

# Comparative evaluation of remineralization efficacy of ELSENZ and SHY-XT toothpastes on initial enamel lesions: A scanning electron microscopic study

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## Abstract

The present study was done to evaluate the remineralization potential of two different commercially available toothpaste, namely, ELSENZ and SHY-XT on initial enamel white spot lesion using Scanning Electron Microscope (SEM). The objective of this study is to evaluate and compare the remineralization efficacy of ELSENZ and SHY-XT toothpaste on initial enamel white spot lesion. This study included a total 12 samples of extracted human permanent anterior teeth that were collected from the Oral and Maxillofacial Department in College of Dental Science Amargadh, Bhavnagar, Gujarat. All samples were equally divided in three groups. In group 1, remineralization was done by ELSENZ (fluoro-calcium phosphosilicate) toothpaste, and in group 2, remineralization was done by SHY-XT (nano-hydroxyapatite and fluoride) toothpaste and group 3 was taken as control group in which deionized water was used. At the end of 12 days, pH cycling procedure, remineralization for all the samples were analysed by the scanning electron microscope. Statistical analysis was done by One Way Analysis of Variance to check the difference between the groups and Post hoc analysis was done by Tukey's test. The result showed that the maximum remineralization occurred in ELSENZ toothpaste group ( $P < 0.00$ ) followed by SHY-XT toothpaste group ( $P < 0.50$ ) and deionized water showed least remineralization potential ( $P < 2.00$ ). From the result of the study, we concluded that ELSENZ toothpaste has more remineralization potential on initial enamel white spot lesion as compared to SHY-XT toothpaste.

**Keywords:** demineralization, fluoridated toothpaste, initial enamel lesion, remineralization

## Introduction

Dental caries is an infectious bacterial oral disease, related to oral flora. Dental plaque and consumption of fermentable sugar can lead to demineralization of dental tissue. Nowadays, caries management has shifted into non-invasive treatment, using remineralizing agents that is effective on demineralized early enamel lesions (Indrapriyadharshini *et al.*, 2018).

The first clinical sign of demineralization is seen as "white spot lesion" that is reversible

to normal enamel surface by using the remineralizing agents. Fluoride is the most commonly used remineralizing agent in the dentistry as fluoridated toothpaste. This fluoridated toothpaste converts hydroxyapatite crystals to fluorapatite crystals which are more resistant to acid challenge (Toda *et al.*, 2008).

The SHY-XT toothpaste contains fluoride, nano hydroxyapatite and potassium nitrate. Potassium nitrate depolarizes the dentinal nerve fibres and prevents the tooth sensitivity. By sealing the open dentinal tubules and establishing a layer over the

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dentin surface, Nano Hydroxyapatite works profoundly to block dentinal tubules and relieve sensitivity while fluoride fortifies tooth enamel. The SHY-XT toothpaste aids in remineralizing the white spot lesion and alleviating discomfort brought on by sensitivity.

The fluoro-calcium phosphosilicate in ELSENZ toothpaste is an enhanced variety of bioactive glass. Over the course of 8 to 12 hours, ELSENZ produces acid resistant fluorapatite and releases fluoride. ELSENZ is a distinctive toothpaste to use for remineralization because of the combined actions of apatite production and ion release. These two toothpastes are different from normal fluoridated toothpaste in terms of having different remineralization contents with fluoride.

Dentifrices containing 1000ppm fluoride have been recommended for children as it is effective in reducing caries (Kielbassa *et al.*, 2009; Mir *et al.*, 1969; Shetty *et al.*, 2016; Wright *et al.*, 2014). However, the use of greater amount of fluoridated toothpaste may increase the risk for fluorosis, especially among children more than 6 years of age as they cannot spit during brushing (Reynolds *et al.*, 2008). Hence, non-fluoride containing dentifrices with remineralizing agents have been developed (Wright *et al.*, 2014). The process of remineralization is constrained by the availability of calcium and phosphate ions. Other than fluoride toothpaste, calcium phosphate-based remineralizing agents are the combination of calcium sucrose phosphate and inorganic amorphous calcium phosphate are commercially available that helps in remineralization of demineralized enamel (George *et al.*, 2015; Reynolds, 2008). In our study, we used experimental toothpastes having remineralizing agents with fluoride.

