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RESEARCH ARTICLE

EVALUATION OF ETCH PATTERNS AND BOND STRENGTH AFTER USING DIFFERENT ENAMEL CONDITIONERS WITH AND WITHOUT DEPROTEINIZING AGENT: AN IN VITRO STUDY

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ABSTRACT

Objective: The objective of this study was to compare etch patterns, shear bond strength and ARI scores using different enamel conditioners with and without deproteinizing agent.

Study Design: A total of 125 human premolars were divided into 5 groups (n = 25) as follows: Group A, B1, C1 in which teeth are etched with 37% phosphoric acid, 10% maleic acid, 20% lactic acid for 20 seconds respectively. In Groups B2 and C2, etchants were same as B1 and C1 but enamel deproteinization with 5.25% sodium hypochlorite was done for 1 minute before enamel etching. Etching patterns were then evaluated in SEM, bond strength was examined with universal mechanical test machine and ARI scores were evaluated to check the bracket debonding interface. Data was analyzed statistically.

Results: Group B2 showed superior SBS, more of type 1 etching patterns and debonding occurring at bracket adhesive interface compared to the control group (A), whereas the Group C1 showed lowest mean SBS, type 4 etching patterns and debonding occurring at the enamel adhesive interface. Group B2 and C2 showed higher SBS and better etching patterns compared to the Group B1 and C1 where deproteinizing agent not was used.

Conclusions: Enamel deproteinization with 5.25% sodium hypochlorite showed improvement in etching patterns and increased shear bond strength of orthodontic brackets bonded with alternative acids to etch the enamel surface.

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INTRODUCTION

It was a cutting edge in dentistry which came up at the end of the 20th century with the advent of aesthetic adhesive dental materials. Buonocore demonstrated an increased adhesion of acrylic resins on enamel treated with 85% phosphoric acid (H₃PO₄). Further research was fundamental to the understanding and acceptance of enamel etching and the adhesion system in dentistry (Espinosa et al., 2008). Phosphoric acid remained the principal enamel etchant since it was first introduced by Buonocore in 1955 and used by Newman in 1965.

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Phosphoric acid concentration between 30% and 40% results in the most retentive etching pattern (Alsulaimani, 2014). Current clinical application of phosphoric acid uses a 37% acid concentration (Olsen et al., 1997). The tensile bond strength between enamel surfaces and restorative dental material is affected by several factors that include acid etching, etching time, pH, acid composition (organic inorganic), acid concentration, enamel morphology, calcium phosphate concentration, enamel site, and rinsing time. In the process of bonding, the microporous enamel surface created by acid etching with phosphoric acid plays a main role in the bond strength of restorative dental materials for primary and permanent teeth (Loyola-Rodriguez et al., 2009). It is speculated that 37% phosphoric acid is very acidic (Mazzoni et al., 2006). Acid etching has been reported to cause enamel loss.

The permanent loss of enamel calcium during the acid etching procedure may render the enamel surface more susceptible to demineralization during and after the orthodontic treatment. Cehreli and Altay observed that regardless of treatment time, etching with 37% phosphoric acid results in irreversible damage of the enamel surface (Cal-Neto and Miguel, 2006). Therefore, to minimize the extent of enamel surface damage, alternate conditioners, such as lactic acid, maleic acid, nitric acid, polyacrylic acid, and ethylenediaminetetraacetic acid, have been used to obtain clinically useful bond strengths by decreasing the depth of enamel dissolution (Alsulaimani, 2014). Espinosa *et al.* (2008) showed that when 5.25% of NaOCl was used as deproteinizing agent before acid etching with alternative etchants, increased the bond strength and produced more of type 1 and type 2 etching patterns while without the use of NaOCl, more of type 3 etch patterns were found. The present study was conducted to test the following hypothesis:

- a) Different enamel etchants will provide different etching patterns and different shear bond strength to enamel.
- b) Improvement in etching patterns, shear bond strength and debonding interface will be seen after the use of deproteinizing agent for 1 minute before the acid etching.

