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Efficacy of novel toothpaste on fluoride availability and enamel microhardness

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ABSTRACT:

Purpose: This study evaluated the remineralization potential of dental enamel after applying Senquel F® toothpaste based on fluoride ion availability, enamel layer thickness, and surface microhardness.

Methods: The toothpaste, Senquel-F®, is a potassium nitrate-based desensitizing dentifrice. 30 human teeth were used for fluoride ion availability and enamel layer thickness, while 35 were used for surface microhardness. The study used freshly extracted permanent teeth, excluding carious, deciduous, and hypoplastic teeth. The toothpaste was brushed individually with Senquel F® gel for 1-2 minutes daily for two weeks. Fluoride ion availability was tested after a single application and continuous use of the toothpaste for two weeks.

Results: There was a significant ($p<0.0001$) rise in fluoride ion concentration. Fluoride ion levels increased by almost 94.4% after a 24-hour period. Additionally, enamel became noticeably thicker ($p<0.0001$). The results are expressed in parts per million (PPM).

Conclusion: The study highlights the importance of toothpaste in promoting dental enamel remineralization.

KEYWORDS: Tooth development, Dentifrice(s), Fluoride(s), Dental hygiene, Remineralization

I. INTRODUCTION

Dentin hypersensitivity (DH) is one of the most common complaints from patients in dental clinics and is a rising concern due to the pain and discomfort associated with it [1]. Prevalence of DH is varied ranging from 1% to 98% [2]. It is characterized by a transient sharp pain following various stimuli, which disappears immediately after removal of the external stimulus. The short sharp pain in response to these stimuli may affect daily activities including eating, drinking, speaking and tooth brushing [1]. Severe DH lasting 6 months or more can induce psychological and emotional changes and may often require management similar to neuropathic pain [3]. A meta-analysis shows significant improvement in oral health related quality of life in patients with DH after treatment [4].

Exposure of dentine is one of the commonest causes of DH, which can be caused by physical, chemical, pathological, biological challenges and/or developmental abnormalities that result in dental and/or periodontal damage or defects leading to enamel exposure [1]. Other factors, such as aging, soft tissue dehiscence, including aggressive brushing can cause gingival recession.

Management of DH includes multiple approaches ranging from patient education, non-invasive restorative treatments, or surgical approach depending on the etiology. Recent advances dealing with in office treatment includes LASER therapy or fluoride iontophoresis. Non-invasive desensitizing agents are most widely used particularly in cases with limited or minimal tooth loss.

In bone and dental hard tissues, calcium phosphates exist primarily as hydroxyapatite, which is thermodynamically most stable form at neutral and slightly acidic pH. When fluoride is present, saliva and saliva-like solutions become supersaturated with respect to fluorapatite across most pH values found in dental plaque. However, the super saturation toward hydroxyapatite is limited to the pH range above 5.6 to 5.8. In the presence of low concentrations of fluoride (such as in saliva or plaque fluid), hydroxyapatite may dissolve below its critical pH. Notably, the critical pH for a mineral composed of fluoride-rich apatite is significantly lower than that for hydroxyapatite.

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Fluoride application through varnish and dentifrice facilitates the formation of fluorapatite. Demineralization- and remineralization experiments with enamel and dentin have demonstrated that fluoride enhances mineral uptake during continuous remineralization and inhibits mineral loss during demineralization [5].

Many desensitizing toothpastes containing fluorides influence the mineralization of enamel in varying proportions. However, the effectiveness of a Sodium fluoride (NaF) and Sodium mono fluorophosphate (SMFP) containing desensitizing toothpaste on the surface microhardness of enamel is not yet established in-vivo. Senquel-F® is a potassium nitrate based desensitizing dentifrice available in India (Dr. Reddy's Laboratories, India).

This study assessed the availability of fluoride ions and enamel surface changes with Senquel-F®, and its effect on surface microhardness of enamel of extracted permanent teeth.

