ORIGINAL RESEARCH

A Comparative Evaluation of the Remineralization Potential of Two Contemporary Bioactive Glass-containing Dentifrices on Artificially Demineralized Human Enamel: An *In Vitro* Study

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Abstract

Background: There is limited literature comparing the remineralization potential of these two dentifrices, Elsenz™, which contains fluoro calcium (Ca) phosphosilicate, and Shy-NM™, which contains Ca sodium phosphosilicate, are a few of the remineralizing agents.

Aim: To assess and compare the remineralization potential of Elsenz™ and Shy-NM™ dentifrices on artificially induced carious lesions on permanent teeth, using the Vickers microhardness measuring method and scanning electron microscope (SEM) connected to energy dispersive X-ray analysis after laboratory stimulation of the oral environment employing the pH cycling model.

Materials and methods: A total of 30 sound human premolar teeth were divided into six groups for both parameters. Group I—Elsenz™ dentifrice, group II—Shy-NM™ dentifrice, and group III—control. The surface microhardness (SMH) of the test specimens was evaluated followed by a scanning electron microscope with energy dispersive analysis (SEM-EDAX). The specimens were tested at baseline, demineralization, and remineralization. The collected data were subjected to statistical analysis.

Results: Surface microhardness following remineralization with Elsenz™ was 359 Vickers hardness number (VHN), and with Shy-NM™ was 312 VHN. Elsenz™ showed significantly higher remineralization compared to Shy-NM™ ($p = 0.002$). The SEM-EDAX of the tooth specimens after remineralization revealed an increase in the Ca weight percentage (wt%) compared with demineralization values, which was statistically significant for both Elsenz™ (45.95 ± 3.55%) and Shy-NM™ (47.24 ± 1.99%), along with an increase in the phosphorus wt%, which was statistically significant for Elsenz™ (20.25 ± 0.95%) compared to Shy-NM™ (19.95 ± 0.59%).

Conclusion: Within the scope of this study, the incorporation of fluoride in bioactive glass (BAG) in Elsenz™ had the potential to remineralize enamel better than Shy-NM™ dentifrice. It can, therefore, be concluded that Elsenz™, when compared with Shy-NM™, would be effective in inhibiting demineralization.

Keywords: Bioactive glass, Elsenz™, Scanning electron microscope with energy dispersive analysis, Shy-NM™, Surface microhardness.

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Introduction

The oral cavity is a combat zone of demineralization and remineralization events.[1] Lowering of the pH of the oral fluids (below 5.5) leads to demineralization, that is, the dissolution of hydroxyapatite (HA) crystals, which is followed by the release of phosphate and calcium (Ca) ions from the tooth surface into oral fluids, leading to dental caries. When pH increases, remineralization results from the supersaturation of Ca and phosphate ions in the oral solution.[2] To reestablish natural equilibrium, either demineralization should be retarded or remineralization must be accelerated.[3]

The demineralization process can be discontinued by creating an environment favorable for remineralization using various remineralizing agents such as casein phosphopeptide (CPP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), bioactive glass (BAG), and Ca sodium phosphosilicate (Novamin) incorporated either into a toothpaste or a topical cream for caries prevention.[4,5]

Bioactive glass substances have the rare capacity to mimic the body's natural mineralization processes while also inducing cell signaling in a way that aids in the recovery of tissue function and form.[5] These are pH-sensitive because they dissolve quicker in acidic than in basic or neutral conditions.[6] The active ingredient is amorphous Ca sodium phosphosilicate, which releases bioavailable Ca, sodium, and phosphate ions in an aqueous environment.[5] The Ca phosphate complexes crystallize to form HA, contributing to the remineralization process.[2]

There is limited literature comparing the remineralization potential of Elsenz™ and Shy-NM™. The present study evaluated and compared these two BAG formulations, fluoro Ca phosphosilicate (Elsenz™), and Ca sodium phosphosilicate (Shy-NM™), on enamel using the Vickers hardness measuring method and scanning electron microscope (SEM) with energy dispersive X-ray spectroscopy after laboratory simulation of the oral environment employing the pH

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cycling model for determining microhardness and remineralizing capacity of the tooth enamel.

