Effect of Xylitol-fluoride and Fluoride Varnish on Remineralization Enamel after Interproximal Reduction In Vitro

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Abstract

Interproximal reduction (IPR) is frequently employed in orthodontic treatment to create space for tooth alignment; however, its effects on enamel integrity warrant further investigation. This in vitro study assessed the impact of fluoride varnish and xylitol-fluoride on enamel microhardness, mineral density, and mineral content after IPR. Seventeen extracted human premolars were prepared, yielding 30 treated surfaces that underwent IPR and subsequent polishing. All specimens were then randomly allocated into three groups: a control group (no treatment; n=10), a fluoride varnish group (Duraphat®; n=10), and a xylitol-fluoride group (Embrace™ Varnish; n=10). Baseline measurements for enamel microhardness, mineral density, and mineral content were obtained prior to treatment. The treated surfaces were subjected to a 14-day pH cycling regimen designed to simulate the acidic challenges present in the oral environment. Enamel microhardness was measured using the Vickers hardness test, mineral density was evaluated via micro-computed tomography (micro-CT), and mineral content was analyzed using Energy Dispersive X-ray Spectroscopy (EDX). The data obtained were statistically analyzed using ANOVA and the post hoc test (P = .05) indicated that both the fluoride varnish and xylitol-fluoride groups exhibited significant improvements in enamel microhardness and mineral density ($p < 0.05$) compared to the control group. Furthermore, EDX results revealed that the percent weight of fluoride was significantly higher in the fluoride varnish group than in the control and xylitol-fluoride groups ($p < 0.05$), whereas calcium phosphorous levels and the calcium phosphorous ratio did not differ significantly among the groups. These findings indicate that the application of fluoride varnish and xylitol-fluoride enhances microhardness and prevents mineral loss and that fluoride varnish provides greater fluoride retention compared to xylitol-fluoride following IPR.

Keywords: *interproximal reduction, fluoride varnish, xylitol-fluoride, microhardness, mineral density, mineral content*

1. Introduction

The primary goal of orthodontic treatment is to achieve a harmonious balance of occlusion, function, esthetics, and long-term stability. One of the most common concerns of the patients is tooth alignment problems, including proclination, crossbite, spacing, malalignment, and crowded teeth. To address these problems, a range of strategies are proposed from gaining space to relieve the mismatch of tooth size and arch-sized sized discrepancies by tooth proclination, arch expansion, tooth removal, arch distalization and reduction of tooth surface (Choudhary et al., 2015).

Interproximal reduction (IPR) is a clinical procedure used in orthodontics to correct disharmonies in dental shape or size and manage minor arch length discrepancies. Reduction of interproximal enamel is typically recommended for the treatment of mild to moderate crowding, the correction of Bolton tooth size discrepancies, and the improvement of dental aesthetics. The armamentarium that is frequently used in IPR includes handpieces or contra-angle-mounted diamond coated discs, air-rotor stripping techniques, and handheld dental manual stripping systems. Handheld manual stripping is a simple method for reducing the proximal surface, typically performed using artery forceps or Mathieu pliers to facilitate interproximal reduction (IPR). This approach offers the advantage of being effective even in cases with tight proximal contacts or malaligned teeth, where motor-driven tools may not be as suitable. Moreover, this technique can be employed in conjunction with other instruments to recontour teeth following IPR. However, the use of

handheld metal strips for posterior teeth poses significant challenges, as the procedure is time-consuming and may result in the formation of a ledge at the gingival margin of the proximal contact (Choudhary et al., 2015).

Despite the advantages of the IPR procedure, there are adverse effects, which are plaque accumulation, tooth damage by overheating, hypersensitivity, and risk of periodontal disease (Livas, Jongsma, & Ren, 2013). In addition to extensive reduction of enamel surface, the root of the tooth might come too close, resulting in root proximity. These contribute to thin interdental alveolar bone which might result in periodontal breakdown and periodontal disease (Zachrisson, Nyøygaard, & Mobarak, 2007).

IPR invariably results in the formation of grooves and furrows in enamel, leading to various issues such as plaque buildup, heightened vulnerability to dentin hypersensitivity, and potential dental caries. The impact of IPR on caries susceptibility remains uncertain. While certain studies have shown that anterior IPR does not elevate the likelihood of dental caries, conflicting findings have emerged regarding posterior IPR and its alleged association with caries risk, with recent research disputing this connection (Jarjoura, Gagnon, & Nieberg, 2006).