A medical literature has revealed till date no scanning electron microscope study has been conducted to compare the remineralizing efficacy of ELSENZ and SHY-XT toothpaste on initial enamel white spot lesion. So this study will be helpful in preventing the progress of demineralized enamel.

## Materials and Method

This study was designed and conducted in the Department of Pediatric and Preventive Dentistry in College of Dental Science and Hospital, Amragadh, Bhavnagar, Gujarat. Ethical clearance was obtained by Institutional Ethics Committee (CODS/IEC/111/2021) and study permission was taken from the Medical Government Collage, Department of Microbiology, Bhavnagar, Gujarat, India.

### Inclusion criteria

- Permanent teeth without caries and without initial enamel white spot lesion.
- Permanent teeth with all four intact surfaces.
- Permanent teeth with non-visible cracks.

### Exclusion criteria

- Teeth with initial enamel white spot lesion.
- Teeth with developmental defects.
- Teeth with hypoplastic enamel.
- Restored teeth / teeth with restoration.

### Preparations

Extracted teeth were kept in the hydrogen peroxide before enamel block preparation.

#### • Preparation of enamel blocks

The crowns of all the incisors were separated from the roots at the cemento-enamel junction using a water-cooled carborundum disc. Enamel blocks of 3 × 3 mm were prepared from a flatter labial surface and embedded in polymethyl methacrylate. The superficial surface of the enamel was ground flat with water-cooled carborundum disc and polished with grit waterproof silicon carbide paper to remove approximately 0.1 mm of the outermost enamel layer and prepared a flat surface then blocks were made in cold cure resins (Chandru *et al.*, 2020) (Figure 1 and 2).

#### • Preparation of demineralizing solution

Demineralizing solution made with distilled water by adding 0.05 mM lactic acid, 2.2 mM of calcium chloride and 2.2 mM of sodium dihydrogen

orthophosphate. Potassium hydroxide pellets was added for the adjustment of pH of the solution to 4.5 (Chandru *et al.*, 2020).

- **Preparation of artificial saliva**

Artificial saliva was prepared in distilled water by adding 3.90 mM of sodium phosphate, 4.29 mM of sodium chloride, 17.98 mM of potassium chloride, 1.1 mM of calcium chloride, 0.08 mM of magnesium chloride, 0.05 mM of sulfuric acid, and 3.27 mM of sodium bicarbonate. The pH of the artificial saliva was set at 7 (Figure 3).

### Sample size

Power analysis for mean difference from constant one sample t-test was conducted in G\*Power (version 3.1.9.7) to determine the sufficient sample size, with a power of study = 0.95, alpha= 0.05 The minimum sample size calculated was 12.

### Methods

In this experimental study total 12 samples were allocated by using random sampling technique (Figure 4).

- 1) Group 1 ( $n = 4$ ): ELSENZ (Fluoro-calcium phosphosilicate) toothpaste.
- 2) Group 2 ( $n = 4$ ): SHY-XT (Nano-hydroxyapatite, and fluoride) toothpaste.
- 3) Group 3 ( $n = 4$ ) (control group): Deionized water.

Demineralizing solution was prepared, and its pH was set at 4.5. After that, all the samples were kept in demineralizing

solution for 2 hours at 37 °C in an incubator for a day followed by washing the samples in deionized water (Chemi pure, deionized water, India) for 2 min then all samples were observed under Scanning electron microscopic to check the morphological changes (Figure 5). This protocol is followed in accordance to the study conducted by Cai *et al.* (2003) and Huang *et al.* (2009).

The evaluation of SEM images was done according to Bonetti *et al.*, (2014) scoring criteria, after that all samples were coated with the experimental toothpaste namely, ELSENZ, SHY-XT and control group was treated with deionized water by using applicator brush (Figure 6). The samples were left stable for 3 min on green cloth on the floor, then all the samples were kept in the artificial saliva for 24 hours and every after 3 days new artificial saliva was prepared. This entire procedure was repeated for 12 days. At the end of 12 days pH cycling, SEM images of all selected samples from each group were taken to analysed remineralization by using Bonetti *et al.* (2014) grading criteria (Table 1).

