Original Article

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Dentistry Section

Comparison of the Remineralisation Potential between Flaxseed Paste, Aloe Vera Gel and Fluoride Toothpaste on Artificially Created White Spot Lesions around Orthodontic Brackets: An In-vitro Study

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ABSTRACT

Introduction: White Spot Lesion (WSL) is one of the major iatrogenic effect at the end of treatment that might reduce both patient's and orthodontist's satisfaction in otherwise promising treatment results. Flaxseed and Aloe Vera (AV) have been used as phytotherapeutic agents because of inherent antimicrobial, anti-inflammatory, antioxidants and healing properties. They were considered in this study for their effectiveness in remineralisation of WSL.

Aim: To evaluate and compare the remineralisation potential of organic flaxseed paste, Aloe Vera gel and fluoride toothpaste on artificially created white spot lesions around orthodontic brackets using Vicker's microhardness assessment, spectrophotometry and Scanning Electron Microscope (SEM).

Materials and Methods: This experimental in-vitro study was undertaken in Department of Orthodontics, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India, in October 2020. Forty eight extracted premolar teeth were exposed to demineralising solution for 48 hours in-vitro and randomly assigned to four groups: Group 1- untreated control, group 2- treated with flaxseed paste, group 3- treated with Aloe Vera gel and group 4-treated with fluoride toothpaste. All groups except control were treated with their respective remineralising paste for 28 days. Vicker's Microhardness Number (VHN) and spectrophotometric values (ΔL, Δa, Δb, ΔE) were evaluated for normal enamel, WSL, remineralisation after 14 and 28 days. The surface characteristics were analysed using SEM. Statistical analysis was performed using repeated measures Analysis of Variance (ANOVA) and post hoc Bonferroni test was for pairwise comparison between groups with significance level $p \leq 0.05$.

Results: Total of 48 extracted teeth were treated and analysed in their respective groups after 28 days. Aloe Vera gel showed highest surface microhardness (SMH) (144.09±6.05 VHN) and has statistically significant difference ($p<0.001$) compared to flaxseed paste (125.28±3.75 VHN) and then the fluoride toothpaste (121.20±5.12 VHN). There was no significant difference (p-value=0.31) between flaxseed paste and fluoride toothpaste. Significant (p-value=0.05) ΔE changes were observed in groups treated with flaxseed paste (16.39) and fluoride toothpaste (15.08) after 28 days. SEM verified mineral gain in all three treatment groups.

Conclusion: All the three groups increased mineral gain. Aloe Vera gel showed promising results by significantly remineralising WSLs. Flaxseed paste and fluoride toothpaste had SMH recovery which was lesser than Aloe Vera gel but, these two groups significantly improved the colour of WSL.

Keywords: Anticariogenic, Dental plaque, Microhardness, Plant polyphenols

INTRODUCTION

White Spot Lesions (WSL) are considered as undesirable outcomes after orthodontic treatment. It has a chalky white appearance which is an optical phenomenon due to mineral loss in the subsurface and surface of enamel [1]. The brackets, wires, bands, excess composite, serve as a housing for plaque and colonisation of aciduric bacteria like *Streptococcus mutans* over a period of time and results in active WSL. They are difficult for the patient to cleanse and are also a limitation to the self-cleansing properties of the oral musculature and saliva [2]. Approximately one-third of orthodontic patients are found to have atleast one WSL [3]. The extent of the risk posed by decalcification during orthodontic treatment is a wide range of 2% to 96% of tooth surfaces [4].

Traditionally fluoride toothpaste has been used for control of WSL. They maintain the plaque fluid supersaturated with fluorapatite, hence moving the balance of caries process towards remineralisation [5]. High concentration fluoride application can create hypermineralised areas on the enamel surface and prevent passage of ions into the deeper affected layers, which is unaesthetic [6]. There is lack of reliable evidence to support the effectiveness of remineralising agents for the treatment of post orthodontic white spot lesions. Swallowing of fluoride could be detrimental to health because of its toxicity especially in children, it could accumulate in the tissues overtime and cause adverse health effects and dental fluorosis [7].

Recently, various organic vegetables and food supplements have shown to promote oral health. Antimicrobial compounds derived from plants can be considered an alternative to chemical agents for plaque control and prevention of demineralisation [8]. Newer findings show that polyphenol component in plants have potential activity in preventing oral diseases and anticariogenic properties [9].

'*Linum usitatissimum*'- Flaxseed has minerals like calcium, magnesium, phosphorous and potassium which can help in surface remineralisation of enamel [10]. The polyphenol compound called lignans in flaxseed possess antibacterial activity against cariogenic bacteria *Streptococcus mutans* [11].