To mitigate the potential drawbacks of IPR, post-reduction enamel polishing can effectively reduce the accumulation of plaque (Jarjoura et al., 2006). Furthermore, certain studies indicate that the use of a remineralizing agent following enamel surface reduction can substantially enhance the integrity of the enamel surface. This process works by preventing enamel demineralization and encouraging remineralization through the regulation of free calcium and phosphate ion activities (Vicente et al., 2017). Remineralizing agents are various options, including 5% sodium fluoride, 8% stannous fluoride, fluoride mouth rinses, 0.05% neutral sodium fluoride mouth rinses, and 1.23% acidulated phosphate fluoride (Choudhary et al., 2015). On the other hand, the non-fluoride group comprises agents such as casein phosphopeptide-amorphous calcium phosphate (ACP-CPP), bioactive glass, sealants, and xylitol, which are also supported by evidence for their potential to remineralize enamel (Bonchev et al., 2019).

Fluoride-containing agents confer multiple benefits in clinical research. They promote enamel remineralization by facilitating the formation of a fluoroapatite structure with enhanced acid tolerance. Additionally, these agents decelerate the demineralization process, reverse early-stage dental caries, and effectively inhibit the proliferation of cariogenic bacteria. Duraphat® (Colgate Palmolive, New York, NY) is the first commercial fluoride varnish widely used in various countries. The fluoride ingredient is 5% sodium fluoride (Milburn et al., 2015). However, it's crucial to acknowledge that each fluoride application comes with its own set of drawbacks. For instance, although fluoride varnish is a widely utilized fluoride treatment, it necessitates a waiting period of at least 30 minutes before consuming food or beverages. Additionally, concerns may arise regarding its appearance, as it can leave a yellowish and sticky residue when instructed to be left overnight before brushing teeth. Additionally, there have been documented instances of allergic reactions linked to the fluoride carrier, specifically the colophony base, which can lead to contact dermatitis. This colophony base is commonly found in fluoride varnish products, underscoring the need for vigilant attention within clinical research environments. The manufacturers' product information typically highlights that fluoride varnish is not recommended for individuals with ulcerative gingivitis or stomatitis, or for those known to have sensitivities to colophony or any other ingredients (Garcia et al., 2017).

Therefore, an alternative treatment as non-fluoride containing agents is introduced. The effectiveness of ACP-CPP, which is commonly used in white spots prevention and caries prophylaxis, is documented to be inferior to fluoride (Giulio et al., 2009). For Xylitol, on the other hand, there is in conclusive evidence regarding to its effectiveness. The mechanisms of xylitol in enamel protection are the reduction of demineralization, enhancement of remineralization of tooth structure and potential to inhibit *S.mutans*, as well as the fact that it contains a sugar alcohol sweetener substance, widely used in food products (Miake, 2003). A study by Arends et al., (1990) investigated the combined effects of fluoride ions and a high xylitol concentration (0.3 mMol of fluoride and 2.63 M of xylitol) and found that this combination exhibits a synergistic effect, which protects enamel by reducing further mineral loss and enhancing enamel protection. Another study by Amaechi, Higham, and Edgar (1998) examined the effects of xylitol, fluoride, and their combination on enamel erosion caused by pure orange juice in vitro, using bovine incisors. Mineral loss was quantified, and the results showed that the combination of xylitol (25% w/v) and fluoride (0.5 ppm) had an

additive protective effect, significantly reducing mineral loss. The study concluded that xylitol and fluoride together provide superior protection against dental erosion due to xylitol's ability to retain calcium and enhance fluoride-induced remineralization. Embrace™ Varnish (Pulpdent Corporation, Watertown, MA) was introduced as a product containing 5% sodium fluoride in combination with Xylitol-coated Calcium/ Phosphate. The manufacturer claims that Embrace™ Varnish provides greater fluoride release, facilitates remineralization, and caries prevention (Milburn et al., 2015). Moreover, it is formulated with a hydrogenated rosin vehicle that reduces allergic reactions, making it a potential option for patients allergic to colophony-based vehicles (Karlberg, Boman, & Nilsson, 1988).

To date, no study has specifically examined the effect of xylitol-fluoride following the IPR procedure, although there was one study on the effect of xylitol-fluoride on enamel caries. Therefore, this study aims to investigate the impact of xylitol-fluoride (Embrace™ varnish) compared with fluoride varnish (Duraphat®) following IPR.

2. Objectives

The purpose of this in vitro study was to compare the effect of xylitol–fluoride varnish (Embrace™ varnish) and fluoride vanish (Duraphat®) on microhardness, mineral density and mineral content of the polished enamel surface after interproximal reduction.