II. MATERIALS AND METHODS

2.1. Study design

This was an experimental in-vitro study.

2.2. Study objectives

This study evaluated the remineralization potential of dental enamel after applying Senquel F® toothpaste based on the fluoride ion availability, enamel layer thickness and surface microhardness.

2.3. Study Setting

The study for fluoride ion availability and enamel layer changes were conducted at Vitely Corp LLP, Ahmedabad, whereas the surface microhardness study was conducted at department of Periodontology, Dr. G.D. Pol Foundation's YMT Dental College and Hospital, Navi Mumbai in collaboration with a private metallurgical laboratory.

2.4. Test product

Senquel-F® (Fast Relief and Enamel Protection desensitizing dentifrice) is a commercially available toothpaste (Dr. Reddy's Laboratories, India), which is a potassium nitrate based desensitizing dentifrice containing KNO3 - 5%, NaF 800 ppm, SMFP 200 ppm and Xylitol - 10% (Batch No.: SNQ-01-(0.16+0.14+5+10)-F-NB1-014).

2.5. Study sample

Total 35 human teeth were used for surface microhardness, out of which 30 human teeth were also assessed for the fluoride ion availability and enamel layer thickness. Freshly extracted permanent teeth were obtained between September 2023 to January 2024 from clinical sites. Carious teeth, deciduous teeth, and those with immature root apices, and hypoplastic defects were not included. Also, teeth with any cracks, attrition, erosion, abrasion, abfraction, resorption, fractured roots and endodontically treated teeth were excluded. Collected teeth were washed with 2.5% sodium hypochlorite (NaOCl), dried and used for further testing.

2.6. Study procedure

The study procedure explains the brushing technique and processes used to determine fluoride ion availability in Senquel F® toothpaste and teeth, enamel layer conditions, and tooth microhardness.

2.6.1. Brushing procedure

Each tooth was brushed individually with Senquel F® gel applied on the enamel surface of the teeth. Brushing was done by a single operator using manual standardized method for 1-2 minutes once in a day for 2 weeks. After brushing, teeth were rinsed with deionized water to remove excess gel.

2.6.2. Procedure for fluoride ion availability in Senquel F® toothpaste

The test was done in three replicates for both total fluoride and total soluble fluoride. Total fluoride is the amount of fluoride ions extracted from the Senquel F® toothpaste sample by acid hydrolysis. Total soluble fluoride is the amount of fluoride ions dissolved in water.

Senquel F® toothpaste sample (1.0669g) weighted and homogenized in 100 mL of deionized water, and 0.25 mL of the suspension was transferred to assay tubes in triplicate for Total Fluoride (TF) analysis. The remaining suspension was centrifuged at 3,000 RPM for 10 minutes at room temperature to remove insoluble fluoride. 0.25 mL of the supernatant was transferred to assay tubes in triplicate to determine the Total Soluble Fluoride (TSF) concentration. For all TF and TSF tubes, 0.25 mL of 2.0 M HCL was added, and tubes were incubated at 45°C for 1 hour; the samples were neutralized with 0.5 mL of 1.0 M NaOH and buffered with 1.0 mL of TISAB buffer after incubation. The tubes were analyzed for fluoride ion availability using a calibrated ion-specific electrode Fluoride ION Meter Model Serene F

(Spectralab, India). Blank samples were tested independently without the presence of toothpaste samples. For the results, blank values were subtracted from test values. The resulting values were then multiplied by the dilution factor, which is 800. Typically, the conversion ratio for fluoride to NaF and SMFP is 2.2. Therefore, the final value was multiplied by 2.2 to obtain the total concentration of NaF and SMFP. The results are expressed in parts per million (PPM), a concentration unit.