'*Aloe barbadensis*'- Aloe Vera mouth rinse was found to reduce plaque and gingivitis in orthodontic patients [12]. AV tooth gel was found to be effective in controlling cariogenic bacteria similar to other commercially available toothpastes [13]. AV gel was also reported

to have remineralising potential, abundance in essential amino acids and deposition of arginine associated to the calcium on the enamel surface was suggested as the mechanism of remineralisation [14]. Polyphenols, including anthraquinones were also thought to be responsible for inducing remineralisation [15].

Till now very few studies have been conducted to evaluate the remineralising potential of natural agents which have additional benefits like anti-inflammatory and antimicrobial action [10,14]. The comparative effect of flaxseed and AV gel on WSLs is not known. Therefore, the purpose of this study is to evaluate and compare the remineralisation potential of organic flaxseed paste, Aloe Vera gel and fluoride toothpaste on artificially created WSLs around orthodontic brackets using Vicker's microhardness assessment, spectrophotometry and Scanning Electron Microscope (SEM).

MATERIALS AND METHODS

This experimental in-vitro study was undertaken in Department of Orthodontics, SRM Dental College, Ramapuram in October 2020. This study was approved by Institutional Review Board and Institutional Ethical Committee: [IRB APPROVAL NUMBER:SRMDC/IRB/2018/MDS/No.106].

Inclusion criteria: Maxillary and mandibular premolar teeth, extracted for orthodontic purpose with intact crowns were included in the study.

Exclusion criteria: Teeth with restoration or decay or damage on buccal surface of tooth that was to be bonded with bracket, teeth with caries, hypocalcification, fluorosis, enamel cracks and teeth pretreated with chemical reagents were excluded from the study.

Sample size estimation: G power software with power of 95% and error of 5% was used for sample size estimation and sample size of 48 was calculated. Hence, 48 maxillary and mandibular premolar teeth were included.

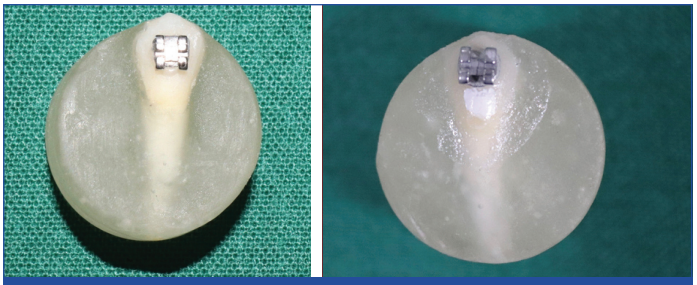
Procedure

The teeth were washed and stored in distilled water until the start of procedure. The buccal enamel surfaces were cleaned with non fluoridated pumice and water, etched with 37% phosphoric acid (EAZETECH, Anabond Stedman) for 30 seconds. Primer (ORTHOFIX, Anabond Stedman) was applied on the etched enamel and cured. Upper premolar brackets (3M-Unitek Gemini) were bonded using composite resin (ORTHOFIX, Anabond Stedman) and were light cured for 40 seconds after removal of excess resin around brackets. The teeth were mounted in acrylic blocks with the buccal surfaces exposed such that the surface was flat and parallel to the base [Table/Fig-1].

Development of artificial white spot lesion: Demineralising solution was prepared by mixing 0.4723 g-calcium nitrate, 0.2722 g-potassium dihydrogen phosphate, 4.5083 g-acetic acid in one litre of distilled water [16]. Fifty percent NaOH was added to adjust the pH of the solution to 4-4.5. Clear acid resistant nail varnish was painted on the entire buccal surface except a 3×3 mm area of window gingival to the bracket. The 48 samples along with two additional teeth were immersed completely in the demineralising solution and placed in an incubator at 37°C for 48 hours [17]. After 48 hours, WSL appearance was visually verified and then the brackets were deboned, and any residual adhesive were removed [Table/Fig-2]. Two additional teeth were sectioned longitudinally and depth of the WSL measured in SEM was about 70 µm [18].

The sample of forty-eight teeth with WSLs were then split into four groups.

1. Group 1 (n=12): Artificial saliva (Control)
2. Group 2 (n=12): Flaxseed paste
3. Group 3 (n=12): Aloe Vera (AV) gel
4. Group 4 (n=12): Fluoride toothpaste



[Table/Fig-1]: Tooth mounted in acrylic resin block with buccal surface exposed.
[Table/Fig-2]: Artificial white spot lesion of 3×3 mm. (Images from left to right)

Artificial saliva solution was prepared by adding 0.75 g- sodium azide, 0.804 g- potassium monohydrogen phosphate, 0.166 g-calcium chloride, 0.59 g- magnesium chloride, 1.02 g- sodium chloride, in one litre of distilled water and the pH was approximatey 7 [17]. The samples in the four groups were placed in four separate containers of artificial saliva solution and placed in an incubator at 37°C to mimic oral environment [Table/Fig-3].