Hypothesis

H_0 (Null Hypothesis): There is no statistically significant difference between xylitol-fluoride and fluoride varnish in terms of microhardness, mineral density, and mineral content after interproximal reduction.

H_1 (Alternative Hypothesis): There is a statistically significant difference between xylitol-fluoride and fluoride varnish in terms of microhardness, mineral density, and mineral content after interproximal reduction.

3. Materials and Methods

Seventeen upper first permanent premolars from human donors were promptly obtained after extraction, without specific owner identification.

Inclusion criteria: The permanent first maxillary Premolars to be extracted for orthodontic purposes were selected.

Exclusion criteria**:** Teeth with proximal restorations, visible or detectable caries, enamel hypoplasia on the proximal surface, or spot lesions on the proximal surface were excluded.

Immediately after extraction, gross debris was removed from the specimens with tab water, before being placed in a 0.1% thymol solution at a temperature of 4°C. Each tooth was rinsed with distilled water and wiped with tissue paper prior to being placed into the plaster block.

The sample size for this study was determined using G*Power version 3.1.9.7. Based on the study by Peng et al., (2016), which investigated the effects of resin infiltration and fluoride varnish on enamel microhardness after interproximal reduction, an effect size of 0.79 was adopted. The sample size calculation was performed using a one-way ANOVA with three groups, setting the significance level (α) at 0.05 and the power (1-β) at 0.80. The analysis indicated that a minimum of 7 specimens per group would be sufficient to detect a statistically significant difference among the groups. To ensure adequate power and account for potential data loss, 10 specimens per group were included in this study.

Ethical approval was achieved form the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COE.No.MU-DT/PY-IRB 2023/0.54.2311).

3.1 Treatment Interventions

All specimens were positioned in plaster blocks and arranged in an arch form with tight proximal contacts. The block comprised 17 teeth, resulting in 15 proximal contacts; therefore, the study encompassed a total of 30 treated enamel surfaces. An investigator performed enamel stripping on all specimens using handheld metal strips (8 mm in size, double-sided, with a grain size of 45 microns; (DynaFlex, Lake St. Louis,

MO 63367). The stripping was executed using a back-and-forth motion, and each metal strip was replaced every 20 strokes until a width of 0.5 mm was achieved, as measured using IPR gauges (Intensiv, Montagnola, Switzerland). Once the target width was reached, all surfaces were polished using Intensiv® Proxopolish handheld strips (15-micron grain) with 20 strokes in both directions.

Interproximal reduction (IPR) was carried out on both the mesial and distal surfaces of each proximal contact in a moist environment to mimic intraoral conditions. The enamel reduction was 0.5 mm per proximal contact (0.25 mm per proximal side). The 30 treated surfaces were divided into three groups:

Control group: 10 treated surfaces with no intervention.

Fluoride varnish group: 10 treated surfaces were treated with 5% fluoride varnish (Duraphat®).

Xylitol-fluoride group: 10 treated surfaces received 5% sodium fluoride with 20% xylitol application (Embrace™ varnish).

3.2 Sample Preparation

The root portion was removed from each tooth using a low-speed saw (Buehler, Lake Bluff, Illinois, USA). The crown was divided into two parts: a mesial part and a distal part. Each tooth was embedded in a PVC block, 1.5 cm in diameter, with the treated surface positioned at the bottom and attached to adhesive tape. Acrylic resin was then poured into the block until it was completely filled, and the specimen was pressed into the top of the acrylic block. Afterward, all specimens were cleaned with distilled water in an ultrasonic cleaner (L&R Sweepzone, L&R Manufacturing Company, USA) for 4 minutes (Martins et al., 2012). The treated surface, which had been coated with either fluoride varnish or xylitol-fluoride, was immersed in artificial saliva for 6 hours and then meticulously cleaned with acetone to remove any excess varnish and debris (Vongsavan, Surarit, & Rirattanapong, 2014).

3.3 pH Cycling Protocol

All specimens underwent pH cycling. The specimens were first incubated in artificial saliva for 11 hours, then placed in the demineralizing solution for 1 hour. This cycle was repeated twice daily for 14 days at 37°C. Fresh solutions were used each time, and the specimens were rinsed with distilled water between cycles (Peng et al., 2016).

Artificial saliva was prepared according to the ISO/TR1027 Standard (pH 6.8) and contained 0.4 g/L NaCl, 0.4 g/L KCl, 0.795 g/L $CaCl_2 \cdot 2H_2O$, 0.78 g/L $NaHPO_4 \cdot 2H_2O$, 0.005 g/L $Na_2S \cdot 2H_2O$, 1.0 g/L urea, and 1.0 g/L water (Peng et al., 2016).