Materials and Methods

A total of 30 freshly extracted sound human permanent premolar teeth were extracted due to orthodontic reasons. The extracted tooth was sectioned into buccal and lingual halves. For the tooth/test specimens assigned for Vickers testing, the tooth specimens were embedded in acrylic resin using a customized metallic mold. The tooth specimens were mounted horizontally in self-cure acrylic resin with the buccal or lingual surface facing upward. While embedding, the exposed part of the specimen was covered with a damp laboratory napkin to avoid dehydration during setting.[7]

The test surfaces of all the specimens assigned for Vickers and scanning electron microscope with energy dispersive analysis (SEM-EDAX) were ground flat and hand-polished with progressively finer grades of silicon carbide (800, 1000, and 1200 grit) in order to obtain a flat surface. To prevent dehydration, the specimens were stored in deionized (DI) distilled water during processing. The specimens were visualized under a stereomicroscope at 10× magnification to eliminate any specimens with obvious cracks or other flaws in the enamel surface or loss of enamel of the polished enamel specimens. Prepared specimens were stored at 100% relative humidity and 4°C until testing. Before testing for demineralization, all the specimens were coated with acid-resistant nail paint, exposing only a standardized window on the buccal surface of enamel (4 × 4 mm). For demineralization, specimens were exposed to the demineralizing solution. Exposure to demineralization produced caries-like lesions in the exposed window.[8,9]

Baseline testing of the specimens was done with Vickers microhardness and SEM-EDAX.

Demineralizing/Remineralizing Study Protocol

The specimens were immersed in the demineralization solution, which was then stirred. The demineralization solution consisted of Ca (2.0 mmol/L Ca nitrate tetrahydrate), phosphate (2.0 mmol/L monopotassium phosphate), and acetic acid (75.0 mmol/L).[10] The demineralization cycle was performed at 37°C for a period of 48 hours. After demineralization, prior to testing, all the specimens were rinsed with DI distilled water, blotted dry with filter paper, and stored in artificial saliva (14.4 mM sodium chloride, 16.1 mM potassium chloride, 0.3 mM magnesium chloride, 2.9 mM dipotassium phosphate, 0.75 mM Ca chloride dihydrate, 0.10 gm/100 mL sodium carboxymethylcellulose, and pH of solution = 7)[11] at 37°C until further use.[9]

Vickers microhardness and SEM-EDAX testing of the specimens were done postdemineralization.

The remineralization protocol of the specimen, which was subjected twice a day (8:00 am, 3:00 pm) over a period of 10 days, is as follows:

At 08:00 am: The specimens were retrieved from artificial saliva. After removal of the specimens from artificial saliva, a battery-operated soft-bristled toothbrush (Oral B™) was used for brushing the specimens with respective remineralizing agents and DI distilled water as control (group I with Elsenz™ dentifrice, group II with Shy-NM™ dentifrice, group III with DI distilled water), for 2 minutes, and gently rinsed with DI water.

From 08:30 am to 3:00 pm: All teeth were soaked in artificial saliva and stored in an incubator at 37°C to simulate physiological oral conditions.

At 3:00 pm: After removal of the specimens from artificial saliva, a battery-powered soft-bristled toothbrush (Oral B™) with respective remineralizing agents/control for 2 minutes was used for brushing of the specimens and gently rinsed with DI water.

From 03:30 pm to 08:00 am: All teeth were again soaked in artificial saliva and stored in an incubator at 37°C.[12]

All the tooth specimens were subjected to the remineralization as per the abovementioned protocol for 10 days. After remineralization, the specimens were subjected to Vickers microhardness and SEM-EDAX testing.

Statistical Analysis

The collected data were analyzed with IBM Statistical Package for the Social Sciences Statistics for Windows, version 23.0 (Armonk, New York: IBM Corp). Descriptive statistics, including the mean and standard deviation (SD), were used to describe the data. To determine significant differences, one-way analysis of variance (ANOVA) with Tukey's *post hoc* test and the Kruskal–Wallis test were employed for multivariate analysis. For repeated measures, repeated measures ANOVA was used, with Bonferroni correction to control the type I error on multiple comparisons, and the Friedman test was utilized. In all the above statistical tools, the probability value of 0.05 was considered significant.