2.6.3. Procedure for fluoride availability test in teeth

Fluoride ion availability in 30 teeth was tested after single application and continuous use of the Senquel F® toothpaste for two weeks. The testing was done before application and at different timepoints (30 seconds, 1 minute, 2 minutes, 5 minutes, 10 minutes, 30 minutes, 1 hr., 4 hr., 24 hr., 72 hr. and 168 hrs.) post-application of Senquel F® toothpaste. The fluoride concentration is expressed in parts per million (PPM) multiplied by 10-3.

Each tooth was separately submerged in an individual container with 5 mL of deionized water for 24 hours. After 24 hours, teeth were removed from deionized water, and fluoride ions were measured from deionized water using a calibrated Fluoride ION Meter Model Serene F (Spectralab, India).

2.6.4. Procedure for enamel layer conditions of teeth

Enamel thickness was measured using calibrated micrometer, while enamel integrity testing was done by visual inspection under light microscopy. To evaluate the condition of the enamel before and after the application of toothpaste, microscopy of each tooth was performed at specific sites marked for comparison. The images of the enamel surface were captured and analysed.

2.6.5. Procedure for enamel surface microhardness

For cleaning and storing of the specimens normal saline and distilled water was used. Airotor hand piece was used for specimen preparation. Teeth samples were stored in artificial saliva (Water, Glycerin, Sorbitol, Propylene Glycol, PEG 40 HCO, Poloxamer, Sodium Benzoate, Sodium CMC, Flavour, Cetylpyridinum chloride, parabens, Xylitol, Xanthan gum, Disodium hydrogen phosphate and Sodium dihydrogen Phosphate) [6].

2.7. Preparation of Enamel blocks:

Enamel blocks were prepared using. Freshly extracted permanent human teeth (n=35) were collected, cleaned using normal saline and 2.5% NaOCl and stored in artificial saliva. Decoronation of teeth was done at cementoenamel junction. A square label with dimension 4x2 mm was placed on buccal/labial surfaces of teeth. All remaining surfaces of teeth were covered in self-cure acrylic.

2.8. pH cycling (Remineralization and demineralization):

30 samples (15 test and 15 control) were subjected to pH cycling conditions using demineralizing and remineralizing solutions. Demineralizing solution contained sodium acetate (0.1mM CH3COONa), potassium chloride (150 mMKCl2), calcium chloride (1.5 mM CaCl2) and potassium dihydrogen phosphate (0.9 mM KH2PO4). The pH was adjusted to 4.5 using hydrochloric acid(0.1mol/l). Slight elevations were corrected with hydrochloric acid(0.1mol/l) to maintain a constant pH of 4.35-4.65 during the demineralization phase. Remineralizing solution contained sodium chloride (0.5g/l), sodium bicarbonate (4.2g/l), sodium nitrate (0.03g/l) and potassium chloride (0.20 g/l). The pH was 8.0 and the solution prepared was similar to saliva.

One pH cycle comprised of demineralization phase for 6 hours at pH=4.5, followed by remineralization phase for 18 hours at pH=8. In between two phases, the samples were immersed in distilled water to rinse off the solutions for 15 seconds and application of the toothpaste under study to test group samples. The processing of the samples was done over a period of 10 days, in which the samples were immersed in a demineralizing solution for 6 hours followed by rinsing thoroughly and remineralizing solution for 18 hours.

For test (n=15) and control (n=15) groups, the microhardness was measured after remineralization, whereas for demineralization group (n=5) microhardness was measured after demineralization. A Vickers Microhardness indenter (Reichert, Austria) was used to measure the microhardness under the load of 100g for 15 seconds at three different positions, each 1 mm apart. The microhardness was measured with a Diamond Indentor at a 136º Angle under a load of 100g. Mean VHN (Vickers hardness number) was calculated.

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2.9. Statistical analysis

Sample size for the microhardness study was estimated using Epi-Info software (Microsoft Windows) and substituting the value in the formula "$n = p\ (100-p)\ /\ z2$". With an effect size of 20%, at alpha 0.05 and 80% power, using a two-sided test, the estimated sample size of 15 in each group led to a total sample size of 30. Additional 5 samples were taken for the demineralization group of the study.