### Statistical Analysis

Statistical analysis was done by One Way Analysis of Variance to check the surface roughness on tooth of difference between the groups. Post hoc analysis was done by Tukey's for the intergroup comparison of surface roughness. Data was analysed by the IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA). A p-value of less than 0.05 was considered as statistically significant.

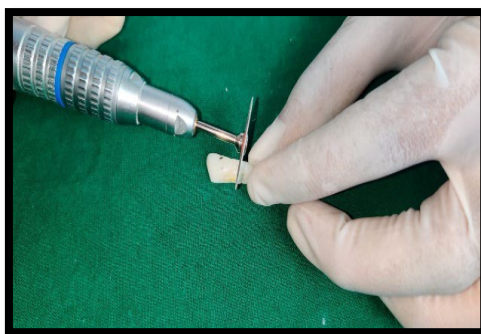


Figure 1. Preparing block for all 12 teeth at cervical area of teeth.



Figure 2. All samples in cold cure acrylic resin.



Figure 3. All the teeth kept in artificial saliva to prevent the dehydration of extracted teeth.



Figure 4. Divided the samples among three groups.

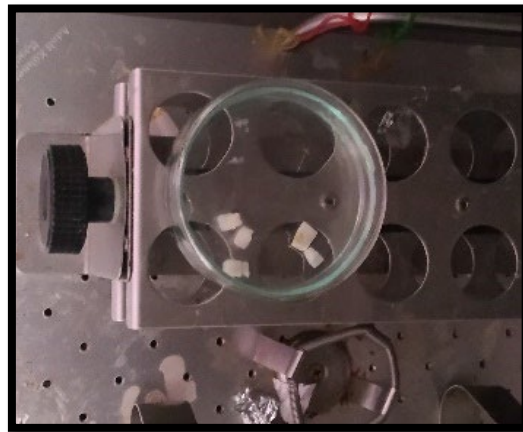


Figure 5. Placed samples in demineralized solution for 2 hours at 37 °C in incubator.

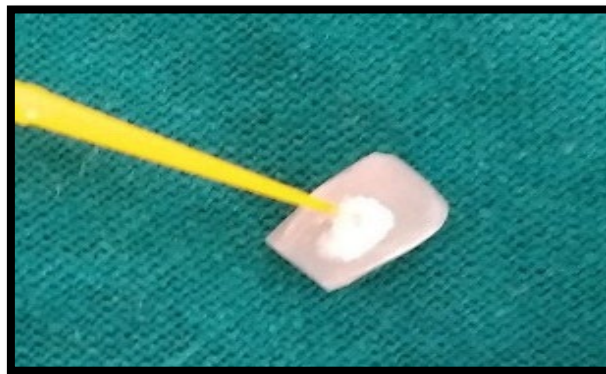


Figure 6. Samples were coated with the experimental toothpaste.

Table 1. Scoring criteria used for the evaluation enamel lesion by SEM images Bonetti *et al.* (2014).

<b>Score 0</b>	Enamel surface remained perfectly intact with no grooves, pits, and porosity.
<b>Score 1</b>	Presence of surface irregularities on enamel surface, without demineralization of prismatic and/or interprismatic enamel.
<b>Score 1</b>	Presence of wrinkles and demineralization of prismatic /interprismatic enamel. Diffuse demineralization involved the rod core, with decomposition of Morphology of prism.

## Result

Table 2 and Figure 7 showed the SEM images under 200x magnification and 2500x magnification in which the teeth surface was seen smooth and intact enamel surface

before demineralization. Table 3 and Figure 8 showed SEM images under 200x magnification and 2500x magnification after demineralization. It revealed the appearance of collapsed rod and uneven enamel surface was seen with increase in porosity.