Results

Comparative Evaluation of SMH of the Tooth Specimens Using Vickers Microhardness Indenter

The mean surface microhardness (SMH) at baseline, after demineralization, and after remineralization for group IA (Elsenz™) was 313.60, 59.73, and 358.96 Vickers hardness number (VHN), respectively. For group IB (Shy-NM™), the values were 314.53, 65.28, and 312.36 VHN, respectively. group IC (control) exhibited values of 316.22, 64.01, and 91.06 VHN, respectively (Table 1 and Fig. 1).

The intragroup comparison revealed a highly statistically significant difference between the SMH at baseline and final SMH values in the Elsenz™ group and Shy-NM™ group (p-value = 0.0005).

Comparative Evaluation of SEM-EDAX of the Tooth Specimens

Calcium Element

The mean Ca wt% of group IIA (Elsenz™) for baseline, after demineralization and remineralization were 39.81 ± 3.16, 35.17 ± 2.27, 45.95 ± 3.55, respectively, Group IIB (Shy-NM™) was 44.58 ± 2.18, 40.32 ± 3.89, 47.24 ± 1.99, respectively, group IIC (control) was 41.33

Table 1: Mean baseline, demineralization, and remineralization SMH values of tested specimens

		N	*Mean ± SD*
Mean baseline SMH	Group IA (Elsenz™)	10	313.60 ± 3.50
	Group IB (Shy-NM™)	10	314.53 ± 4.32
	Group IC (control)	10	316.22 ± 2.76
Mean SMH after demineralization	Group IA (Elsenz™)	10	59.73 ± 3.03
	Group IB (Shy-NM™)	10	65.28 ± 1.96
	Group IC (control)	10	64.01 ± 4.15
Mean SMH after remineralization	Group IA (Elsenz™)	10	358.96 ± 37.61
	Group IB (Shy-NM™)	10	312.36 ± 24.73
	Group IC (control)	10	91.06 ± 12.65

± 1.37, 37.67 ± 2.45, 38.20 ± 2.12, respectively (Table 2). The mean Ca atomic percentage (at%) of group IIA (Elsenz™) for baseline after demineralization and remineralization were 23.81 ± 2.67, 21.44 ± 1.15, 29.04 ± 3.56, respectively, group IIB (Shy-NM™) was 28.04 ± 2.07, 24.90 ± 3.35, 29.50 ± 1.17, respectively, group IIC (control) was 25.21 ± 1.18, 21.89 ± 1.76, 22.84 ± 3.62, respectively.

Phosphorous Element

The mean phosphorus (P) wt% of group IIA (Elsenz™) for baseline, after demineralization, and after remineralization was 18.92 ± 1.11, 18.24 ± 1.32, and 20.25 ± 0.95, respectively. For group IIB (Shy-NM™), the values were 20.02 ± 0.42, 18.78 ± 0.59, and 19.95 ± 0.59, respectively. Group IIC (control) exhibited values of 20.04 ± 0.67, 14.15 ± 9.46, and 18.81 ± 0.87, respectively. The mean P at% of group IIA (Elsenz™) for baseline, after demineralization, and after remineralization was 14.63 ± 1.26, 14.25 ± 0.76, and 16.47 ± 1.12, respectively. For group IIB (Shy-NM™), the values were 28.04 ± 2.07, 14.75 ± 0.87, and 19.95 ± 0.59, respectively. Group IIC (control) exhibited values of 25.21 ± 1.18, 14.19 ± 0.53, and 14.97 ± 0.92, respectively.

Discussion

Dentifrices containing bioavailable stabilized calcium, fluoride, and phosphate ions are remineralizing systems that instigate subsurface remineralization (mineral gain) rather than surface deposition of minerals and are effective for caries prevention.[13]

The parameters considered in this study were hardness, evaluated using Vickers microhardness, and surface properties with elemental analysis, conducted using an SEM-EDAX.[14]

Baseline microhardness values were between 300 and 400 VHN (like that of sound enamel).[9] Specimen standardization and allotment of specimens to the test groups prevented any sample bias. No significant difference was observed in the SMH values at baseline between the groups ($p = 0.270$). The mean SMH values postdemineralization ranged from 54.6 to 69.5 VHN, respectively. Postdemineralization, the microhardness of all the enamel specimens was found to decrease considerably.