3.1. Fluoride ions in Senquel F® toothpaste

The total NaF and SMFP (Sodium Monofluorophosphate) content in Senquel F® toothpasteis presented in table 1. The results show that the Senquel F® toothpaste sample has a high solubility of fluoride ions, as the total soluble fluoride values are close to the total fluoride values. This suggests that the fluoride ions in the Senquel F® toothpaste are quickly released into the oral cavity and interact with the enamel surface. The results demonstrated that the rough enamel surface became smoother after the application of Senquel F-Fast Relief & Enamel Protection Gel toothpaste under pH cycling conditions.

The null hypothesis that there is no difference in enamel microhardness before and after the application of Senquel F® toothpaste, was tested using t-test. A p-value of less than 0.05 was the threshold for statistical significance. Statistical analyses were performed using SAS® statistical software (Version: 9.4; SAS Institute Inc, USA).

III. RESULTS

Table 1: Fluoride ion availability in Senquel F® toothpaste (PPM).

	NaF + SMFP (PPM)	
	Cumulative Soluble content	Cumulative Total content
Replicate I	732.2	880.0
Replicate II	649.4	924.0
Replicate III	1008.5	1043.7
Average content	796.7	949.2
NaF: Sodium fluoride; PPM: Parts per million; SMFP: Sodium Monofluorophosphate		

3.2. The fluoride availability in teeth

Table 2 presents the levels of fluoride concentration prior to brushing (pre-application), directly after application, and consecutive two weeks following application. The fluoride concentration exhibited a gradual increase, commencing at 1-minute post-application and reaching its maximum levels after 24 hours. After 2 weeks of regular application, there was a notable increase in the concentration of fluoride ions by (p<0.0001) 160 %.

3.3. Enamel thickness and integrity

The enamel thickness significantly increased (p<0.0001) from pre-application to 2 weeks post- application with Senquel F® application (table 2). Results of the enamel integrity testing done by visual inspection under light microscopy are presented in figure 1. The results showed that the application of toothpaste improved the enamel condition.

Table 2: Fluoride ion concentration and enamel thickness of teeth (PPM).

	Post single application		**Post 2 weeks application**		**Paired t-test***	
	Mean (SD)	% change	Mean (SD)	% change	t	p
Fluoride ion (X 10^{-3} PPM)						
Pre-application (n=30)						
▪ 24 hrs.	234.4 (125.7)	-	234.4 (125.7)	-	-	-
Post-application (n=30)						
▪ 30 Sec.	165.4 (114.9)	-29.4%	228.2 (152.9)	-2.6%	5.197	<0.

						0001
▪ 1 Min.	58.2 (54.1)	-75.2%	71.6 (59.2)	-69.4%	6.274	<0.0001
▪ 5 Min.	55.6 (30.2)	-76.3%	74.4 (37.6)	-68.3%	5.134	<0.0001
▪ 10 Min.	84.1 (48.1)	-64.1%	133.4 (133.8)	-43.1%	2.787	<0.0001
▪ 30 Min.	127.9 (55.9)	-45.4%	178.7 (69.8)	-23.8%	5.482	<0.0001
▪ 1 hr.	106.3 (45.4)	-54.7%	156.9 (75.7)	-33.1%	4.981	<0.0001
▪ 4 hrs.	149.0 (95.1)	-36.4%	193.2 (104.7)	-17.6%	6.637	<0.0001
▪ 24 hrs. (repeat application)	331.0 (136.7)	41.2%	455.7 (143.6)	94.4%	6.067	<0.0001
▪ 72 hrs.	-	-	127.6 (79.7)	-45.6%	-	-
▪ 168 hrs.	-	-	171.2 (48.7)	-27.0%	-	-
Enamel thickness (n=30)	4.18 (0.92)	-	4.28 (0.93)	-	7.593	<0.0001
* Single Vs 2 weeks application						

p: probability; SD: Standard deviation

Figure 1: Microscopic image (Image resolution) of teeth surface before and weeks post application of Senquel F® toothpaste