[Table/Fig-3]: Treated samples placed in incubator at 37°C.

Remineralisation procedure: All the teeth in control group were not treated with any agents. Edible raw organic flaxseed (Nutriwish®) was ground into fine powder and mixed with deionised distilled water to make flaxseed paste with concentration of 1 gm/mL [10]. Hundred percent pure AV gel extract (Cunega, India) was acquired, stored at 4°C and fresh gel was obtained every two weeks. Fluoridated toothpaste- Colgate total 12 (Colgate-Palmolive, India) was used that contained Sodium Fluoride 0.22% w/w 1000 ppm. Each block was removed from the artificial saliva solution, treated with its designated product evenly on the buccal surface using a separate toothbrush for each group to avoid contamination and placed back without rinsing. This treatment was done twice a day for three minutes and artificial saliva solution was freshly replaced after each day.

Vicker's microhardness assessment: The Surface Microhardness (SMH) was measured using Vicker's microhardness tester (Micro Vicker's hardness tester, 10 g-1000 g, MH6, Everone) for normal enamel at baseline, after WSL, at the end of 14 days and 28 days of treatment. Three indentations about 100 µm apart were performed with a load of 100 g exercised onto the surface of the specimens in the middle third for 10 seconds and the average of three readings of Vicker's hardness number (VHN) were taken [19].

Spectrophotometric evaluation: Digital spectrophotometer (Vita Easyshade Advance 4.0) was used to measure L*, a* and b* after grouping of the samples. L is the degree of lightness in a colour from 0- absolute black to 100- absolute white, a is the red- green chromacity; +a*-measure of redness, -a*- measure of greenness and b is the blue- yellow chromacity; +b*- measure of yellow, -b*-measure of blue [20].

The samples were placed in a uniform black background and assessment was done in an area without any light leak. The tip of the spectrophotometer was placed on the buccal surface of demineralised enamel and simultaneously adjacent normal enamel was also assessed. Similarly, spectrophotometer readings were obtained after remineralisation at the end of 14 and 28 days. Three measurements were made and average was calculated. ΔE represents the Euclidean distance between the initial and final L*, a*,

and b* values calculated using the formula $\Delta E=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$ [17]. The ΔL, Δa, Δb and ΔE quantified the difference from normal enamel and enamel at various intervals of time including WSL, remineralised enamel at 14 days and 28 days.

Scanning Electron Microscope (SEM): On the 29th day, buccal surface sections of one random remineralised sample from each group was cleaned, dehydrated by placing in 100% ethanol for one hour and then air dried. The sections were mounted in a platform using carbon tape and placed in a sputtering machine (Emitech, SC7620 sputter coater) for gold sputtering to facilitate conduction of electricity and aid in high quality images. The sputtered sections were examined under SEM (Tescan, Vega3) with acceleration voltage standardised to 20 kV and samples were projected at 1000x magnification.

STATISTICAL ANALYSIS

Statistical analysis was performed using International Business Management (IBM) Statistical Package for Social Sciences (SPSS) software package version 26.0. (IBM Corp). Paired t-test was done for comparison between normal and demineralised enamel. Statistical analysis of hardness value was done within groups using repeated measures ANOVA and post hoc Bonferroni test was performed for pairwise comparison between groups. Non parametric Friedman test was performed to evaluate changes in ΔL, Δa, Δb and ΔE within groups and multiple analysis Bonferroni test was done for comparison between groups. Significance was set at the 0.05 level.

RESULTS

Vicker's microhardness assessment: Paired t-test displayed statistically significant (p<0.001) difference in the mean hardness value of normal enamel (269.13±24.06) Vicker's Pyramid Number (HV) and enamel with WSLs (45.53±15.45) HV indicating that there is a decrease in the hardness of enamel when subjected to demineralisation [Table/Fig-4]. Repeated measures ANOVA showed statistically significant changes (p<0.001) in surface microhardness of normal enamel, demineralised enamel and remineralised enamel after 14 days and 28 days in all the four groups [Table/Fig-5].

Post hoc Bonferroni test was performed for multiple comparison of remineralisation values at the end of 14 and 28 days between the control and treatment groups [Table/Fig-6]. During initial remineralisation (14 days), fluoride toothpaste showed the maximum SMH (69.71±9.64) HV followed by AV gel (60.90±5.86) HV and flaxseed paste (50.03±3.63) HV and all the groups showed greater SMH than control (42.56±1.95) HV. However, at 28 days, AV gel had the maximum hardness value (144.09±6.05) HV and was significantly higher than other three groups (p<0.001) [Table/Fig-5]. There was no significant difference (p=0.315) between flaxseed paste (125.28±3.75) HV and fluoride toothpaste (121.20±5.12) HV and both showed similar remineralisation potential after 28 days [Table/Fig-6].