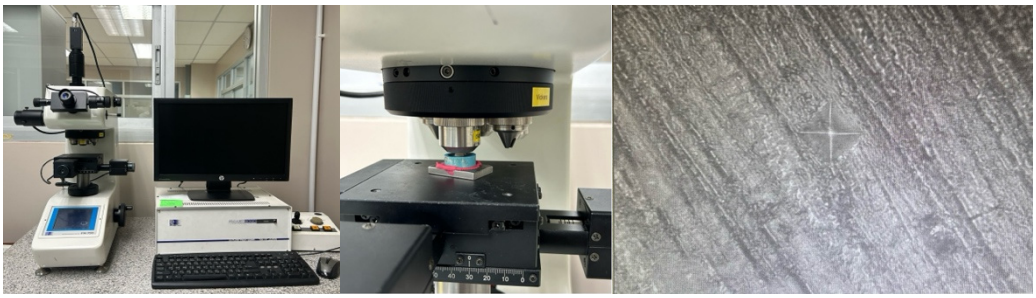
The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid, and 1 M KOH, which was used to adjust the pH to 4.4. The solution was then diluted with distilled water to a 5% concentration (Peng et al., 2016). After the 14-day period, all specimens were cleaned with distilled water in an ultrasonic cleaner for 4 minutes to remove any remaining substrate and to terminate further chemical reactions.

Measurement

Microhardness

Microhardness was measured using the Vickers hardness test (ARS 9000, Future-Tech Corp., Tokyo, Japan) in Figure 1. Three indentations were applied to each specimen under a force of 100 grams for 10 seconds each (Chuenarrom, Benjakul, & Daosodsai, 2009), and the mean surface microhardness was calculated. The mean microhardness and the percentage reduction in microhardness were compared among the three groups. The percentage reduction in microhardness was calculated using the following formula:

$$\frac{\text{Pre pHcycling} - \text{Post pHcycling}}{\text{Pre pHcycling}} \text{X100}.$$

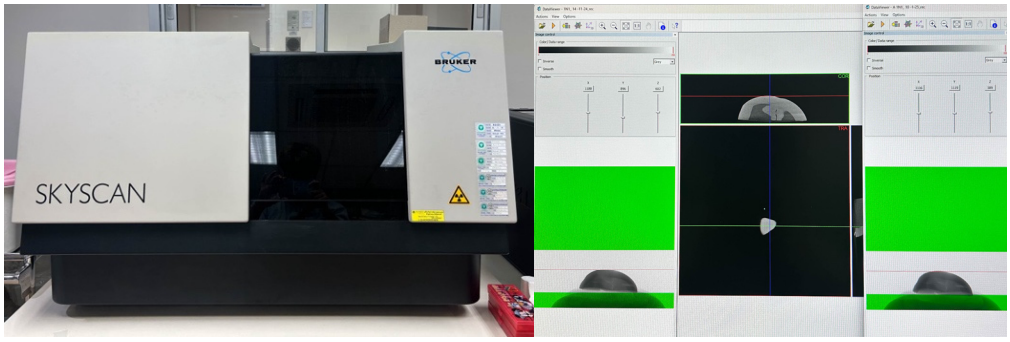


(A) **(B)** **(C)**

Figure 1 Microhardness testing using the Vickers hardness test (ARS 9000, Future-Tech Corp., Tokyo, Japan). (A) The Vickers hardness test setup; (B) Device-base parallel specimen orientation; and (C) The Vickers hardness indentation on the treated surface

Mineral Density

Mineral density was measured using a micro-CT scanner (Bruker®, Skyscan1173) in Figure 2. A reference area measuring 1.06 × 1.06 mm² at a depth of 120 µm was used to assess each sample both before and after the pH cycle. Place the specimen on wax and press it until the IPR surface is parallel to the ground. Mark reference points on both the tooth and the specimen holder to accurately define the measurement location. The percentage of mineral density loss due to acid attack was calculated using the following formula: $\frac{\text{Pre pHcycling} - \text{Post pHcycling}}{\text{Pre pHcycling}} \text{X100}$.



(A) **(B)**

Figure 2 Mineral density examination. (A) A micro-CT scanner (Bruker®, Skyscan1173); (B) Specimen positioning for mineral density analysis

Mineral Content

Calcium, phosphorous, and fluoride weight percentages were examined using Energy Dispersive X-ray Spectroscopy (EDX) (Vicente et al., 2017) in figure 3. The Ca/P ratio, which serves as an indicator of remineralization, was calculated using the formula: Ca/P ratio = $(\frac{\text{Ca weight \%}}{40.08\ \text{g/mol}})$ x $(\frac{30.97\ \text{g/mol}}{\text{P weight \%}})$.