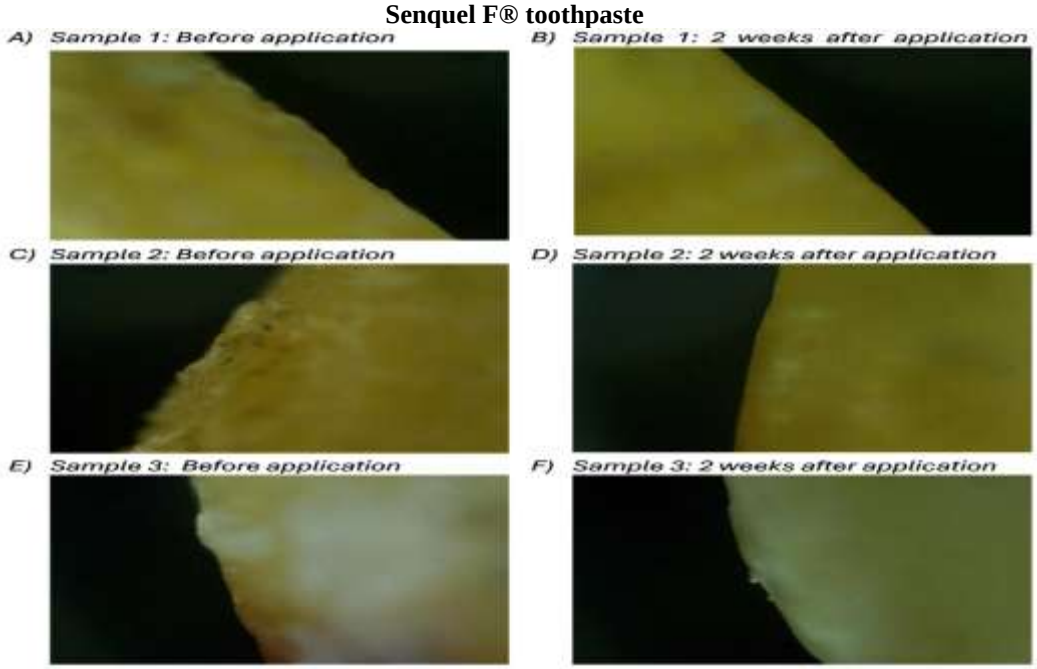

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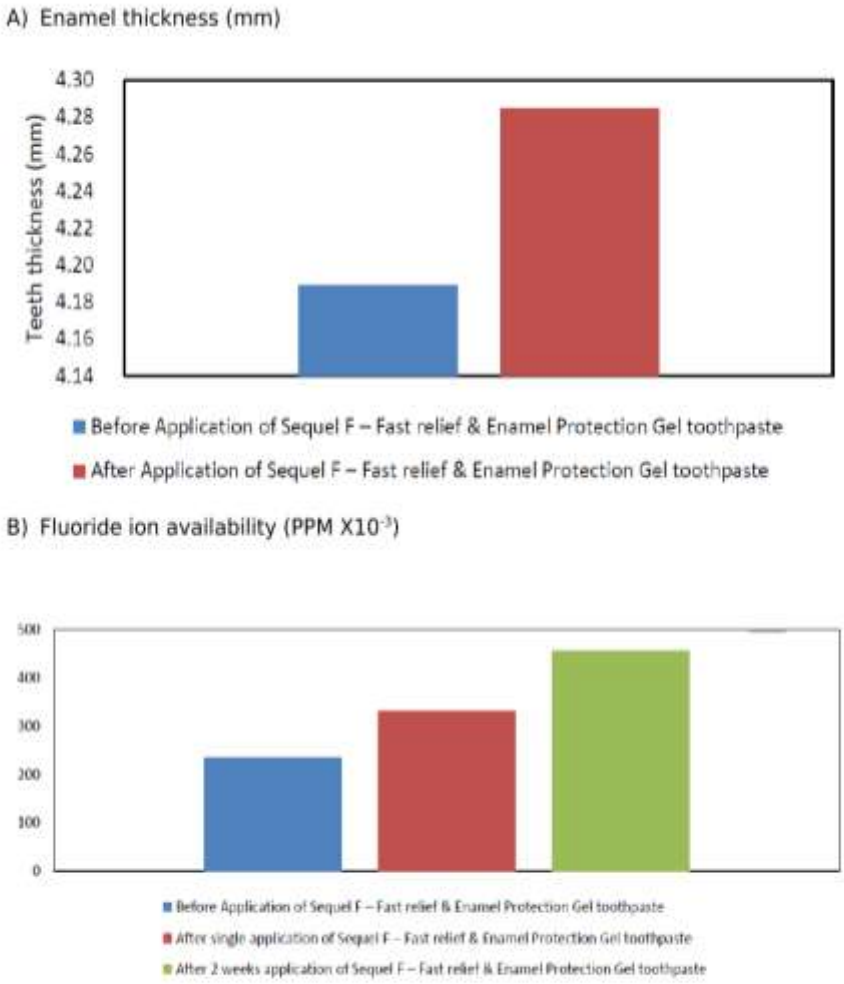
Figure 2: Aggregate values of 30 teeth thickness and fluoride ion availability before and after application of Senquel F – Fast relief & Enamel Protection Gel toothpaste.