The posttreatment increase in SMH observed in the present study was considered indicative of remineralization and could be interpreted as a clinically meaningful outcome. An increased VHN was observed, which indicated remineralization.[15]

Table 2: Mean baseline, demineralization, and remineralization of SEM-EDAX, Ca, and P (wt, at%) values of tested specimens

		N	*Element*		*Mean ± SD*
Baseline SEM-EDAX	Group IIA (Elsenz™)	4	Ca	wt%	39.81 ± 3.16
				at%	23.81 ± 2.67
			P	wt%	18.92 ± 1.11
				at%	14.63 ± 1.26
	Group IIB (Shy-NM™)	4	Ca	wt%	44.58 ± 2.18
				at%	28.04 ± 2.07
			P	wt%	20.02 ± 0.42
				at%	16.29 ± 0.74
	Group IIC (control)	4	Ca	wt%	41.33 ± 1.37
				at%	25.21 ± 1.18
			P	wt%	20.04 ± 0.67
				at%	15.82 ± 0.66
Demineralization SEM-EDAX	Group IIA (Elsenz™)	4	Ca	wt%	35.17 ± 2.27
				at%	21.44 ± 1.15
			P	wt%	18.24 ± 1.32
				at%	14.25 ± 0.76
	Group IIB (Shy NM™)	4	Ca	wt%	40.32 ± 3.89
				at%	24.90 ± 3.35
			P	wt%	18.78 ± 0.59
				at%	14.75 ± 0.87
	Group IIC (control)	4	Ca	wt%	37.67 ± 2.45
				at%	21.89 ± 1.76
			P	wt%	14.15 ± 9.46
				at%	14.19 ± 0.53
Remineralization SEM-EDAX	Group IIA (Elsenz™)	4	Ca	wt%	45.95 ± 3.55
				at%	29.04 ± 3.56
			P	wt%	20.25 ± 0.95
				at%	16.47 ± 1.12
	Group IIB (Shy-NM™)	4	Ca	wt%	47.24 ± 1.99
				at%	29.50 ± 1.17
			P	wt%	19.95 ± 0.59
				at%	15.82 ± 0.75
	Group IIC (control)	4	Ca	wt%	38.20 ± 2.12
				at%	22.84 ± 3.62
			P	wt%	18.81 ± 0.87
				at%	14.97 ± 0.92