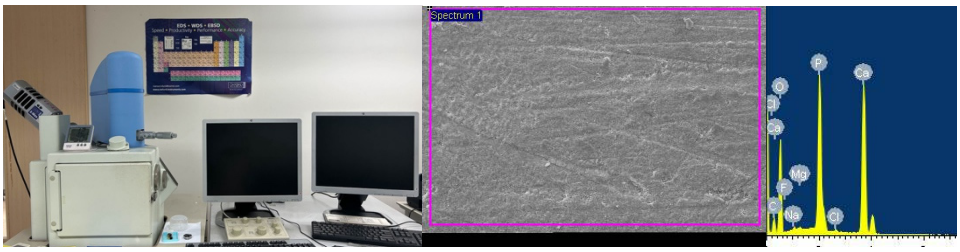


Figure 3 Mineral content analysis. (A) The Energy Dispersive X-ray Spectroscopy (EDX) device; (B) Scanning electron microphotographs of the specimen (C) The mineral content analysis of the specimen

Statistical Analysis

All groups were tested for normality using the Shapiro-Wilk test, which confirmed a normal distribution in all groups. The control, fluoride varnish, and xylitol-fluoride groups were compared using one-way ANOVA ($p < 0.05$). When the ANOVA results were significant, further analysis was performed using the Bonferroni post hoc test.

4. Results and Discussion

4.1 Results

Microhardness

All specimens were assessed for microhardness immediately after proximal stripping to serve as a baseline prior to pH cycling. No statistically significant differences were observed among the control, fluoride varnish, and xylitol-fluoride groups before pH cycling. After pH cycling, all three groups exhibited a decrease in mean microhardness. One-way ANOVA revealed statistically significant differences in mean microhardness between the control and fluoride varnish groups ($p = 0.00$) and between the control and xylitol-fluoride groups ($p = 0.00$). However, the fluoride varnish and xylitol-fluoride groups showed no statistically significant difference in mean microhardness ($p = 0.084$), as shown in Figure 4.

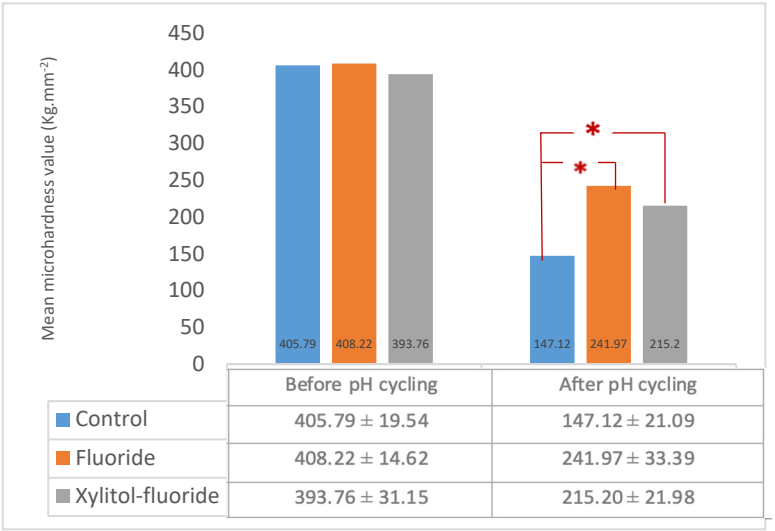


Figure 4 Mean microhardness of enamel among the groups subjected to pH cycling. An asterisk (*) indicates statistically significant differences between groups

Fluoride varnish showed the lowest percentage microhardness reduction, followed by xylitol-fluoride and control, respectively. Statistical analysis indicated that the control group was significantly different from the intervention groups, while no statistical difference was observed between the fluoride varnish group and the xylitol-fluoride group, as shown in Table 1.

Table 1 Mean Comparison of the Percent Reduction of Microhardness Among the Control, Fluoride Varnish, and Xylitol-Fluoride Groups

		Reduction of microhardness (p-value)	**Reduction of microhardness (Percent)**
Control	Fluoride vanish	0.00* (p < 0.05)	63.62 ± 5.52
	Xylitol-Fluoride	0.00* (p < 0.05)	
Fluoride vanish	Control	0.00* (p < 0.05)	40.62 ± 8.60
	Xylitol-Fluoride	0.066 (p > 0.05)	
Xylitol-Fluoride	Control	0.00* (p < 0.05)	44.91 ± 8.35
	Fluoride vanish	0.066 (p > 0.05)	

An asterisk (*) indicates statistically significant differences between groups

Mineral Density

All specimens were measured for mineral density before pH cycling for baseline evaluation, and no statistically significant differences were observed in the mean mineral density before pH cycling. After pH cycling, the mineral density was measured again. The calculation of mineral density loss after the specimens underwent pH cycling showed that the control group exhibited the highest percentage of mineral density loss compared to the intervention groups. A statistically significant difference was observed between the control and fluoride varnish groups (p = 0.00) and between the control and xylitol-fluoride groups (p = 0.00), as shown in Table 2.

