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ORIGINAL RESEARCH ARTICLE

Effect of Resin Infiltration and Various Etching Techniques on the Bond Strength of Sound and Demineralized Enamel

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Abstract

Purpose: The objective was to assess the effect of various etching techniques on the shear bond strength(SBS) of resin infiltrated sound/ demineralized enamel.
Materials and Methods: A total of 180 extracted human upper incisors' enamel surfaces were utilized. Half of these specimens underwent artificial demineralization, which involved exposing to an acidic buffer with a pH of 4.8 (14 days). The remaining half of the specimens were kept in their original sound condition. Subsequently, both the sound/ demineralized specimens were divided into 6 groups according to the presence (I)/absence (NI) of resin infiltrant and etching methods (35% phosphoric acid (PA), 9% hydrofluoric acid (HFA), and Er,Cr:YSGG laser(L) (n=15): Following different etching procedures, an etch-and-rinse adhesive system was used, before the application of a nanohybrid composite to the enamel surfaces. Then all specimens were submitted to SBS test (MPa). Three-way ANOVA and Bonferroni test were used for statistical analysis (p=0.05).
Results: PA showed the highest SBS in the I groups compared with L and HFA groups, both in demineralized and sound enamel ($p<0.05$). On sound enamel, L showed higher SBS than HFA ($p<0.05$). On demineralized enamel, L showed similar values with HFA($p>0.05$). In the NI groups, L showed similar SBS to HFA on both demineralized and sound enamel($p>0.05$).
Conclusions: Resin-infiltrated enamel can be etched by 35% phosphoric acid without jeopardize bond strength.

Key words: Shear bond strength; Resin infiltration; Etching; Laser; Composite resin

Introduction

Enamel demineralization is one of the earliest stages of caries lesions that occurs as a result of the plaque remaining on the tooth surface longer than the critical process. Initial enamel demineralization is expressed as a "white spot lesion" (WSL) as a consequence of the optical illusion caused by mineral loss [1,2].

WSL is the reversible stage of dental caries. Initial enamel caries have been traditionally treated with the use of remineralizing and antibacterial agents, such as topical fluoride, chlorhexidine gluconate, ozone therapy. However, the success of these treatment approaches is closely related to the patient's cooperation [3,4].

The resin infiltration treatment has been introduced as a minimally invasive technique for noncavitated carious lesions. The technique provides penetration of a low viscosity resin into the lesion with minimal removal of the hypermineralized surface layer [5,6]. The resin has a comperable refractive index (RI 1.51) to that of the sound enamel (RI 1.62); therefore, the resin infiltration of WSLs can also mask the whitish appearance [7].

Recently, it was questioned whether a resin infiltration treatment can also be applied prior to a composite resin restoration [8–10]. For minimally invasive treatment of initial enamel lesions with partially cavitated areas, both resin infiltration and restoration might be required. This application also may be required for the treatment of WSLs, which is frequently observed around the orthodontic brackets. In clinical circumstances of rebonding the brackets, it may be necessary to apply composite resin to the resin-infiltrated areas. In cases where aesthetic restoration is applied to the resin-infiltrated teeth, the bond strength of the composite resin will also be questioned.

The enamel etching protocol with 35-40% phosphoric acid (PA) before the bonding procedures is most commonly used today [11]. It has been reported in the literature that etching composite resin with 4-10% hydrofluoric acid (HFA) may be successful in repairing composite resins [12,13].

Erbium lasers also represent an alternative method for etch-

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ing. Given their nature of being well absorbed by hydroxyapatite, Erbium lasers can form microretentive surfaces for adhesive infiltration [14,15]. It also has been reported that the laser-etched enamel turns into a structure which exhibits more resistance to acid attacks [16,17].

The aim of this in vitro study is to assess the effect of three different etching methods, i.e., hydrofluoric acid, phosphoric acid and laser, on the SBS of resin-infiltrated sound and demineralized enamel. The null hypothesis of this in vitro study is that 1) application of the resin infiltrant does not improve the bond strength of sound and demineralized enamel, and 2) the SBS of resin infiltrant does not differ depending on different etching techniques.