The percentage of SMH recovery (%SMHR) for all the four treatment groups at the end of remineralisation after 28 days was calculated [Table/Fig-7]. Highest recovery rate was seen in AV gel with 43.18%, followed by flaxseed paste with 35.66% and fluoride toothpaste

Characterstics studied	Mean hardness value {in Vicker's Pyramid Number (HV)}	N	Standard deviation (HV)	Standard error mean (HV)
Normal enamel	269.133	48	24.0619	3.4730
Artificial white spot lesion	45.5322	48	15.44547	2.30618

Paired samples test-Comparative analysis	Paired differences					T	df	p-value (2-tailed)
	Mean (HV)	Standard deviation (HV)	Standard error mean (HV)	95% Confidence interval of the difference				
				Lower	Upper			
Normal enamel-artificial white spot lesion	222.66250	29.20846	4.21588	214.18125	231.14375	52.815	47	**<0.001**

[Table/Fig-4]: Paired t test comparing surface microhardness of normal enamel and artificial white spot lesion.
HV: Vickers Pyramid Number; p-value <0.05 considered significant

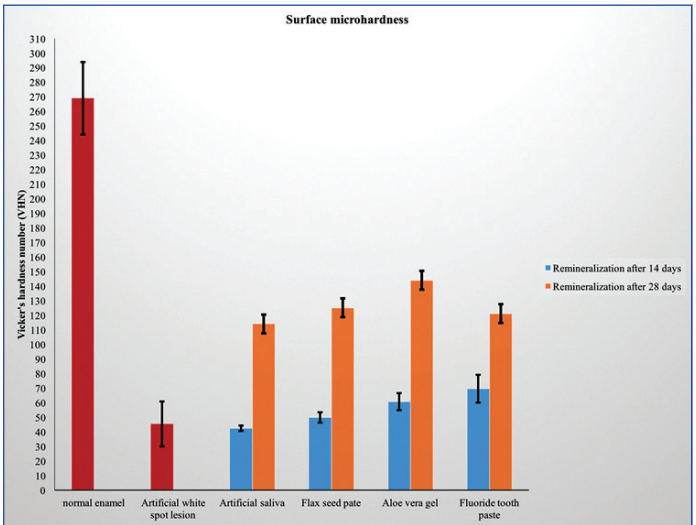
	Groups	Mean HV (VHN)	Standard deviation HV (VHN)	N
Normal enamel		269.133	24.0619	48
Artificial white spot lesion		45.5322	15.44547	48
Remineralisation after 14 days	1- Group-1	42.5625	1.94832	12
	2- Group-2	50.0333	3.62713	12
	3- Group-3	60.9000	5.86283	12
	4- Group-4	69.7083	9.64013	12
	Total			48
Remineralisation after 28 days	1- Group-1	114.2042	4.86689	12
	2- Group-2	125.2792	3.75224	12
	3- Group-3	144.0958	6.04842	12
	4- Group-4	121.2000	5.11695	12
	Total			48

Tests of within-subjects effects					
Groups	Type III Sum of squares	df	Mean square	F	Significance
1- Group-1	435828.112	1.083	402323.830	934.450	**<0.001**
2- Group-2	399704.447	1.096	364798.819	897.566	**<0.001**
3- Group-3	386907.205	1.203	321620.781	692.328	**<0.001**
4- Group-4	319588.631	1.414	226046.830	604.549	**<0.001**

[Table/Fig-5]: Repeated measures ANOVA for comparison of changes in surface microhardness between normal enamel, artificial white spot lesion and remineralized enamel after 14 days and 28 days within the groups.
HV: Vickers pyramid number; VHN: Vicker's hardness number; p-value <0.05 considered significant

Dependent variable	(I) Group	(J) Group	Mean difference (I-J) Vicker's Pyramid Number (HV)	Standard error (HV)	p-value	95% Confidence interval	
						Lower bound	Upper bound
Remineralisation after 14 days	1-Group-1	2-Group-2	-7.47083	2.45167	0.023	-14.2444	-0.6973
		3-Group-3	-18.33750	2.45167	<0.001	-25.1110	-11.5640
		4-Group-4	-27.14583	2.45167	<0.001	-33.9194	-20.3723
	2-Group-2	3-Group-3	-10.86667	2.45167	<0.001	-17.6402	-4.0931
		4-Group-4	-19.67500	2.45167	<0.001	-26.4485	-12.9015
	3-Group-3	4-Group-4	-8.80833	2.45167	0.005	-15.5819	-2.0348
Remineralisation after 28 days	1-Group-1	2-Group-2	-11.07500	2.04667	<0.001	-16.7296	-5.4204
		3-Group-3	-29.89167	2.04667	<0.001	-35.5462	-24.2371
		4-Group-4	-6.99583	2.04667	0.008	-12.6504	-1.3413
	2-Group-2	3-Group-3	-18.81667	2.04667	<0.001	-24.4712	-13.1621
		4-Group-4	4.07917	2.04667	0.315	-1.5754	9.7337
	3-Group-3	4-Group-4	22.89583	2.04667	<0.001	17.2413	28.5504