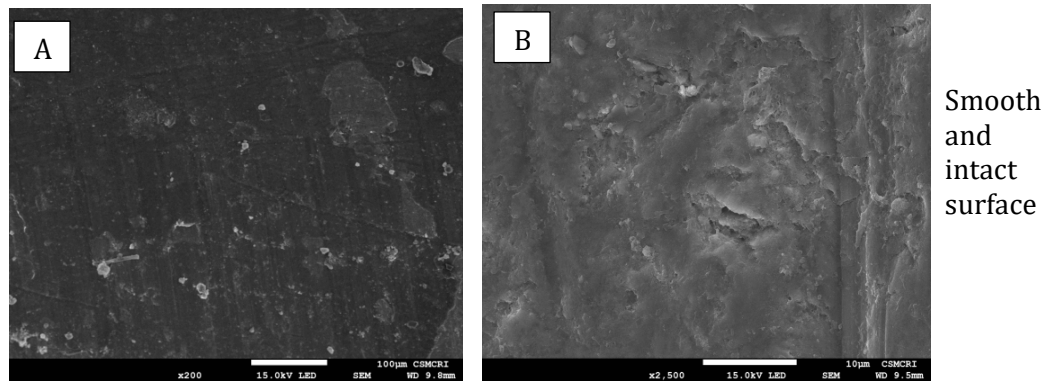


Figure 7. Smooth and intact enamel surface. A) SEM image of enamel before demineralization (under 200x magnification). B) SEM image of enamel before demineralization (under 2500x magnification).

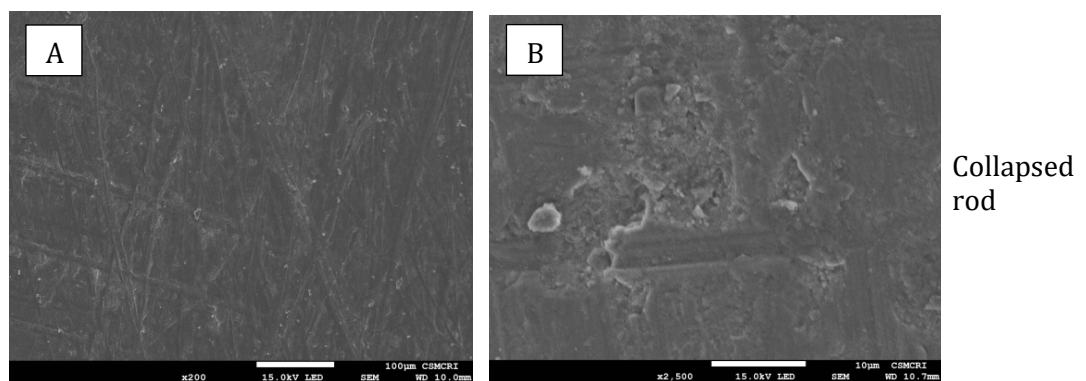


Figure 8. Appearance of collapsed rod and uneven enamel surface was seen with increase in porosity. A) SEM image of enamel after demineralization for 24 hours (under 200x magnification). B) SEM image of enamel after demineralization for 24 hours (under 2500x magnification).

Table 2. Comparison of the three materials under scanning electron microscope using Bonetti *et al.* (2014)'s rating before demineralization.

	200x Magnification	2500x Magnification
GROUP 1	Grade 0	Grade 1
GROUP 2	Grade 0	Grade 2
GROUP 3	Grade 0	Grade 1

Table 3. Comparison of the three materials under scanning electron microscope using Bonetti *et al.* (2014)'s rating after demineralization.

	200x Magnification	2500x Magnification
GROUP 1	Grade 1	Grade 2
GROUP 2	Grade 0	Grade 1
GROUP 3	Grade 1	Grade 2

After 12 days of remineralization by application of ELSENZ toothpaste (Fluoro-calcium phosphosilicate), SHY-XT Toothpaste (Nano -hydroxyapatite and fluoride), the morphological changes were observed in SEM under 200x magnification and 2500x magnification (Figure 9, Figure

10). Group 1 and Group 2 revealed layers of surface deposition of minerals obliterating the defects, filling up the rods and interrod region, showing an uneven yet more homogeneous surface compared to the deionized water on enamel surface of teeth (Figure 11).