MATERIALS AND METHODS

One hundred and twenty-five extracted human premolars were collected from the Department of Oral and Maxillofacial Surgery. The teeth extracted were from the patients whose treatment plan needed orthodontic extractions and were collected with the informed consent of the patients. The sample size was estimated and this study was designed to have power of 90%. The exclusion criteria for selection of the samples were the teeth with caries, cracks, erosion, fluorosis or hypocalcification, and restored teeth. A total of 125 human premolars were, divided into 5 groups ($n = 25$), and denominated as follows:

Group A ($n=25$): The enamel surface was etched with 37% Phosphoric acid (3M Unitek) applied with a microbrush for 20 seconds, washed with water and air spray for 20 seconds, then dried with oil free compressed air.

Group B1 ($n=25$): The enamel surface was etched with 10% Maleic acid (99.9% From Loba Chemie, Diluted in the Pharmacology Lab at Surendera Dental College and Research Institute) applied with a microbrush for 20 seconds, washed with water and air spray for 20 seconds, then dried with oil free compressed air.

Group B2 ($n=25$): The enamel surface was treated with 5.25% sodium hypochlorite applied with sterile cotton pellet for one minute, washed, then dried with water for 10 seconds and etched with 10% Maleic acid (99.9% From Loba Chemie, Diluted in the Pharmacology Lab at Surendera Dental College and Research Institute) applied with a microbrush for 20 seconds, washed with water and air spray for 20 seconds, then dried with oil free compressed air.

Group C1 ($n=25$): The enamel surface was etched with 20% Lactic acid (99.9% From Loba Chemie, Diluted in the Pharmacology Lab at Surendera Dental College and Research Institute) applied with a microbrush for 20 seconds, washed

with water and air spray for 20 seconds, then dried with oil free compressed air.

Group C2 ($n=25$): The enamel surface was treated with 5.25% sodium hypochlorite applied with sterile cotton pellet for one minute, washed, then dried with water for 10 seconds and etched with 20% Lactic acid (99.9% From Loba Chemie, Diluted in the Pharmacology Lab at Surendera Dental College and Research Institute) applied with a microbrush for 20 seconds, washed with water and air spray for 20 seconds, then dried with oil free compressed air.

The orthodontic brackets (MBT brackets, 0.022" slot, American Orthodontics) for premolars were fixed in the centre of the crown using Transbond XT Adhesive (3M Unitek). Excess adhesive was removed from the teeth with a probe, and each bracket was then light polymerized with a LED appliance (Woodpecker, Guilin Woodpecker Medical Instrument company Ltd, Guangxi, China) for 20 s (5 s on each side). After bonding, all samples were stored in distilled water with 0.1% thymol solution. Each tooth was mounted in acrylic resin in the respective mould with roots embedded upto cervical line in the acrylic portion to enhance the control and manipulation of the specimens.

Thermocycling and Shear bond strength testing

The test samples were subjected to 500 thermocycles at temperature (5°C to 55°C) before testing. The dwell time was 30 seconds for each bath as advised by International Organisation for Standardization before testing to mimic thermally stressed accelerated aging. 20 samples from each group were subjected to a shear load in a universal testing machine model (Star testing systems model no. 9036 TD) with a knife-edged blade at a crosshead speed of 1 mm/min. The force was applied parallel to the long axis of the tooth on top of each orthodontic bracket base, and the shear load was recorded at the point of failure. The force per unit area required to dislodge the bracket was recorded in Newtons for each specimen and divided by the surface area of the bracket base to estimate the SBS in megapascals (MPa).

Assessment of adhesive remnant index

The enamel surfaces were examined with a stereomicroscope under 40× magnifications to determine the amount of composite remaining, and then they were classified according to adhesive remnant index (ARI). The ARI scores ranged from 1 to 5 with 1 indicating all the composite remained on the tooth as well as the impression of the bracket base, 2 indicating more than 90% of the composite remained, 3 indicating more than 10% but less than 90% of the composite remained on the tooth, 4 indicating less than 10% of composite remained on the tooth surface and 5 indicating that no composite remained on the enamel.

