

Effectiveness of Various Remineralizing Agents on White Spot Lesions after Orthodontic Treatment: A Comparative Study

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ABSTRACT

Aim and objective: To evaluate and compare the ability of three commercially available remineralizing agents on the surface microhardness (SMH) of enamel after induction of white spot lesions (WSLs) by demineralization.

Materials and methods: About 80 sound mandibular human premolars that were later removed as a part of orthodontic treatment were employed in this research under the inclusion criteria. After cleaning and disinfecting the teeth, their crowns were mounted in acrylic resin and painted with nail varnish, not including a 3 mm × 3 mm window in the middle of the buccal surface. Each tooth was soaked in 15 mL of a demineralizing solution at a pH of 4.5 that was prepared for this study and left in a place for 10 subsequent days to permit the formation of WSLs after which the SMH was again assessed. The premolar teeth were then randomly allocated and equally divided into one of the four groups of 20 each depending on the treatment they received as Group 1: control group, Group 2: Colgate sensitive Pro-Relief toothpaste group, Group 3: Amflor group, and Group 4: Enafix group. After 15 days, the microhardness of all samples was estimated and alterations in the same were noted. Scanning electron microscopy (SEM) was used to evaluate the surface morphology.

Results: Enhanced and highest mean SMH was present in the Colgate sensitive Pro-Relief group (53.26 ± 0.10) followed by Enafix group (47.72 ± 0.21), Amflor group (44.84 ± 0.66), and control group (39.52 ± 0.32), respectively after application of remineralizing agents. Except for Group 3 vs Group 4, statistically significant differences ($p < 0.05$) were noted in all the groups.

Conclusion: All the three agents employed in this study, that is, Colgate sensitive Pro-Relief toothpaste, Amflor, and Enafix improved the SMH of teeth after the therapy given over 15 days compared to the microhardness following demineralization.

Clinical significance: White spot lesions often occur on labial surfaces of teeth after orthodontic treatment. Hence, these lesions pose esthetic challenges thereby affecting treatment satisfaction perceived by the patient. As esthetics and appearance are of foremost significance after orthodontic treatment, elimination protocols for remineralization of WSLs are of utmost importance employing economic means.

Keywords: Amflor, Colgate sensitive Pro-Relief, Enafix, Fluoride, Remineralization, White spot lesions.

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INTRODUCTION

White opaque regions owing to mineral loss beneath the surface enamel on clinical examination are defined as white spot lesions (WSLs).¹ The process of decalcification is initiated by the persistence of bacteria on the surface enamel for prolonged periods.² As organic acids generated by bacterial flora seep into the interprismatic areas of enamel, it results in dissolving of the apatite crystals, loss of ionic calcium and phosphorus with ensuing demineralization.²

In subjects receiving orthodontic treatment, their prevalence ranges from 5 to 97% following debonding.^{1,3} In these patients, white opaque regions frequently arise in areas inaccessible for brushing due to the presence of bands, brackets, elastics, hooks, and springs.⁴ Dental caries causing bacteria namely, *Streptococcus mutans* and *Lactobacillus* are mainly implicated in the development of WSLs following fixed orthodontic appliance therapy.⁵

White spot lesions occur on labial surfaces of maxillary lateral incisors, canines, premolars, and central incisors, in the descending order of their frequency.⁶ Hence, these lesions pose esthetic challenges thereby affecting treatment satisfaction perceived by the patient.³

Arrays of techniques have been suggested to reduce the occurrence and severity of WSLs. Maintenance of scrupulous oral hygiene, fluoride-containing remineralizing agents, such as toothpaste, dentifrices, varnishes, adhesives, antibacterial

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mouthwash, such as chlorhexidine, xylitol gums, derivatives of casein, such as casein phosphopeptide-amorphous calcium phosphate, and self-amassing peptides are a few of the methods used in the prevention of WSLs.⁷⁻¹¹ Other implicated treatment strategies include resin infiltration, bleaching, or microabrasion.⁹