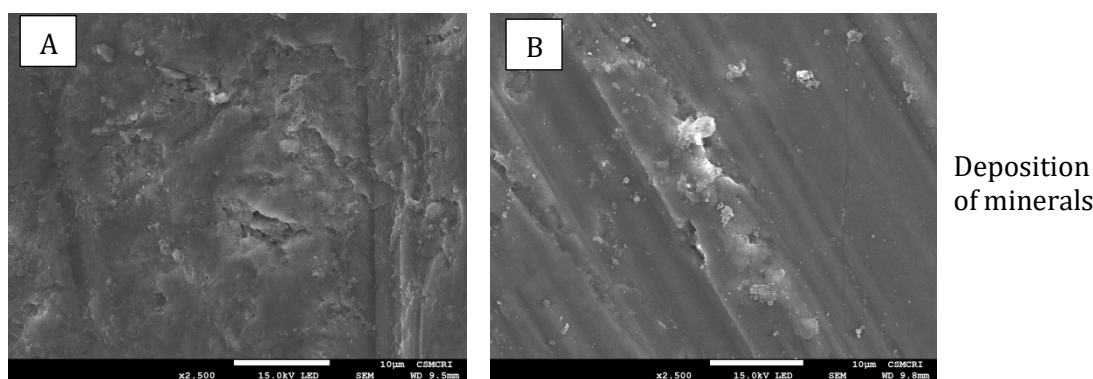


Figure 9. Layers of surface deposition of minerals obliterating the defects, filling up the rods and interrod region, showing an uneven yet more homogeneous surface. A) Enamel surface after application of ELSENZ toothpaste (Under 200x magnification). B) Enamel surface after application of ELSENZ toothpaste (Under 2500x magnification)

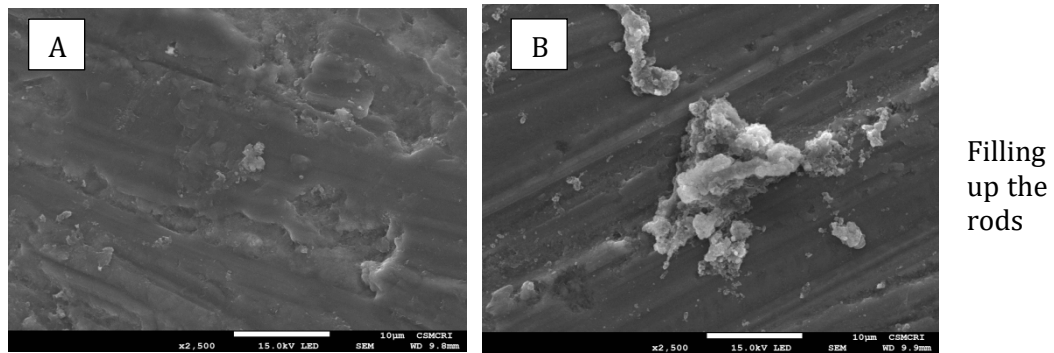


Figure 10. Layers of surface deposition of minerals obliterating the defects, filling up the rods and interrod region, showing an uneven yet more homogeneous surface. A) Enamel surface after application of SHY- XT toothpaste (Under 200x magnification). B) Enamel surface after application of SHY-XT toothpaste (Under 2500x magnification)

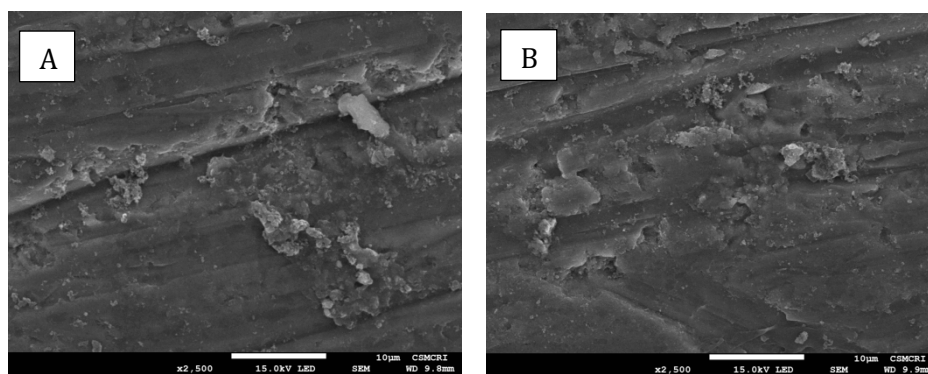


Figure 11. Layers of surface deposition of minerals obliterating the defects, filling up the rods and interrod region, showing an uneven yet more homogeneous surface. A) Enamel surface after application of deionized water (Under 200x magnification). B) Enamel surface after application of deionized water (Under 2500x magnification).