Scanning electron microscope examination

Five premolars from each group were used for ultra-structural examination of the etched enamel surfaces using a scanning electron microscope (JEOL, JSM-6100 LV, USA). Each etched buccal surface was observed under SEM and photomicrograph was taken in the centre of the test surface at 2000X magnification. The teeth were pre-treated with the respective acid, then rinsed with water for 10 s, and dried. The

teeth were separated from the acrylic moulds and gold sputtering was done separately for each group. A total of 25 photomicrographs (5 for each experimental group) were taken. Under the scanning electron microscope, all the etched surfaces resulted in different etch patterns where enamel prism cores preferentially removed with peripheries relatively intact shows type 1 etching patterns, peripheral regions of the prisms removed leaving relatively unaffected prism cores shows type 2 etching patterns, regions in which the pattern of etching could not be related to prism morphology shows type 3 etching patterns, prismless enamel displaying no rod or prism patterns shows type 4 etching patterns and flat and smooth lacking micro irregularities shows the type 5 etching patterns. The second invigilator who was experienced in SEM analysis analyzed the etching patterns seen on the buccal surface and they were recorded. Hence both the assessing invigilators were blinded. After recording the pictures, they were evaluated.

Statistical analysis

Statistical analyses were performed with the SPSS (SPSS Version 23 for window, release 7.5.1, Chicago, USA). Descriptive statistics that included mean, standard deviation, median, minimum and maximum values were calculated for all five groups. Analysis of variance was applied to determine whether significant differences existed among the groups. Post hoc Tukey's test was used to compare the mean between the individual groups. Chi-square test was used for assessing ARI scores. The level of significance adopted was 5% ($p < 0.05$).

RESULTS

Scanning Electron Microscope

Group A showed both type 1 and type 2 etching patterns with more of type 2 etching patterns. Group B1 showed more of type 3 etching patterns. Group C1 showed less well-defined patterns indicating more of type 4 etching patterns. With the use of deproteinizing agent for one minute before acid etching, Group B2 showed type 1 etching patterns and Group C2 showed type 2 etching patterns. On comparing the SEM images after etching, Group B2 showed the best etching patterns, followed by Group A, Group C2, Group B1 and Group C1 showed the etch patterns which were less favourable (Figure 1-5).

Universal Testing Machine

The highest SBS was recorded for Group B2 (10.09 ± 0.47 MPa), followed by Group A (9.62 ± 0.41 MPa) and then Group B1 (7.25 ± 0.56 MPa) which was almost similar to Group C2 (7.23 ± 0.28 MPa). Group C1 had the least bond strength with the values (5.9 ± 0.36 MPa). The ANOVA test showed significant difference between the groups with respect to the median load ($p < 0.01$) (Table 1). To find out the difference between the groups, Turkey HSD Post-hoc Test was carried out and results were given in (Table 2). The significant differences were found between all the groups except Group B1 and Group C2 which showed non-significant difference between the bond strength.

Analysis of ARI Scores: The ARI was used to determine the bond failure location between the different adhesives and etching techniques. The results of the Chi Square test comparing the scores indicated the presence of significant

differences between the various groups ($p < 0.03$). Group B2, A, C2 had bond failure at the composite-bracket interface, whereas Group B1 and C1 revealed bond failure primarily at the enamel-composite interface (Table 3).

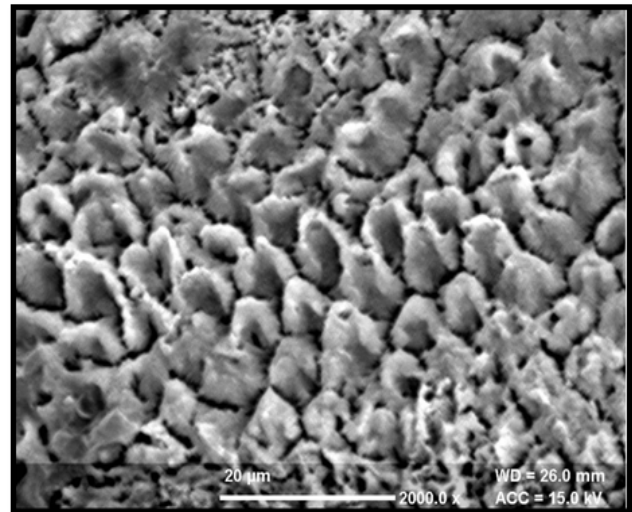


Figure 1. Type 1 and Type 2 etching patterns, with more of type 1 etching patterns seen after etching the enamel surface with 37% phosphoric acid