Recently few agents have been introduced that aid in remineralization by checking the surrounding microenvironment. These include Colgate sensitive Pro-Relief toothpaste, Amflor, and Enafix. Colgate sensitive Pro-Relief toothpaste contains 8% arginine, insoluble calcium carbonate, and 1450 ppm fluoride that is optimal for enabling remineralization of dental hard tissues.¹² Amflor toothpaste contains 3.85% amine fluoride while Enafix is a complex mixture of calcium sucrose phosphate with inorganic amorphous calcium phosphate (ACP).⁵ Though all the above agents have a disparity in their chief constituents, they have exhibited satisfactory results on remineralization of enamel. Furthermore, the literature search revealed no comparative study between the three agents. Thus, this *in vitro* study was undertaken to evaluate and compare the ability of these commercially available remineralizing agents on the surface microhardness (SMH) of enamel after induction of WSLs by demineralization.

MATERIALS AND METHODS

This *in vitro* research was carried out in the department of orthodontics and dentofacial orthopedics. About 80 healthy mandibular human premolars that were later removed as a part of orthodontic treatment were employed in this research. The absence of dental caries, fractures, cracks, discoloration, developmental anomalies, and restorations constituted the inclusion criteria. The teeth thus selected were meticulously cleaned with an ultrasonic scaler and pumice paste. Following this, the premolars were subjected to disinfection employing 0.1% thymol solution for 2 days and then soaked in saline for storage.

Sample Preparation

The crowns of the premolars were mounted in acrylic resin and painted with nail varnish not including a 3 mm × 3 mm window in the middle of the buccal surface, after which they were again immersed in saline. Utilizing the Vickers microhardness evaluating machine, the SMH was estimated over three assorted points chosen on the premolar tooth under a fixed load for 20 seconds.

Demineralization Process

Each tooth was soaked in 15 mL of a demineralizing solution at a pH of 4.5 that was prepared for this study (2.2 mM CaCl₂, 2.2 mM NaH₂PO₄, 1 ppm NaF, 100 mM NaCl, 50 mM acetic acid, and 0.02% NaN₃). The teeth were left in the demineralizing solution for 10 subsequent days at room temperature to permit the formation of WSLs. The Vickers microhardness evaluating machine was again employed to assess the SMH.

These 80 sound premolar teeth were then randomly allocated and equally divided into one of the four groups of 20 each.

The samples in each group were soaked in artificial saliva solution for 15 days. During this time each group received different treatment as follows:

Group 1: Control Group

The 20 teeth that constitute this group obtained no external treatment except for being immersed in the artificial saliva solution that was changed twice a day.

Group 2: Colgate Sensitive Pro-Relief Toothpaste Group

The 20 teeth comprising this group were subjected to the application of Colgate sensitive Pro-Relief toothpaste (Colgate Palmolive India Ltd, Mumbai, India) using a paintbrush for 5 minutes once in 12 hours for 15 days. The toothpaste was gently wiped. The sample was then placed in fresh artificial saliva solution without being rinsed.

Group 3: Amflor Group

Amflor (Group Pharma Ltd, Mumbai, India) was applied on the 20 premolars of this group using a paintbrush for 5 minutes once in 12 hours for 15 days. After gentle wiping, the sample was placed in a fresh artificial saliva solution without being rinsed.

Group 4: Enafix Group

Enafix (Group Pharma Ltd, Bengaluru, India) was applied on the 20 premolars belonging to this group using a paintbrush for 5 minutes once in 12 hours for 15 days. After gentle wiping, the sample was placed in a fresh artificial saliva solution without being rinsed.

After 15 days, the microhardness of all samples was estimated and alterations in the same were noted. A complete procedure was carried out by a single investigator and a blinding procedure was followed.

Assessment of Surface Morphology

A two-sided tape with carbon adhesive was utilized to place the sample on a holder. After this; the samples were taken to the scanning electron microscopy (SEM) vacuum chamber where samples were air-dried. All the samples were thoroughly viewed under ×2000 magnification at 20 kV to evaluate the surface morphology.

Statistical Analysis

SPSS version 20 was employed to perform data analysis. The mean and standard deviation were calculated. The comparisons between different remineralization agent groups were measured using One-way analysis of variance (ANOVA). The *p*-value less than 0.05 was considered statistically significant.