[Table/Fig-6]: Post Hoc Boneferroni test used in comparison of remineralization surface microhardness at the end of 14 and 28 days between the control and treatment groups. p-value <0.05 considered significant



[Table/Fig-7]: Changes in Surface Microhardness (SMH) from normal enamel to White Spot Lesion (WSL) and remineralization after 14, 28 days in four groups.

with 33.84% and least recovery rate was seen in artificial saliva with 30.71%.

Spectrophotmetric evaluation: There is a statistically significant change in the ΔL, Δa, Δb and ΔE parameters in all four groups from demineralisation, remineralisation at 14 and 28 days measured as difference from normal enamel [Table/Fig-8]. All the four groups showed decrease in ΔE value at the end of 28 days [Table/Fig-9]. Fluoride toothpaste showed a significant decrease in ΔE value of remineralised enamel at 14 days (p=0.003) and 28 days (p=0.005) compared to demineralised enamel (31.51±12.65). However, there was no significant difference (p=0.530) between remineralisation at 14 days (15.89±8.79) or remineralisation at 28 days (15.08±5.93) [Table/Fig-8,10].

Flaxseed paste group showed a statistically significant decrease (p=0.006) in ΔE values from demineralised enamel (27.11±6.49) to remineralisation at 14 days (22.52±8.72) and further decreased at 28 days (16.39±8.23) suggesting the remineralised enamel colour to be changing closer towards the colour of baseline normal enamel. The colour change by 28 days from demineralised enamel was not statistically significant in AV gel (p=0.937) and artificial saliva (p=0.583) [Table/Fig-8,10].

Intergroup comparison showed no significant difference (p=1.00) in ΔE between the flaxseed and fluoride groups at the end of 28 days suggesting similar decrease in ΔE values [Table/Fig-9,11].

SEM Examination

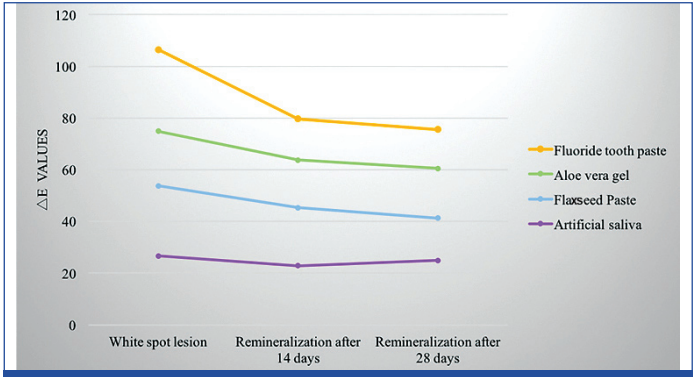
Sound enamel showed smooth, uniform surface with homogenously arranged enamel crystals and regular appearance of enamel rods [Table/Fig-12a]. The demineralised surface in artificial WSL was highly porous with loss of enamel structure and disorganised crystal arrangement [Table/Fig-12b]. In Artificial saliva, predominantly porosities were evident on the surface with faint lines of sound enamel morphology (arrows) in and around the porosities [Table/Fig-12c]. Flaxseed paste showed irregular globular structures along with porosities in-between areas of remineralisation (arrows) which were thick and more frequent [Table/Fig-12d]. In AV gel predominantly intact enamel prismatic arrangement and ideally oriented, orderly mineralisation pattern was accomplished (arrows) with very minute areas of dissolution [Table/Fig-12e]. Fluoride Toothpaste also showed oriented mineralisation pattern (arrows) with slight interprismatic core dissolution [Table/Fig-12f].

Groups			Mean	Std. Deviation	Minimum	Maximum
Group-1	After demineralisation	ΔL	24.7250	9.07555	13.20	38.20
		Δa	-4.9917	1.37011	-7.40	-2.00
		Δb	-6.7167	4.54669	-16.00	-0.60
		ΔE	26.7197	8.32853	15.98	38.63
	Remineralisation after 14 days	ΔL	21.5833	9.77072	9.30	44.50
		Δa	-3.4167	1.44778	-5.70	-0.60
		Δb	-4.3000	4.75968	-13.10	2.90
		ΔE	22.8476	9.58342	10.81	44.95
	Remineralisation after 28 days	ΔL	22.7500	6.60874	13.20	33.60
		Δa	-4.3833	1.85366	-6.90	-1.50
		Δb	-7.9083	5.29055	-17.60	-0.50
		ΔE	24.9427	7.08555	14.85	34.43