3.4. Surface microhardness

The microhardness values (VHN) before and after the application of Senquel F® are presented in Table 3. At baseline, the test and control groups exhibited similar VHN values ($p>0.05$; data not shown). Paired comparisons between pre-application and 2 weeks post-application revealed that the VHN values did not change significantly ($p=0.808$). However, there was a significant reduction in hardness observed in the control group and the demineralization-only group ($p<0.0001$). The surface microhardness improved significantly after application of the Senquel F® toothpaste for consecutive 2 weeks.

The difference in VHN values obtained for pre and post application was significant with $p=0.038$ for between test and control group and $p=0.003$ for test Vs demineralization only group

Table 3: Surface microhardness (VHN) in three groups.

	Pre-application	**Post-application***	**Change**	**Paired t-test**	
	Mean (SD)	Mean (SD)	Mean (SD)	t	p
Control group (n=15)	351.0 (20.73)	267.4 (53.68)	83.6 (72.89)	-5.627	<0.0001
Test group (n=15)	319.6 (29.34)	317.2 (23.85)	2.4 (13.74)	-0.246	0.808

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Demineralization group (n=5)	363.8 (22.89)	239.0 (19.73)	124.8 (26.88)	-9.234	<0.0001
One-Way ANOVA	(F, p)	(F, p)	(F, p)		
	(4.247, 0.04)	(6.120, 0.015)	(9.346, 0.004)		
Post-hoc Tukey's test	p	p	p		
Test Vs Control	0.150	0.111	0.038		
Test Vs Demineralization	0.037	0.012	0.003		
Control Vs Demineralization	0.697	0.446	0.357		
* 2 weeks					

ANOVA: Analysis of variance; F: ANOVA statistic; p: probability; SD: Standard deviation; VHN: Vickers hardness number