Table 4 showed the comparison of the three materials under scanning electron microscope using Bonetti *et al.* (2014)'s rating criteria after remineralization. SEM observation under 200x magnification and 2500x magnification showed that there was

decrease irregularities, rough and wrinkles on the demineralized enamel surface after remineralization. There were no discernible enamel rods or prismatic material but there were calcified deposits with crystals of fluorhydroxyapatite in them.

Table 4. Comparison of the three materials under scanning electron microscope using Bonetti *et al.* (2014)'s rating after remineralization.

	200x Magnification	2500x Magnification
GROUP 1	Grade 0	Grade 0
GROUP 2	Grade 0	Grade 1
GROUP 3	Grade 0	Grade 2



Table 5 showed mean baseline score of surface roughness according to Bonetti *et al.* (2014) criteria that was not statistically significant among the groups. The difference between the groups after demineralization was also not statistically significant with a p-

value of 1.17. After remineralization there was statistically significant change in the score in relation to the control group ( $2.00 \pm 0.00$ , 95% CI). Table 6 showed that there was statistically significant difference between ELSENZ toothpaste and deionized water.

Table 5. Mean and standard deviation of surface roughness on tooth in different stages of the study.

Stage of the study	Magnification	Group	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Before demineralization	200	SHYXT	4	.00	.000	.00	.00
		ELSENZ	4	.00	.000	.00	.00
		Deionized water	4	.00	.000	.00	.00
		Total	12	.00	.000	.00	.00
	2500	SHYXT	4	1.00	.000	1.00	1.0
		ELSENZ	4	1.50	.707	-4.85	7.85
		Deionized water	4	1.00	.000	1.00	1.00
		Total	12	1.17	.408	.74	1.60
After demineralization	200	SHYXT	4	.00	.000	.00	.00
		ELSENZ	4	.00	.000	.00	.00
		Deionized water	4	.50	.707	-5.85	6.85
		Total	12	.17	.408	-.26	.60
	2500	SHYXT	4	2.50	.707	-3.85	8.85
		ELSENZ	4	2.50	.707	-3.85	8.85
		Deionized water	4	2.50	.707	-3.85	8.85
		Total	12	2.50	.548	1.93	3.07
After remineralization	200	SHYXT	4	.00	.000	.00	.00
		ELSENZ	4	.00	.000	.00	.00
		Deionized water	4	.00	.000	.00	.00
		Total	12	.00	.000	.00	.00
	2500	SHYXT	4	.50	.707	-5.85	6.85
		ELSENZ	4	.00	.000	.00	.00
		Deionized water*	4	2.00	.000	2.00	2.00
		Total	12	.83	.983	-.20	1.87

Table 6. Intergroup comparison of surface roughness on tooth at various stages and magnifications.

Dependent Variable	(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before demineralization (x2500)	SHYXT	ELSENZ	-.500	.408	.518	-2.21	1.21
		Deionized water	.000	.408	1.000	-1.71	1.71
	ELSENZ	SHYXT	.500	.408	.518	-1.21	2.21
		Deionized water	.500	.408	.518	-1.21	2.21
	Deionized water	SHYXT	.000	.408	1.000	-1.71	1.71
		ELSENZ	-.500	.408	.518	-2.21	1.21
After remineralization (x200)	SHYXT	ELSENZ	.000	.408	1.000	-1.71	1.71
		Deionized water	-.500	.408	.518	-2.21	1.21
	ELSENZ	SHYXT	.000	.408	1.000	-1.71	1.71
		Deionized water	-.500	.408	.518	-2.21	1.21
	Deionized water	SHYXT	.500	.408	.518	-1.21	2.21
		ELSENZ	.500	.408	.518	-1.21	2.21
After demineralization (x2500)	SHYXT	ELSENZ	.000	.707	1.000	-2.95	2.95
		Deionized water	.000	.707	1.000	-2.95	2.95
	ELSENZ	SHYXT	.000	.707	1.000	-2.95	2.95
		Deionized water	.000	.707	1.000	-2.95	2.95
	Deionized water	SHYXT	.000	.707	1.000	-2.95	2.95
		ELSENZ	.000	.707	1.000	-2.95	2.95
After remineralization (x2500)	SHYXT	ELSENZ	.500	.408	.518	-1.21	2.21
		Deionized water	-1.500	.408	.069	-3.21	.21
	ELSENZ	SHYXT	-.500	.408	.518	-2.21	1.21
		Deionized water*	-2.000*	.408	.033	-3.71	-.29
	Deionized water	SHYXT	1.500	.408	.069	-.21	3.21
		ELSENZ*	2.000*	.408	.033	.29	3.71

\*The mean difference is significant at the 0.05 level.