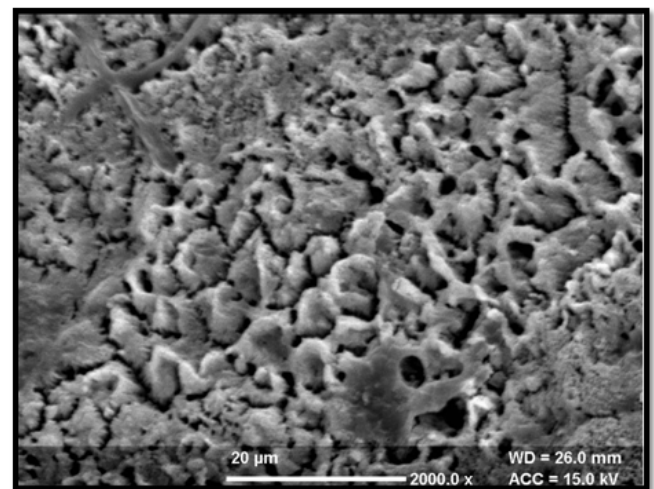


Figure 2. Type 3 etching patterns seen after etching the enamel surface with 10% maleic acid

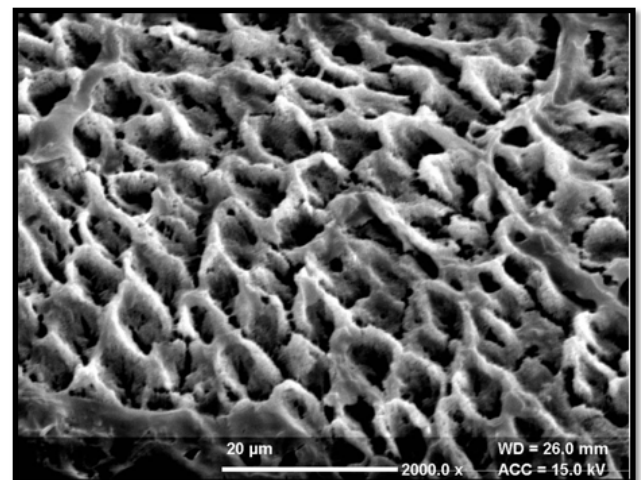


Figure 3. Type 1 etching patterns seen after treating the enamel surface with deproteinizing agent before enamel etching with 10% maleic acid

Table 1. Comparison of shear bond strengths of all the groups.

Groups	N	Bond strength (MPA)		Min	Max	Median	Anova test	p value
		Mean	SD					
Group A	20	9.62	0.41	7.89	10.04	8.5	344.73	<0.01*
Group B ₁	20	7.25	0.56	6.57	8.76	7.2		
Group B ₂	20	10.09	0.47	8.32	11.26	10.3		
Group C ₁	20	5.9	0.36	5.1	6.4	5.7		
Group C ₂	20	7.23	0.28	6.1	8.2	7.1		

*p <0.05: statistically significant

Table 2. Tukey HSD Post-hoc Test showing the mean value difference in each group

Groups	Mean difference	Confidence interval (95%)		p value
		Lower	Upper	
Group A vs Group B ₁	-2.37	-2.7453	-1.9947	<0.01*
Group A vs Group B ₂	0.47	0.0947	0.8453	0.0066*
Group A vs Group C ₁	-3.72	-4.0953	-3.3447	0.3697*
Group A vs Group C ₂	-2.39	-2.7653	-2.0147	<0.01*
Group B ₁ vs Group B ₂	2.84	2.4647	3.2153	<0.01*
Group B ₁ vs Group C ₁	-1.35	-1.7253	-0.9747	<0.01*
Group B ₁ vs Group C ₂	-0.02	-0.3953	0.3553	0.9999
Group B ₂ vs Group C ₁	-4.19	-4.5653	-3.8147	0.8526*
Group B ₂ vs Group C ₂	-2.86	-3.2353	-2.4847	<0.01*
Group C ₁ vs Group C ₂	1.33	0.9547	1.7053	<0.01*

*p < 0.05: statistically significant

Table 3. Analysis of Adhesive Remnant Index Scores

	Score1	Score2	Score3	Score4	Score5	Chi square	p value
Group A	6	8	3	2	1	17.33	0.03*
B ₁	4	4	6	4	2		
B ₂	8	5	3	3	1		
C ₁	3	4	6	6	1		
C ₂	3	6	5	4	2		