Group-2	After demineralisation	ΔL	25.4917	7.70684	10.00	36.30
		Δa	-4.8775	2.34933	-8.00	-0.80
		Δb	-3.4500	5.59277	-14.50	7.10
		ΔE	27.1134	6.48583	15.64	37.68
	Remineralisation after 14 days	ΔL	20.9167	9.78504	4.90	34.00
		Δa	-3.0750	2.31600	-6.30	0.90
		Δb	-3.5667	5.21385	-13.40	7.20
		ΔE	22.5283	8.72379	8.51	35.05
	Remineralisation after 28 days	ΔL	12.9500	8.99571	0.70	30.10
		Δa	-2.5583	2.50978	-6.30	3.20
		Δb	-6.4167	6.20847	-16.50	8.70
		ΔE	16.3876	8.23440	3.56	32.13
Group-3	After demineralisation	ΔL	20.2750	8.14317	12.40	34.30
		Δa	-3.8000	1.90502	-7.00	-0.30
		Δb	-.5500	3.83939	-4.30	6.60
		ΔE	21.1172	7.92054	13.60	34.73
	Remineralisation after 14 days	ΔL	16.9833	10.44908	5.00	38.20
		Δa	-2.5333	2.24229	-5.90	0.80
		Δb	-4.2833	4.52304	-10.10	4.30
		ΔE	18.4153	10.31437	6.30	38.66
	Remineralisation after 28 days	ΔL	15.8667	9.13727	1.70	33.40
		Δa	-3.8417	2.40282	-7.50	0.40
		Δb	-8.2333	4.60342	-13.70	0.60
		ΔE	19.1250	8.72512	2.44	34.42
Group-4	After demineralisation	ΔL	30.1583	13.69057	2.50	61.10
		Δa	-5.4167	1.57528	-7.40	-1.40
		Δb	-2.8000	4.56309	-8.20	7.20
		ΔE	31.5147	12.65073	8.69	61.78
	Remineralisation after 14 days	ΔL	13.5308	10.13024	-0.03	32.80
		Δa	-1.8750	1.81665	-4.80	0.50
		Δb	-6.0333	1.89081	-9.00	-3.10
		ΔE	15.8906	8.79228	5.85	33.77
	Remineralisation after 28 days	ΔL	10.9083	7.61511	0.30	24.10
		Δa	-2.1333	1.72275	-5.70	.30
		Δb	-8.3917	3.27149	-13.90	-4.40
		ΔE	15.0802	5.93032	6.44	24.80

Groups	Chi-square	Df	Asymptotic Significance (Asymp. Sig.)
1-Group-1	108.947	11	<0.001
2-Group-2	115.550	11	<0.001
3-Group-3	107.162	11	<0.001
4-Group-4	116.808	11	<0.001

[Table/Fig-8]: Friedman Test for comparison of changes between ΔL, Δa, Δb and ΔE of artificial white spot lesion and remineralized enamel after 14 days and 28 days from normal enamel within the groups.
N=12 teeth in each group. All parameters and sub-parameters measured for all the teeth in that Group; L is the degree of lightness in a colour from 0- absolute black to 100- absolute white; a : Red- green chromacity; b: blue- yellow chromacity



[Table/Fig-9]: Changes in ΔE of artificial WSL and remineralized enamel after 14 days and 28 days from normal enamel within the groups.

DISCUSSION

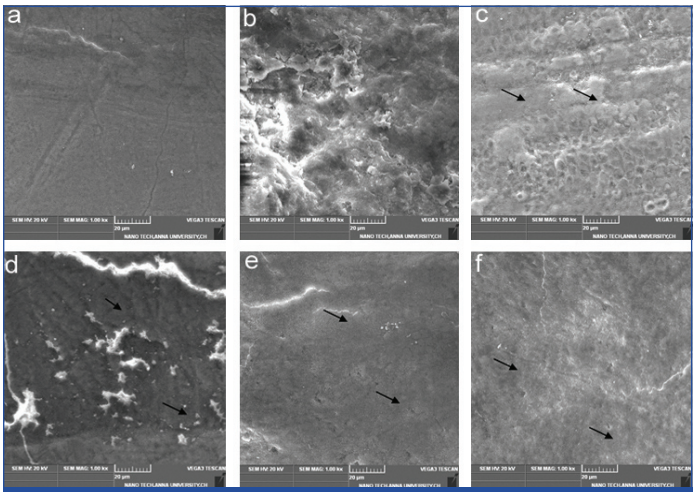
Topical application of various remineralising agents were efficient in promoting remineralisation in the present study. WSLs are the earliest macroscopic evidence of enamel caries and has been one of the inevitable clinical problems seen in most of the patients during orthodontic treatment [21]. There is constant need for preventive programs to prevent peri-bracket demineralisation. Fluoride toothpastes, mouth rinses, varnishes are the most popular methods followed until today for prevention and remineralisation of WSLs. Casein Phosphopeptide-Amorphous Calcium Phosphate Complexes (CPP-ACP), Tooth mousse, resin infiltration methods, subsurface sealants, laser therapy etc., are also some of the systems prescribed to promote remineralisation [22].