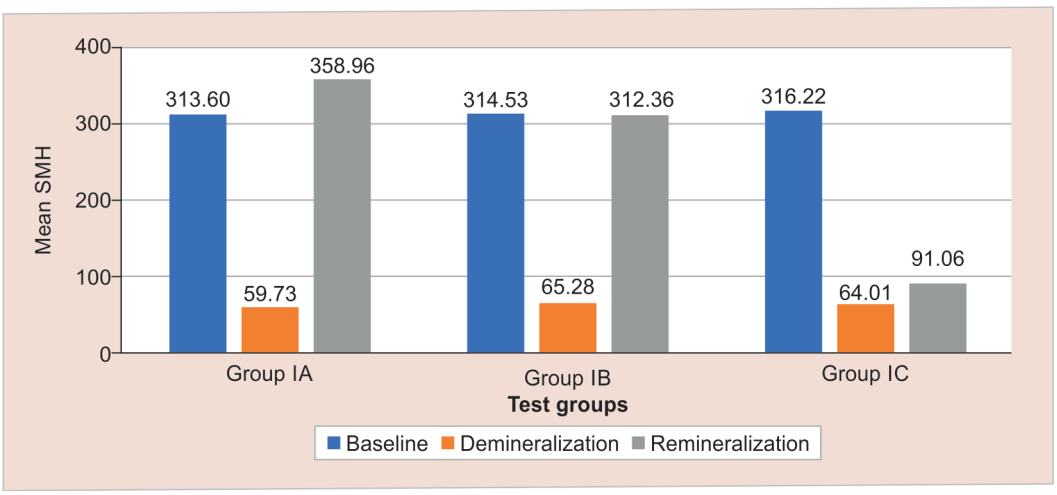


Fig. 1: Intergroup and intragroup comparison of mean SMH of test groups

Kaur et al. found a distinct difference between baseline microhardness (sound teeth) and remineralization values, which was observed in the present study.[11] The results of the present study were in agreement with the work of Farooq et al.,[16] Meshki et al.,[17] and Srivastava et al.,[18] who also inferred those bioactive containing dentifrices when compared with other denitrifies showed enhanced remineralization.[9]

Scanning electron microscope images of baseline enamel specimens revealed a smooth homogeneous appearance (intact enamel, Fig. 2A). SEM images of postdemineralized enamel specimens revealed a porous interprismatic and prismatic enamel appearance—Fish scale (Fig. 2B).[15] SEM images after remineralization revealed mineral deposits, which were appreciable and more prominent in group I (Elsenz™, Fig. 2C) compared to group II (Shy-NM™, Fig. 2D). No evident mineral deposits were observed in group III (control group).

This finding is in agreement with Huang et al.,[19] who used SEM to assess the impact of nano-HA concentration on remineralization of initial enamel lesions. The author found that nano-HA crystals were continuously deposed on demineralized enamel.[13]

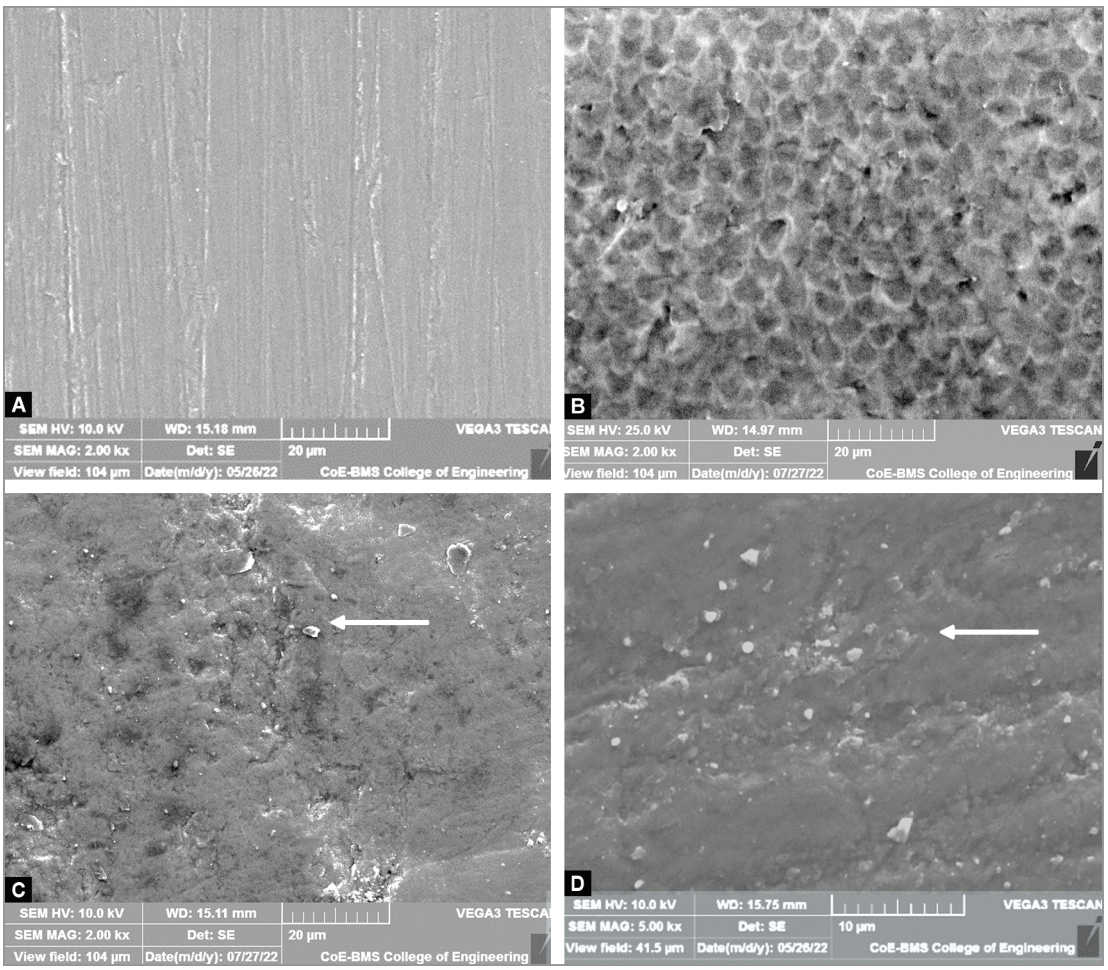
In the present study, while testing remineralization potential using SEM-EDAX for topographical and elemental analysis,