IV. DISCUSSION

Senquel F® is a potassium nitrate based desensitizing dentifrice which contains KNO3 (5%), NaF (800 ppm), SMFP (200 ppm) and Xylitol (10%), developed as a low-abrasive dentifrice using a 13-micron silica abrasive (Fluorocoat™). The lower size of silica particles ensures better fluoride and KNO3 availability at the damaged enamel site [7]. FluorocoatTM is a combination of two distinct fluorides (NaF and SMFP) purposed for two distinctive actions. These fluorides are in the dissolved form and processed under controlled homogenization at higher temperatures, resulting in finer and uniform Fluorocoat™ particles. NaF can cause fast F+ release and fluorapatite layer formation [8], whereas SMFP is designed for 24-hour F+ release and fluorapatite layer protection [2]. A fine and uniform Fluorocoat™, combined with KNO3 adheres to silica particles (13 microns) in Senquel F®, serves as carriers, and this interaction enhances the availability of fluoride and KNO3 at the targeted site. Potassium nitrate (KNO3) is a desensitizing agent which provides rapid relief from sensitivity in 30 seconds [9,10]. KNO3 (5%) containing toothpaste have shown to provide reduction in sensitivity scores on visual analogue scale ($p<0.001$) [10]. It provides reduction in tooth sensitivity scores by 62.5% after 4 weeks of therapy[11]. As against this sodium calcium phosphosilicate containing dentifrice shows less reduction (42.8%) in VAS scores at week 4 for cold stimuli [12]. The effects of KNO3 are further supported with chemical and physical protection provided by Xylitol and low abrasive silica to the fluorapatite layer formed. Xylitol helps protect fluorapatite layer from further damage by inhibiting plaque and supplementing the action of fluoride [2,8].

This study evaluated the effects of the 2-week application of Senquel F® – Fast Relief and Enamel Protection Gel toothpaste on freshly extracted human teeth. We observed high concentrations of soluble fluoride in Senquel F® toothpaste which is available for release in the oral cavity and available for action on teeth. Thus, the Senquel F® toothpaste effectively transported fluoride ions to the enamel and sustained a significant level of fluoride availability for a full 24 hours. This has potential clinical implications in terms of a long-lasting effect on teeth.

We also observed significant ($p<0.0001$) increase in the surface microhardness after 2 weeks of Senquel F® topical application. The reduction in surface roughness with Senquel F® can be attributed to the toothpaste's formulation, which likely facilitates remineralization of the enamel surface. This can be attributed to the high fluoride content which facilitates the remineralization process. Fluoride topical application stimulates remineralization and inhibit demineralization [13]. It has several other effects, including glycolysis prevention (by acidifying the cytoplasm of cells and inhibiting enolase enzyme) and delay of tooth decay. Antibacterial activity of fluoride alters the cariogenicity of Streptococcus mutans in large doses [14].

The fluoride varnish-treated teeth have shown to have superior remineralization than non-treated teeth [15]. These results align with our study, as the average enamel thickness of teeth before and after application of Senquel F toothpaste increased by 0.096. Toothpastes with potassium salts with varying fluoride concentrations (1,100–1,500 ppm F) are found to control DHS [16–18]. NaF and SMFP present in the Senquel F® results in faster sensitivity relief while dentinal occlusion agents like sodium calcium phosphosilicate take longer to exhibit similar sensitivity relief [11,12]. Xylitol aids in remineralization when combined with Fluoride

and protects the enamel by inhibiting plaque formation [19]. It is reported to reduce the levels of periodontopathic bacteria and plaque [20]. Quantitative light-induced fluorescence (QLF) techniques show that a combination of fluoride and 5% xylitol in the dentifrice can help in enamel remineralization [21].

In our study, the Senquel-F application smoothened the teeth surfaces, and improved discoloration. The low abrasive silica (13 micron) ensures safer cleaning of enamel without causing further damage to the fluorapatite layer; thus, help increase surface hardness.

The pH cycling used in our study simulates the real-life conditions of the oral cavity, and hence our findings can be generalized. The results also show that the increased fluoride availability and the enamel layer thickness significantly indicate a positive impact on the enamel strength and durability. Microscopy images show that Senquel F® smoothens the rough surface, reduces discoloration, and eliminates the caries on the enamel surface.

V. CONCLUSION

Senquel F® toothpaste (Sodium fluoride 800ppm Sodium Monofluorophosphate 200ppm) topical application improves surface microhardness, enamel thickness and provide active fluoride ions in oral cavity, suggesting that it would potentially provide effective and long-term benefits in treatment of dentine hypersensitivity.

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