Test statistics				
Groups		ΔE Remineralization after 14 days - ΔE Demineralized enamel	ΔE Remineralization after 28 days - ΔE Remineralization after 14 days	ΔE Remineralization after 28 days - ΔE Demineralized enamel
Group-1	Z	-1.804b	-0.863b	-0.549b
	Asymp. Sig. (2-tailed)	0.071	0.388	0.583
Group-2	Z	-2.746b	-2.353c	-2.824b
	Asymp. Sig. (2-tailed)	0.006	0.019	0.005
Group-3	Z	-.392b	-0.471b	-0.078c
	Asymp. Sig. (2-tailed)	0.695	0.638	0.937
Group-4	Z	-2.981b	-0.628b	-2.824b
	Asymp. Sig. (2-tailed)	0.003	0.530	0.005

[Table/Fig-10]: Comparison of changes in ΔE within groups.
a. Wilcoxon Signed Ranks Test; b. Based on negative ranks; c. Based on positive ranks; Asymp. Sig.: Asymptotic Significance; p-value <0.05 considered significant

Multiple comparisons							
Bonferroni							
Dependent variable	(i) Groups	(J) Groups	Mean difference (I-J)	Standard error	Significance (p-value)	95% Confidence interval	
						Lower bound	Upper bound
ΔE demineralised enamel	1-Group-1	2-Group-2	-.39363	3.7316	1.000	-10.7035	9.9162
		3-Group-3	5.6025	3.7316	0.842	-4.7073	15.9124
		4-Group-4	-4.7949	3.7316	1.000	-15.1048	5.5149
	2-Group-2	3-Group-3	5.9961	3.7316	0.691	-4.3136	16.3060
		4-Group-4	-4.4013	3.7316	1.000	-14.7111	5.9085
	3-Group-3	4-Group-4	-10.3974*	3.7316	0.047	-20.7073	-.0876
ΔE remineralisation at 14 days	1-Group-1	2-Group-2	.3193	3.8277	1.000	-10.2559	10.8946
		3-Group-3	4.4323	3.8277	1.000	-6.1429	15.0076
		4-Group-4	6.9570	3.8277	0.456	-3.6182	17.5323
	2-Group-2	3-Group-3	4.1129	3.8277	1.000	-6.4623	14.6883
		4-Group-4	6.6377	3.8277	0.539	-3.9375	17.2130
	3-Group-3	4-Group-4	2.5247	3.8277	1.000	-8.0505	13.1000
ΔE remineralisation at 28 days	1-Group-1	2-Group-2	8.5551*	3.0910	0.049	.0152	17.0950
		3-Group-3	5.8177	3.0910	0.399	-2.7221	14.3577
		4-Group-4	9.8624*	3.0910	0.016	1.3225	18.4024
	2-Group-2	3-Group-3	-2.7373	3.0910	1.000	-11.2773	5.8025
		4-Group-4	1.3073	3.0910	1.000	-7.2326	9.8472
	3-Group-3	4-Group-4	4.0447	3.0910	1.000	-4.4952	12.5846

[Table/Fig-11]: Multiple analysis Bonferroni test used for Comparison of changes in ΔE between four groups.



[Table/Fig-12]: SEM images at 1000x magnification of Enamel surface a) Sound enamel, b) Artificial WSL, after 28 days of remineralization- c) Artificial saliva, d) Flaxseed paste e) Aloe Vera gel f) Fluoride toothpaste.

In recent times, there is preference for organic agents due to their immense bio characteristics, cost-effectiveness, availability and a wider safety margin [23]. Some plant polyphenols are able to regulate the demineralisation-remineralisation cycle of enamel [24]. Flaxseed and Aloe Vera gel are two such natural agents rich in polyphenols. Flaxseed extract had been used successfully for treatment of xerostomia [25] and they had been found to have antimicrobial effect on periodontal and cariogenic pathogens [11]. Similarly, Aloe Vera gel has numeral benefits and had been used to promote oral health due to its antimicrobial and healing properties [26,13]. However, the studies in literature of the remineralising potential of organic ingredients are very few [10,14,15].

Vicker's microhardness assessment is a simple, non destructive method and an appropriate tool to evaluate remineralisation potential. In this study, the microhardness of the teeth after demineralisation was approximately 45 which is within acceptable baseline SMH ranging from 25 to 50 VHN [27].