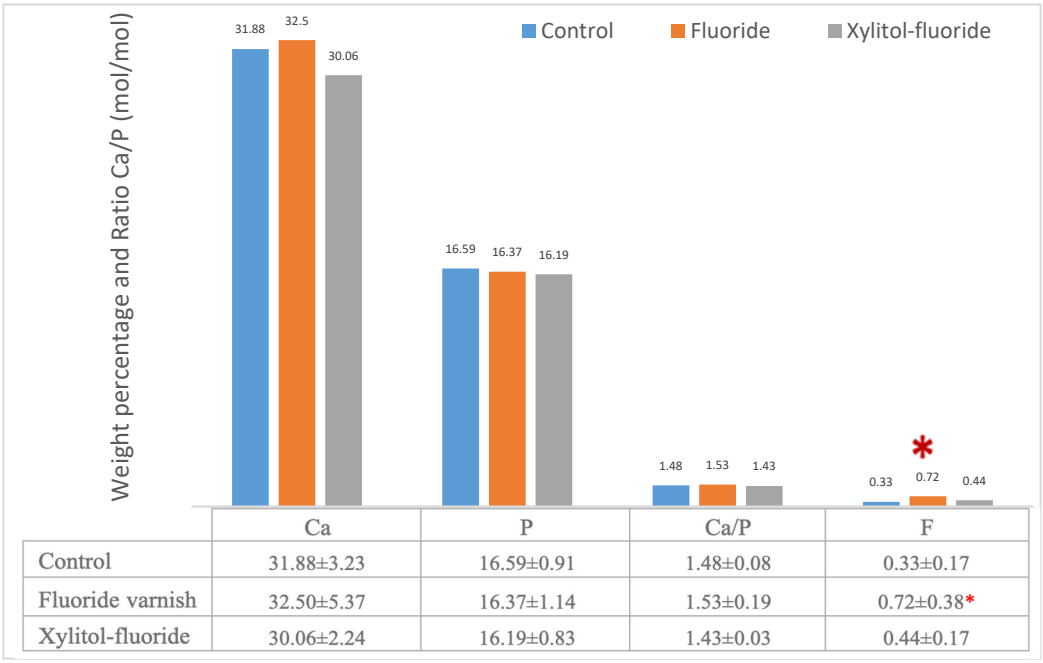
Table 2 Comparison of the percentage mineral density loss among the three groups after pH cycling

		Mineral density loss (p-value)	**Mineral density loss (Percent)**
Control	Fluoride vanish	0.00* (p < 0.05)	39.30±5.50
	Xylitol-Fluoride	0.00* (p < 0.05)	
Fluoride vanish	Control	0.00* (p < 0.05)	2.61±4.53
	Xylitol-Fluoride	0.12 (p > 0.05)	
Xylitol-Fluoride	Control	0.00* (p < 0.05)	7.63±1.63
	Fluoride vanish	0.12 (p > 0.05)	

An asterisk (*) indicates statistically significant differences between groups

Mineral Content

Calcium (Ca), phosphorus (P), the calcium/phosphorus ratio (Ca/P), and fluoride (F) were determined to assess the mineral content before pH cycling. No statistically significant differences in the percent weight of Ca, P, Ca/P, and F were observed before pH cycling. After pH cycling, one-way ANOVA showed no statistically significant differences in Ca ($p = 0.27$), P ($p = 0.65$), or the Ca/P ratio ($p = 0.18$) among the three groups. However, the fluoride measurement exhibited a statistically significant difference between the control group and the fluoride varnish group ($p = 0.00$), as shown in Figure 5.