Material and Methods

1. Specimen Preparation

The study protocol was designed and performed according to the principles of the Helsinki Declaration. In this research, 180 anonymous, extracted, permanent upper central incisor human teeth were used. After being separated from the root under water cooling, the crowns of teeth were encapsulated by acrylic resin (Meliodent, Heraeus Kulzer, Germany) with the buccal surfaces left exposed. The specimens were flattened and polished using 800, 1200, 2400-grit silicon carbide paper, respectively.

Experimental groups were determined according to the following criteria:

- 1. Demineralized enamel (D) / Sound enamel (S)
- 2. Resin infiltrant (ICON) application (I) / No resin infiltrant application (NI)
- 3. Different etching methods [Phosphoric acid (PA), Hydrofluoric acid (HFA), Laser (L)]

Figure 1 represents the flow chart of the experimental design. Materials used in this study are listed in Table 1.

For the demineralized enamel groups, 90 specimens were employed randomly. The enamel surfaces were coated with an acid-resistant nail varnish, leaving a window (4 mm × 4 mm) positioned in the incisal third of the teeth. To form artificial WSLs, specimens were immersed into demineralization solution (pH 4.8; 50 mM CH_3COOH, 5 mM NaN_3, 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 0.1 ppm NaF) at 37.5°C. Every day, the pH level was checked and calibrated with acetic acid if necessary. At the end of 15 days, a random sample was selected, and the nail varnish was removed. The white opaque lesion was detected by visual inspection.

2. Application of Resin Infiltrant Treatment

In this study, the resin infiltrant treatment was applied to 45 teeth of the artificially demineralized group and 45 teeth of the non-demineralized group. The specimens underwent resin infiltration following the guidelines provided by the manufacturer. Briefly, the enamel surface was treated with 15% hydrochloric acid gel (Icon-Etch, DMG, Hamburg, Germany) for 2 min, rinsed with water and air-dried for 30 s. The specimen was then desiccated using 99% ethanol (Icon-Dry, DMG, Hamburg, Germany) for 30 s followed by drying with air. The resin infiltrant (Icon-Infiltrant, DMG, Hamburg, Germany) was performed for 3 min, and light-cured for 40 s (Woodpecker, Type B, Curing Light, China). The resin infiltrant was re-applied for 1 min and light-cured for 40 s. Lastly, polishing discs (Sof-Lex, 3M ESPE, USA) were used to resin-infiltrated specimens while being continuously cooled with water.

Thermocycles were submitted to the specimens (5000 times, 5°C-55°C) (SD Mechatronik Thermocycler THE-1100, Germany) prior to bonding procedures.

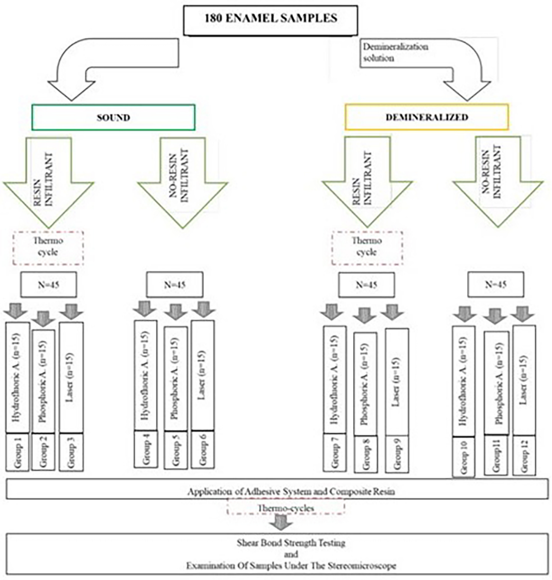

Figure 1. Experimental flow diagram

3. Etching Procedures and Composite Application

3.1. Etching procedures

The sound/demineralized and resin infiltrant application/no resin infiltrant treatment groups were randomly divided into three subgroups.