Remineralization mean values for group IIB (Shy-NM™) dentifrice demonstrated the highest calcium wt% value of 47.24 ± 1.99, followed by group IIA (Elsenz™) dentifrice 45.95 ± 3.55, while the group IIC (control) group demonstrated the least value 38.20 ± 2.12. A highly significant difference was observed between Elsenz™ and Shy-NM™ dentifrice ($p = 0.018$).

Elsenz™ dentifrice demonstrated the highest calcium at% value of 29.04 ± 3.56, followed by Shy-NM™ dentifrice at 29.50 ± 1.17, while the control group demonstrated the lowest value at 22.84 ± 3.62. A highly significant difference was observed between Elsenz™ and Shy-NM™ dentifrice ($p = 0.018$). Elsenz™ dentifrice demonstrated the highest phosphorus wt% value of 20.25 ± 0.95, followed by Shy-NM™ dentifrice at 19.95 ± 0.59, while the control group demonstrated the lowest value at 18.81 ± 0.87. A statistically significant difference was observed between Elsenz™ and Shy-NM™ dentifrice ($p = 0.018$).

Elsenz™ dentifrice demonstrated the highest phosphorus wt% value of 16.47 ± 1.12, followed by Shy-NM™ dentifrice at 15.82 ± 0.75, while the control group demonstrated the lowest value of 14.97 ± 0.92. No statistically significant difference was observed between Elsenz™ and Shy-NM™ dentifrice ($p = 0.174$) and the control group (p-value = 0.105).

Elsenz™ has a phosphate content that is nearly three times higher than that of traditional BAG. The High phosphate content in BAG helps to form apatite faster than currently available BAG.[20]



Figs 2A to D: Representative images SEM-EDAX specimens of (A) baseline—intact enamel; (B) demineralization—Fish scale appearance; (C) remineralization—arrows indicate mineral deposits seen in group IIA; (D) Group IIB

The findings of the present study are comparable with those of:

Sivaranjani et al.,[4] who concluded that Elsenz™ dentifrice showed greater remineralization potential compared with the other dentifrices. Also, Suryani et al.,[15] and Aidaros et al.,[13] concluded that the incorporation of BAG technology in dentifrice showed maximum effect on the demineralized enamel surface and better remineralization compared to the other dentifrices.

This study was carried out under *in vitro* conditions on premolar teeth. Further *in vivo* studies on primary and permanent teeth need to be conducted in order to further evaluate the effectiveness of remineralization of dentifrice-containing BAG.

Within the limitations and based on the results of the present study, test specimens treated with Elsenz™ demonstrated higher remineralization potential. Test specimens of the control group demonstrated lower remineralizing potential when compared with other test groups treated with BAG dentifrice. It can be concluded that BAG dentifrice containing fluoride is more effective in remineralizing incipient carious lesions.

Conclusion

The results of the present study suggest that both dentifrices containing BAG showed effective remineralization potential and can be considered for preventing enamel demineralization by increasing resistance against acid attack and promoting remineralization of carious lesions. In the present study, Elsenz™ demonstrated superior remineralizing potential in comparison with Shy-NM™; however, further clinical studies have to be conducted in order to further evaluate and approve the same.

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