	Ca	P	Ca/P	F
Control	31.88±3.23	16.59±0.91	1.48±0.08	0.33±0.17
Fluoride varnish	32.50±5.37	16.37±1.14	1.53±0.19	0.72±0.38*
Xylitol-fluoride	30.06±2.24	16.19±0.83	1.43±0.03	0.44±0.17

Figure 5 Mean weight percentages of enamel among the groups after pH cycling. An asterisk (*) indicates statistically significant differences between groups. Abbreviations: Ca, calcium; P, phosphorus; Ca/P, the calcium/phosphorus ratio; and F, fluoride

4.2 Discussion

IPR was used to resolve mild to moderate crowding by reducing the interproximal surfaces of enamel. The findings indicated that stripped enamel surfaces were more prone to demineralization, as evidenced by a drop in microhardness after the pH cycle. The application of available remineralizing agents was advised to counteract the potential adverse effects of interdental stripping by promoting remineralization of enamel surfaces. These procedures facilitated the deposition of calcium and phosphate ions into demineralized enamel, leading to a net gain in minerals. Several remineralizing agents, such as fluoride varnish (Rossouw, & Tortorella, 2003), were suggested for restoring early enamel structural loss. Thus, this study evaluated the impact of xylitol-fluoride (Embrace™ varnish) compared with fluoride varnish (Duraphat®) and a control group on enamel surface characteristics following IPR and an erosive challenge induced by acidic exposure during pH cycling.

To simulate the acid exposure encountered in the oral environment, in vitro pH-cycling models were developed to mimic the dynamic processes of mineral loss due to demineralization and mineral gain from artificial saliva. The key elements of the pH-cycling design included the test substrate, the demineralization solution, the remineralization solution, treatment times, and specimen analysis. For human enamel, employing a demineralization solution with a pH of 4.4 and a remineralization solution with a pH of 7.0 over a 14-day period was considered appropriate for evaluating microhardness (Stookey et al., 2011). This study

assessed remineralization based on three parameters: microhardness, mineral content, and mineral density. The Vickers hardness test (ARS 9000, Future-Tech Corp., Tokyo, Japan) was used to evaluate changes in microhardness, which indicated the effectiveness of demineralization protection, as increased hardness suggested enhanced mineral remineralization and enamel strengthening. Studies by Peng et al., (2016) and Mohd Said et al., (2016); Vongsavan, et al., (2014) also used microhardness measurements to obtain information on enamel softening and mineral loss following acid-induced demineralization. Next, mineral content, which refers to the concentration of inorganic components in enamel, was analyzed using Energy Dispersive X-ray Spectroscopy (EDX). The analysis focused primarily on calcium, phosphorus, and fluoride. Enamel consisted of both organic and inorganic components, with calcium and phosphorus serving as the key inorganic elements essential for the formation of hydroxyapatite crystals. Similarly, fluoride ions could incorporate into the enamel structure to form fluorapatite crystals. The study examined mineral content as an indicator of the integrity of the enamel's chemical composition, which reflected its remineralizing ability (Ben Mohimd et al., 2019). Lastly, mineral density evaluation, which represented the mass of minerals per unit volume of enamel, reflected its overall mineralization status by quantifying the extent of mineral loss and gain and provided the ability to analyze the depth of the demineralized lesion (Hoxie et al., 2023). In this study, mineral density loss was assessed using a micro-CT scanner (Bruker® Skyscan 1173), similar to the approach used by Peng et al., (2016), who employed micro-CT to analyze mineral volume loss in enamel following IPR.

This study accepts the null hypothesis, indicating that there is no statistically significant difference between the xylitol-fluoride and fluoride varnish groups in terms of microhardness and mineral density. Regarding mineral content, calcium, phosphorus, and the calcium/phosphorus ratio, the null hypothesis is also accepted. However, for fluoride content, the null hypothesis is rejected, as a statistically significant difference was found between the xylitol-fluoride and fluoride varnish groups. Based on the results of this study, the mean microhardness, percentage of microhardness reduction, and percentage of mineral density loss showed statistically significant differences between the intervention groups (fluoride varnish and xylitol-fluoride) and the control group. Specifically, the fluoride varnish group demonstrated the highest average microhardness following acid exposure in the post-pH cycle, followed by the xylitol–fluoride group, while the control group exhibited the lowest microhardness values. These results were consistent with previous research that highlighted fluoride's effectiveness in promoting remineralization and protecting enamel after reduction procedures (Vicente et al., 2017). Additionally, a study by Peng et al., (2016) demonstrated that both the percentage of microhardness reduction and mineral density loss after the pH cycle were lower in the fluoride varnish group compared to the control group, emphasizing that fluoride varnish effectively remineralized enamel surfaces after IPR. This effect can be further explained by the observation that under acidic conditions, enamel minerals such as calcium and phosphate dissolved from the enamel into the surrounding solution. Once the pH returned to normal, calcium and phosphate re-precipitated as hydroxyapatite crystals on the enamel surface-a process known as remineralization. In the presence of fluoride ions from fluoride varnish, remineralization occurred not only as hydroxyapatite formation but also as fluorapatite formation, which inhibited ion dissolution and strengthened the tooth surface under lower pH conditions compared to hydroxyapatite. These results emphasized the importance of applying fluoride-based remineralization strategies after interproximal reduction (Simmer et al., 2020).