## Discussion

Remineralization of initial enamel white spot lesions is an important concept for prevention of demineralization. Fluoridated toothpaste converts the hydroxyapatite crystals of enamel into fluoroapatite crystals which is more resistant to acid challenge (Martins *et al.*, 2011). As a result, fluorides remineralization activity only rises in the presence of enough free calcium and phosphate ions (Petrou *et al.*, 2009). Fluoride free or fluoride supplemented products have been developed as an alternative to fluoride because of the calcium phosphate dependent effect of fluoride and other issues such as toxicity and the danger of fluorosis. In the current investigation, the *ex vivo* efficacy of two fluoridated toothpastes ELSENZ and SHY-XT was compared (Cai *et al.*, 2003).

Titty *et al.* (2018) compared the remineralizing effect of sodium monofluorophosphate, amine fluoride-containing dentifrices, calcium sucrose phosphate, and inorganic amorphous calcium phosphate-containing dentifrice were found to be equivocal in their ability to remineralize early enamel lesions. This was determined by comparing the remineralizing effectiveness of calcium sucrose phosphate and fluoride dentifrices. Tulumbaci (2019) compared the efficacy of different remineralization agents on treating incipient enamel lesions of primary and permanent teeth. It was seen that Clinpro 5000 ppm fluoride is more efficient in remineralizing incipient enamel lesions compared to the deionized water, Colgate Cavity Protection, Sensodyne Rapid Relief, GC MI Paste Plus and Sensodyne Repair and Protect agents tested. In our study, ELSENZ toothpaste showed more remineralization effect as compared to SHY-XT toothpaste this can be due to presence of remineralizing agents with more fluoride content in ELSENZ toothpaste.

Chandru *et al.* (2020) observed that Colgate sensitive plus toothpaste with Pro Argin™ showed more remineralization potential because of presence of arginine, calcium carbonate and sodium

monofluorophosphate. Arginine alone may be insufficient to remineralize demineralized lesions or to provide resistance to further acid challenge as compared to our study the ELSENZ toothpaste contain fluoro calcium phosphosilicate, the is actively protected teeth against acid attack for up to 12 hours after brushing, which is 6 time longer than other fluoride toothpaste, so it can be used as a noninvasive means of managing early enamel carious lesions.

In the current investigation, SEM was used to assess the enamel surfaces after various treatments. The teeth were incubated in a demineralization solution for two hours at 37°C, which could have accelerated the development of the first enamel lesion on the tooth surface and dramatically increased porosity compared to sound enamel (Figure 8). This provides a bigger surface area for the subsequent response of enamel mineral and enables for higher penetration of solution ion components (Figure 9). Fluoro-calcium phosphosilicate is an improved variety of bioactive glass is a component of ELSENZ toothpaste. This creates fluorapatite that is resistant to acids and releases fluoride over the course of 8 to 12 hours. The early enamel white spot lesions are encouraged to remineralize by the combined actions of apatite production and ion release (Figure 10.) Fluoride nano hydroxyapatite and potassium nitrate are both prevalent constituents in SHY-XT toothpaste. Potassium nitrate, a substance that depolarizes dentinal nerve fibers, alleviates sensitivity-related pain. Fluoride strengthens tooth enamel, whereas nano hydroxyapatite develops a layer on the surface of the dentin to significantly lessen dentin sensitivity. The SHY-XT toothpaste lessens pain associated with sensitivity and repairs damaged tooth enamel (Lv *et al.*, 2007).

In present study, it was seen that deionized water has limited remineralization potential because of its relatively poor mineral nucleation ability. This finding is similar to the findings of Robinson *et al.* (2017).

## Conclusion

Both experimental toothpastes have potential to remineralize the initial enamel lesions so both can be used as a non-invasive method of managing initial enamel white spot lesion, but ELSENZ toothpaste showed more remineralization effect as compared to SHY-XT toothpaste; so, ELSENZ toothpaste can be considered better option than SHY-XT toothpaste for remineralization of demineralized enamel surface.

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