Though remineralisation had been extensively studied, the aesthetic changes produced by remineralising agent is not frequently studied. Increasing the translucency of opaque enamel by remineralisation at the subsurface level will allow light to be absorbed similar to sound enamel. At many instances, WSL may remineralise but the opaque nature might still prevail leading to unsightly appearance. The ΔE value aids in determination of remineralising agent that produces

remineralisation which is most aesthetically similar to sound enamel. ΔE average of 3.3 was reported to be aesthetically acceptable and any difference above this limit is highly perceptible [28].

In-vitro models are the most conventional techniques in caries research and artificial caries like lesion production was first described by Featherstone JD in 1983 [29]. Demineralising solution in this study was prepared as described by Featherstone pH model [16]. In this study, longitudinal section showed that the depth of the white spot lesion produced was around 70 μm similar to lesion described by White DJ in 1987 [18]. The time period for assessment of remineralisation was chosen in accordance with previous study as two weeks and four weeks [10].

Koulourides T et al., described inherent natural remineralisation potential of saliva [30]. In this present study also, remineralisation had occurred in the artificial saliva group but it was lesser than the other three groups at 28 days. Spectrophotometer evaluation showed no improvement in the colour of the WSL and SEM images also showed very less traces of remineralisation and more porosities. This might be because the mineral gain in artificial saliva is thought to be only superficial. Cochrane NJ et al., suggested that there is requirement of supplementing the tooth with additional remineralising agents along with natural remineralisation for repair of deeper layers [31].

The samples treated with flaxseed paste showed microhardness similar to that of fluoride toothpaste at 28 days and SEM images showed more frequent areas of remineralisation than control. There are no studies mentioning about the surface microhardness or changes in surface morphology in literature. Flaxseed paste was studied only on the basis of colorimetric parameters by Raffur MA et al., [10]. They observed that flaxseed paste was effective in reducing the lightness, chroma and increased hue. The exact reason is not known and they attributed the remineralisation potential to the enormous mineral content. ΔE change of remineralised enamel at 28 days (16.39±8.23) was statistically significant (p=0.005) compared to WSL.

Silva TM et al, concluded that commercially available AV tooth gel was similar to sodium fluoride toothpaste in increasing SMH [14] and Teresa Al Haddad et al., in their in-vitro study showed that AV remineralised similar to 1450 ppm fluoride toothpaste [15]. Similarly, AV gel group in the present study increased the SMH significantly and was greater compared to flaxseed paste and fluoride toothpaste. The exact mechanism of action of Aloe Vera on the demineralised surface still remains unclear and the previous studies have attributed the presence of aminoacids like arginine or polyphenol components to induce remineralisation [14,15]. SEM images correlated well with the SMH showing uniform and oriented remineralisation pattern. However, Aloe Vera gel could not produce a significant change in colour similar to normal enamel in this study and there are no previous studies evaluating this parameter.

Raffur MA et al., [10] and Silva TM et al., [14] had compared the natural agents with sodium fluoride toothpaste and the same fluoride toothpaste containing 1000 ppm of sodium fluoride was used in this study. Fluoride toothpaste showed greater increase in microhardness at the end of 14 days but at the end of 28 days the microhardness was similar to flaxseed paste and was lesser than AV gel and the colour change produced was also similar to flaxseed paste. SEM images also showed uniform pattern of remineralisation with slight areas of dissolution.

Aloe Vera or flaxseed can be incorporated into mouthwash or toothpaste and a safe, effective and economical product can be introduced and clinical trials can be performed. In orthodontics, further studies can be carried out on the effect of these natural agents on prevention of peri-bracket demineralisation by means of incorporation into either varnish, primer or composite resin or by coating of brackets or elastomeric ties.

Limitation(s)

Oral environment cannot be reproduced accurately with in-vitro studies, so in-vivo studies are required for more definitive results. Further observation intervals are required to evaluate whether the agents produce remineralisation similar to sound enamel structure.

CONCLUSION(S)

Within the limitations of this study, the authors could conclude that Aloe Vera gel significantly increased the SMH of enamel and was the highest among all groups. It produced uniform mineralisation on the surface although there was no significant improvement in colour. Flaxseed paste and fluoride toothpaste showed significant improvement in the colour of enamel compared to Aloe Vera, but the SMH recovery was lesser. Aloe Vera gel and flaxseed paste could be considered as effective natural remineralising agents. The use of natural based product as therapeutic agent against the chemical synthetic products will have equal potential while negating the unwanted side effects and are promising to human health. The natural agents largely occur and could be used at a reasonable cost in the preparation of specific remedies. The fluoride free remineralising agents will also be safer for use in young children with white spot lesions due to poor oral hygiene maintenance.

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