*p < 0.05: statistically significant

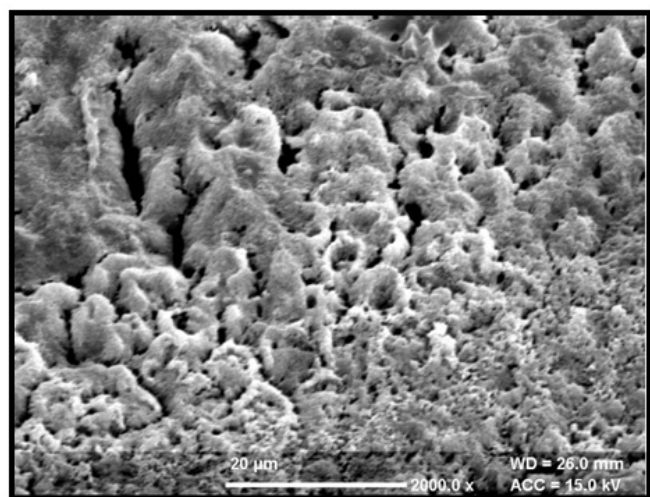


Figure 4. Type 4 etching patterns seen after etching the enamel surface with 20% lactic acid

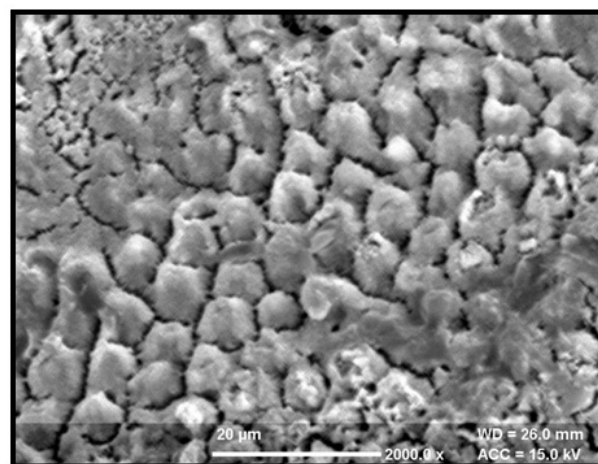


Figure 5. Type 2 etching patterns seen after treating the enamel surface with deproteinizing agent before enamel etching with 20% lactic acid

DISCUSSION

The use of lactic acid as an alternative to phosphoric acid for enamel etching has been documented in the literature (Alsulaimani, 2014). 10 % maleic acid has also been used as a conditioner that could etch dentine as well as be used for enamel bonding. Therefore, maleic acid has been suggested as alternative conditioner that can maintain a clinically useful bond strength while decreasing the depth of enamel dissolution, thus decreasing enamel surface damage (Sinhoreti et al., 1998; Hermsen et al., 1993).

However, the etch patterns created by these alternative acids have been found to be inconsistent. So, elimination of organic substances from the enamel surface before acid etching providing a better acid etching pattern on enamel has been advocated. Deproteinization of the enamel surface before bracket bonding was first proposed by Justus et al. (2010). 5.25% sodium hypochlorite was used for this purpose. According to De-Deus et al. (2011), NaOCl eliminates the organic matter present on the enamel surface by dissolving it, and thus, it can be speculated that increased shear bond strength might be achieved by deproteinizing the enamel

surface with 5.25% NaOCl. The weaker acids such as 10% maleic acid and 20% lactic acid can be used along with NaOCl to get the favourable etch patterns and bond strength equivalent to conventional 37% phosphoric acid etching. Many studies have been done comparing alternative mild acids with phosphoric acid, however no study has been conducted comparing these acids with conventional phosphoric acid used along with deproteinizing agent. So, the present study was done where maleic and lactic acids were used with and without deproteinizing agents and compared to conventional phosphoric acid in terms of bond strength, etch patterns and adhesive remnant index.

