of each enamel block [23].

### Statistical Analysis

Data were statistically analyzed using one-way ANOVA to compare remineralization effectiveness among groups. Post-hoc Tukey's test was used for pairwise comparisons.

A p-value < 0.05 was considered statistically significant [24].

## III. Result

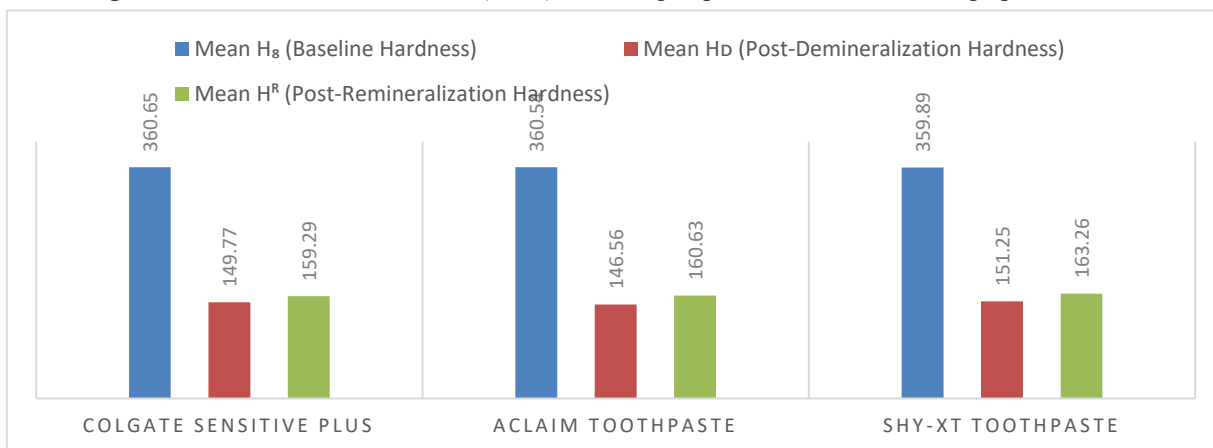
This in-vitro study evaluated and compared the remineralization potential of three dentifrices—Colgate Sensitive Plus, Aclaim, and SHY-XT—on artificially demineralized enamel lesions using Vickers microhardness testing at three stages: baseline ( $H_s$ ), post-demineralization ( $H_d$ ), and post-remineralization ( $H^R$ ).

At baseline, all enamel samples showed comparable hardness values, averaging around 360 VHN, indicating initial uniformity. Following 10 days of artificial demineralization, there was a significant reduction in surface hardness across all groups. The average hardness dropped to 149.77 in the Colgate group, 146.56 in the Aclaim group, and 151.25 in the SHY-XT group, confirming effective formation of white spot lesions. After 14 days of remineralization treatment, all three dentifrices resulted in a statistically significant increase in surface hardness ( $p < 0.001$ ). Among them, SHY-XT demonstrated the highest and statistically significant post-remineralization hardness (163.26 VHN), followed by Aclaim (160.63 VHN) and Colgate Sensitive Plus (159.29 VHN).

**Table no 1:** The mean hardness values (VHN) for each group are summarized in the table below:

Remineralizing Agent	Mean $H_s$ (Baseline Hardness)	Mean $H_d$ (Post-Demineralization Hardness)	Mean $H^R$ (Post-Remineralization Hardness)	p-value	t-value
Colgate Sensitive Plus	360.65	149.77	159.29	< 0.001*	3.14
Aclaim Toothpaste	360.54	146.56	160.63	< 0.001*	4.45
SHY-XT Toothpaste	359.89	151.25	163.26	< 0.001*	2.72

**Fig no 1:** The mean hardness values (VHN) for each group are summarized in the graph below:



## IV. Discussion

The marked decrease in surface microhardness following the demineralization protocol confirms the successful creation of artificial white spot lesions (WSLs). All three tested dentifrices produced a statistically significant recovery in enamel hardness, aligning with previous findings that support their remineralization efficacy (25,26).

Among the groups, Group III (SHY-XT) exhibited the highest post-remineralization surface hardness values. This enhanced performance can be attributed to the synergistic action of fluoride, nano-hydroxyapatite (nHAp), and potassium nitrate, which collectively promote the sustained release and deposition of calcium, phosphate, and fluoride ions onto demineralized enamel (27,28). These ions facilitate the reconstruction of enamel's crystalline structure, thereby improving acid resistance and contributing to more robust remineralization (29).

Group II (Aclaim) demonstrated the second-highest increase in microhardness, significantly more than Group I (Colgate Sensitive Plus). Aclaim, containing only nHAp, showed strong remineralizing potential owing

to its structural resemblance to natural enamel apatite. This similarity allows nHAp to integrate into the enamel surface and promote subsurface lesion repair (30,31). Although it lacks fluoride, Aclaim's performance underscores the standalone capability of nHAp in early lesion remineralization. Moreover, its fluoride-free composition may be preferable in populations where systemic fluoride exposure is a concern, such as in regions with endemic fluorosis (30).

Group I (Colgate Sensitive Plus), while effective, showed comparatively lower remineralization potential. This could be due to its dependence on Pro-Argin™ technology, which primarily occludes dentinal tubules through arginine-calcium-phosphate complexes, without substantially contributing to enamel matrix reconstruction (31). Although beneficial in reducing hypersensitivity, its remineralization capacity appears limited when compared to agents incorporating nHAp and fluoride (27).

## V. Conclusion

This in-vitro study confirms that all tested dentifrices promote significant enamel remineralization, with SHY-XT showing the highest efficacy due to its synergistic combination of fluoride and nano-hydroxyapatite. Aclaim also demonstrated strong potential, while Colgate Sensitive Plus showed comparatively lesser effects. These findings support biomimetic agents as effective alternatives in managing WSLs.

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