However, this study found no significant differences between the xylitol–fluoride and fluoride varnish groups in terms of mean microhardness, percentage of microhardness reduction, and mineral density loss after the pH cycle. Similarly, Vongsavan et al., (2014) evaluated the effects of xylitol combined with fluoride versus fluoride varnish on bovine teeth and also reported no significant difference in mean microhardness values between specimens treated with fluoride varnish and those treated with xylitol–fluoride. In the study by Cardoso et al., (2014), the effect of xylitol-containing varnishes, with or without fluoride, on the remineralization of artificial enamel caries lesions in bovine teeth was investigated. Treatments included varnishes containing 10% and 20% xylitol alone, 10% and 20% xylitol combined with 5% sodium fluoride (NaF), as well as two commercial fluoride varnishes (Duofluorid™ and Duraphat™). The results indicated that surface remineralization significantly improved in the Duraphat™, 10% xylitol + fluoride, and 20%

xylitol + fluoride groups. Subsurface mineral recovery was observed only in the Duraphat™, Duofluorid™, and 20% xylitol groups. Notably, the xylitol-fluoride combination used in our study (20% xylitol + 5% NaF) matches the formulation used in one of Cardoso et al.'s groups.

In contrast, Mohd Said et al., (2016) found that the mean microhardness of enamel treated with xylitol–fluoride (Embrace™ Varnish), which was also used in our study(20% xylitol + 5% NaF), was lower than that of enamel treated with fluoride varnish (Duraphat®). The authors concluded that although xylitol–fluoride varnish was effective in enhancing surface enamel remineralization, it did not significantly improve subsurface remineralization. They suggested that the presence of fluoride might inhibit the diffusion of xylitol into the enamel, thereby reducing its remineralizing efficacy (Mohd Said et al., 2016).

After pH cycling, statistical analysis revealed that calcium ($p = 0.27$), phosphorus ($p = 0.65$), and the calcium/phosphorus ratio ($p = 0.18$) did not differ significantly among the groups. This finding may be explained by the remineralization process mediated by artificial saliva, which precipitates calcium and phosphate ions onto the enamel surface once the pH returns to normal (Ben Mohimd et al., 2019). Notably, fluoride levels were significantly higher in the fluoride varnish group compared with both the xylitol-fluoride and control groups, thereby reinforcing the role of fluoride varnish in facilitating the formation of fluorapatite highly resistant crystalline structure within enamel (Vicente et al., 2017). Our study did not observe a statistically significant difference in fluoride levels between the control and xylitol-fluoride groups. In agreement with Piesiak-Panczyszyn et al., (2023), who evaluated fluoride ion release using an ion-specific electrode, our results indicated that xylitol-fluoride (Embrace™ Varnish) exhibited lower fluoride release compared with fluoride varnish (Duraphat®). This outcome may be attributed to the evaluation intervals, which were set at 2-week periods. Such differences in assessment timing could have influenced the fluoride release capacity of the xylitol-fluoride, potentially resulting in a decline in its effectiveness over time. Furthermore, Milburn et al., (2015) reported that xylitol-fluoride (Embrace™ Varnish) released ten times the amount of fluoride compared with fluoride varnish within the first 4 hours, although this release diminished significantly after 2 days.

A limitation of this study is that it was conducted in a controlled laboratory setting, which may not fully replicate the complexities of the oral environment, including salivary flow, bacterial biofilm, and dietary influences. The absence of these factors may impact the applicability of the findings to real clinical scenarios. Further clinical research should aim to address these limitations by incorporating in vivo designs and a broader range of assessment methods to enhance the clinical relevance of the findings.

Nevertheless, these findings indicate that xylitol-fluoride is effective in counteracting acid exposure, supporting enamel remineralization, and preventing further demineralization after proximal reduction, similar to fluoride varnish. However, xylitol-fluoride did not enhance fluoride ion incorporation into fluorapatite.

5. Conclusion

Fluoride varnish and xylitol-fluoride varnish have demonstrated the capacity to protect enamel against acid attacks. From a clinical perspective, both agents may serve effectively as finishing treatments following interproximal reduction (IPR), thereby enhancing enamel durability and resistance to demineralization.

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