The bond strength of lactic acid alone was found to be lowest as compared to maleic acid. This was in accordance to the studies done by Hughes *et al.* (2000), Ballal (2009), Ayad (1996) and Luque *et al.* (2012). The reason for the above finding was due to the formation of weak bonds of lactic acid to enamel, gentle rinsing with distilled water almost completely removing the acid as seen by X-Ray photoelectron spectroscopy in the study done by Yoshioka *et al.* (2002). They also found that amount of Ca & P extracted from hydroxyapatite powder was more for maleic acid than lactic acid. The fact that acids adhere to or decalcify hydroxyapatite crystals appears to depend largely upon the dissolution rate of the respective calcium salts in their respective acidic solutions. The less soluble their respective salts, the more stable their bond to hydroxyapatite (HAp) and less they decalcify Hap. The lactic acid salts were less soluble in their respective acid solution compared to maleic acid and therefore, their ability to decalcify hydroxyapatite crystals of enamel was less than maleic acid.³³

The bond strengths of both lactic acid & maleic acid increased after adding deproteinizing agent. This was in accordance to study done by Hasija *et al.* (2017), where the authors found that 5% NaOCl showed maximum bond strength compared to the other deproteinizing agent and was an effective enamel bond enhancer. The ARI was used to determine the bond failure location between the different adhesives and etching techniques. Bishara *et al.* maintained that bond failure at the bracket adhesive interface or within the adhesive is more desirable and safer than the failure at the adhesive enamel interface because enamel fracture and crazing have been reported at the time of bracket debonding (Bishara *et al.*, 2001).

The results of the present study indicated that Group B2, (etching with 10% maleic acid after deproteinizing agent) showed superior SBS, more of type 1 etching patterns and debonding occurring at bracket adhesive interface compared to the control group (A), whereas the Group C1 showed lowest mean SBS, type 4 etching patterns and debonding occurring at the enamel adhesive interface. The results of one-way ANOVA showed highly significant difference with the use of deproteinizing agent in SBS of the groups ($p < 0.01$). Group B2 and C2 showed higher SBS and better etching patterns compared to the Group B1 and C1 where deproteinizing agent not was used. 5.25% sodium hypochlorite eliminates the organic substances which allows the acid etchant to penetrate more effectively into enamel creating type 1 & 2 etching pattern as seen in Group B2 & C2 which was in accordance to the studies done by Espinosa *et al.* (2008), Pithon *et al.* (2011), Pereira *et al.* (2012) and Justus *et al.* (2009). Sodium hypochlorite (NaOCl) has an antibacterial effect and does not

damage healthy tissue or tooth structure. Interpreting the chemical reactions, it can be observed that sodium hypochlorite acts as an organic and fat solvent degrading fatty acids, transforming them into fatty acid salts (soap) and glycerol (alcohol), that reduces the surface tension of the remaining solution. Sodium hypochlorite neutralizes amino acids forming water and salt. With the exit of hydroxyl ions, there is a reduction of pH. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as solvent, releases chlorine that, combined with the protein amino group, forms chloramines. Hypochlorous acid (HOCl) and hypochlorite ions (OCl) lead to amino acid degradation and hydrolysis. The chloramination reaction between chlorine and the amino group (NH) forms chloramines that interfere in cell metabolism. Chlorine (strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulfhydryl group) of essential bacterial enzymes (Estrela *et al.*, 2002). The use of 5.25% sodium hypochlorite (NaOCl) as a deproteinizing agent may be a possible strategy to optimize adhesion by removing organic elements of both the enamel structure and the acquired pellicle before acid etching (Espinosa *et al.*, 2008). The results of this study showed maleic acid with deproteinizing agent had the greatest bond strength, improved etching patterns and favourable site of debonding as assessed by ARI scores and therefore, recommended for clinical usage.

Conclusion

This study was done to determine the effect of different acids and deproteinizing agent on the etching patterns, SBS and ARI scores of the bonded premolars and it can be concluded:

1. The addition of deproteinizing agent with the alternate conditioners such as maleic and the lactic acid provided more of type 1 and type 2 etching patterns.
2. The SBS was increased with the use of deproteinizing agent before etching as compared to the SBS samples where deproteinizing agent was not used.
3. Maleic acid with deproteinization could be a clinically useful alternative enamel conditioner.

Conflict of interest: The authors declare that they have no conflict of interest related to this study. The study was self-funded by the authors.

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