RESULTS

Table 1 depicts the assessment of SMH of the samples of the various groups made based on the remineralizing agents used prior to treatment with the solution employed for demineralization. Colgate sensitive Pro-Relief group portrayed the highest mean SMH (60.22 ± 0.07) succeeded by the Enafix group (51.12 ± 0.21), Amflor group (47.92 ± 0.04), and control group (45.80 ± 0.12) in the descending order. No statistically significant differences were established among the study groups.

Table 1: Comparison of mean surface microhardness of remineralizing agents groups before the immersion into demineralizing solution

Groups	N	Mean ± std. deviation (μ)	F	p-value
Group 1: Control	20	45.80 ± 0.12		
Group 2: Colgate sensitive Pro-Relief	20	60.22 ± 0.07	21.218	0.596
Group 3: Amflor	20	47.92 ± 0.04		
Group 4: Enafix	20	51.12 ± 0.21		

Table 2: Comparison of mean surface microhardness of remineralizing agents groups after immersion into demineralizing solution

Groups	N	Mean \pm std. deviation (μ)	F	p-value
Group 1: Control	20	37.24 \pm 0.41	21.914	0.328
Group 2: Colgate sensitive Pro-Relief	20	40.50 \pm 0.23		
Group 3: Amflor	20	37.20 \pm 0.01		
Group 4: Enafix	20	38.64 \pm 0.05		

Table 3: Comparison of surface microhardness of remineralizing agents groups after 15 days

Groups	N	Mean \pm std. deviation (μ)	F	p-value
Group 1: Control	20	39.52 \pm 0.32	23.026	0.001
Group 2: Colgate sensitive Pro-Relief	20	53.26 \pm 0.10		
Group 3: Amflor	20	44.84 \pm 0.66		
Group 4: Enafix	20	47.72 \pm 0.21		

Table 4: Comparison of surface microhardness of remineralizing agents using Tukey's HSD (honestly significant difference) test

Group	Compared with	Mean difference (I - J)	Sig.
Group 1	Group 2	-13.74	0.001
	Group 3	-5.32	0.001
	Group 4	-8.2	0.001
Group 2	Group 1	13.74	0.001
	Group 3	8.42	0.001
	Group 4	5.54	0.001
Group 3	Group 1	5.32	0.001
	Group 2	-8.42	0.001
	Group 4	-2.88	0.08
Group 4	Group 1	8.2	0.001
	Group 2	-5.54	0.001
	Group 3	2.88	0.08

Tables 2 delineates the comparative assessment of SMH values of the samples of the various groups made based on the remineralizing agents used after treatment with the solution employed for demineralization. Colgate sensitive Pro-Relief group once again had the greatest difference of the mean SMH (40.50 \pm 0.23) pursued by the Enafix group (38.64 \pm 0.05), Amflor group (37.20 \pm 0.01), and control group (37.24 \pm 0.41), respectively. Also, the statistical tests employed revealed no significant differences between the groups studied.

Table 3 represents the comparative evaluation of SMH of the various groups based on remineralizing agents used after 15 days. Enhanced and highest mean SMH was present in the Colgate sensitive Pro-Relief group (53.26 \pm 0.10) followed by Enafix group (47.72 \pm 0.21), Amflor group (44.84 \pm 0.66), and control group (39.52 \pm 0.32) in that order. ANOVA test depicted that these differences were statistically significant.

Table 4 illustrates manifold assessments amid the studied groups. Except for Group 3 vs Group 4, statistically significant differences ($p < 0.05$) were noted in all the groups.

DISCUSSION

Demineralization of enamel after orthodontic therapy frequently concerns clinicians and patients. Irregularities resulting from orthodontic brackets, wires, springs, and bands cause intensification of plaque accretion on tooth surfaces. These appliances also limit the maintenance of oral hygiene, muscle movement, and self-cleansing action of saliva.¹³

One of the most objectionable side effects of multiple bracket orthodontic therapy is the formation of WSLs on debonding. The elimination of these WSLs is a key challenge in enhancing esthetics.¹⁴

The role of fluorides in the prevention of WSLs is well documented as fluoride ions alter the metabolism of bacteria in plaque by inhibiting enzymatic processes, production of acids, and reducing demineralization through promoting remineralization in initial stages especially at low concentrations.¹⁵

To evaluate and utilize this property of fluorides, this *in vitro* study was undertaken to assess and compare the ability of three commercially available remineralizing agents on the SMH of enamel after induction of WSLs by demineralization. These agents included Colgate sensitive Pro-Relief toothpaste, Amflor, and Enafix. Colgate sensitive Pro-Relief toothpaste contains 8% arginine, insoluble calcium carbonate, and 1450 ppm fluoride that is optimal for enabling remineralization of dental hard tissues.¹² Amflor toothpaste contains 3.85% amine fluoride while Enafix is a complex mixture of calcium sucrose phosphate with inorganic ACP.⁵ Though all the above agents differ in their chief constituents, they have exhibited satisfactory results on remineralization of enamel.

Our results indicated that the SMH of teeth in all the groups was higher after the therapy given over 15 days compared to the microhardness following demineralization. All the three agents utilized in this research that is Colgate sensitive Pro-Relief toothpaste, Enafix, and Amflor enhanced the SMH when compared to the control group.

This in accordance with the study conducted by Alsubhi et al. who found that in contrast to the control group Colgate sensitive Pro-Relief toothpaste improved the SMH of enamel.¹² The results of this research are also in concordance with the findings of Kakkar et al. who found that both Enafix and Amflor are economic means to avoid demineralization of enamel.⁵

Although the agents employed in the study improved the SMH in contrast to the control, these differences were statistically significant. Previous studies by Farzanegan et al., Alsubhi et al., and Kakkar et al. found no statistical differences in the improvement of the SMH although the SMH in the treatment groups was higher than in the control group.^{4,5,12}

The current study found that the highest recorded difference in the value of surface hardness was for Colgate sensitive Pro-Relief toothpaste group followed by the Enafix group. The least increase in microhardness was noted for the Amflor group. This also is in accordance with the study of Alsubhi et al. who found a high increase in surface hardness with Colgate sensitive Pro-Relief toothpaste group in their study. Also according to the study conducted by Kakkar et al., Amflor depicted greater SMH than Enafix which is in accordance with the findings of the present study.^{5,12}

Various other products have been studied and compared in the prevention of WSLs. A study was conducted by Tahmasbi et al. to contrast the remineralizing possibility of fluoride, MI Paste Plus, and Remin Pro while treating WSLs. They found that MI Paste Plus,

0.05% NaF mouthwash, and to a smaller degree Remin Pro were effective methods for treating WSLs.¹³

Mohanty et al. established by their research that Novamin remineralization toothpaste exhibited significant remineralizing ability in the prevention of artificially induced enamel subsurface lesion surrounding the brackets after 10 days of remineralization stage as employed in this study.⁷

Lopatiene et al. performed a systematic review to upgrade the knowledge of all evidence in preventing WSLs after orthodontic therapy. They found that the employment of fluoride, as well as casein products in eliminating WSLs during and succeeding fixed orthodontic therapy, is significantly valuable. They also noted that the casein phosphopeptide-amorphous calcium phosphate surpassed the favorable effects of fluoridated agents in reducing spots from demineralization.⁶ Farzanegan et al. in their study also found ACP as an equally effective method in the color enhancement of WSLs at a proposed use of 0.05% ACP, 0.5% ACP, or 0.05% fluoride solutions on a daily basis.⁴ In our knowledge, this is the first study that has been performed to compare the remineralization potential of three easily available and economic fluoride-containing agents that is Colgate sensitive Pro-Relief toothpaste, Amflor, and Enafix.

Our interpretations of the study results are however subject to few limitations. An *in vitro* model cannot completely imitate the actual oral atmosphere, thus causing a limitation. Furthermore, the change in SMH is also related to the pH values of saliva in every individual. More controlled clinical trials with long-term follow-up are recommended to ascertain best clinical practice.

CONCLUSION

All the three agents employed in this study that is Colgate sensitive Pro-Relief toothpaste, Amflor, and Enafix improved the SMH of teeth after the therapy given over 15 days compared to the microhardness following demineralization. We found that the highest recorded difference in the value of surface hardness was for Colgate sensitive Pro-Relief toothpaste group followed by the Enafix group. The least increase in microhardness was noted for the Amflor group.

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