The etching groups are as follows:

- a. Hydrofluoric Acid (HFA)
- b. Phosphoric Acid (PA)
- c. Laser (L)

a. Hydrofluoric Acid Etching Group

Ultradent Porcelain Etch gel (Ultradent Products, Inc., South Jordan, UT, USA) containing 9% hydrofluoric acid (HF) was applied for 60 s, rinsed with water and dried by air syringe.

b. Phosphoric Acid Etching Group

Ultra-Etch (Ultradent Products, Inc., South Jordan, UT, USA) containing 35% phosphoric acid was used for 30 s. The enamel surfaces rinsed with water, and slightly air-dried.

c. Laser Etching Group

An Er,Cr:YSGG laser (Waterlase MD, Biolase Technology Inc., San Clemente, CA, USA) were treat the surfaces functioning at a wavelength of 2780 nm with a pulse of 140 µs. A sapphire tip was used as recommended by the manufacturer for etching MC6 (600 µm in diameter and 6 mm in length). A power setting of 2 W (80% air and 60% water) at 20 Hz was used, and enamel surfaces were subject to the laser for 10 s in a sweeping motion with $80 J/cm^2$ energy density, achieving an approximately 3 mm × 3 mm laser-etched enamel surface area.

Table 1. Materials used in the study

	Material	Company	Batch Number	Composition
Phosphoric Acid	Ultra-Etch	Ultradent Products, Inc., (South Jordan, UT, USA)	BDZPW	35% phosphoric acid
Hydrofluoric Acid	Porcelain Etch	Ultradent Products, Inc., (South Jordan, UT, USA)	1-800-552-5512	9% hydrofluoric acid
Adhesive System	AdperTM Single Bond 2 (pH= 4.3)	3M ESPE (St. Paul, MN, USA)	N811881	BisGMA, GDMA, UDMA, HEMA, nanofillers, water, ethanol, methacrylate functional copolymer of polyacrylic and polyitaconic acids
Resin Infiltrant	ICON	DMG (Hamburg, Germany)	758432	ICON Etch: 15% hydrochloric acid, pyrogenic silicic acid, surface active substance
				ICON-Dry: 99% ethanol
				ICON-Infiltrant: TEGDMA based resin matrix, initiators
Composite Resin	Filtek Z250	3M ESPE (St. Paul, MN, USA)	N729198	Organic Matrix Composition: Bis-GMA, Bis-EMA, UDMA, TEGDMA Inorganic Filler Particles: 60.0% (by volume) Zirconia and Silica particles (0.01-3.5 µm)

3.2. Application of composite resins and the thermal cycle

Immediately after the etching of each specimen, the Adper Single Bond 2 (3M ESPE, St. Paul, Minn., USA) etch & rinse adhesive system was applied according to the manufacturer's instructions. A microhybrid composite, (Filtek Z250,3M ESPE, St. Paul, Minn., USA) was placed to the enamel surface using a transparent plastic hollow cylinder (diameter:2 mm, length:2 mm) and light-cured for 20 s (Woodpecker, Type B, Curing Light, China).

After removal of the plastic molds, each sample group was kept in distilled water at room temperature for 24 h. Then, all samples were aged at 50°C and 550°C in a thermal cycling device (immersion time = 25 seconds, transfer time = 10 seconds) for 5000 cycles.

4. Shear bond strength test

The shear bond strength (SBS) was tested using the universal test machine (Lloyd Lrx ,Lloyd Instruments, USA). A shear force subjected to the adhesive interface (crosshead speed:1 mm/min. After applying the shear force, the load at fracture was recorded, and subsequently calculated in Megapascals (MPa).

5. Failure mode analysis

Failure types of the fractured specimens were determined with a stereomicroscope (Leica MZ 21, Leica Microsystems, Germany) under x20 magnification. Failure mode was considered as cohesive failure if at least part of either the enamel or composite, as adhesive failure if at the interface, and as mixed failure if it occurred both at the interface and composite.

6. Statistical analysis

The data were statistically evaluated by three-way ANOVA. Bonferroni test was used for multiple comparisons at the 5% significance level. Analysis of the data was performed using the IBM Statistical Package for the Social Sciences (SPSS 22 Software for Windows).

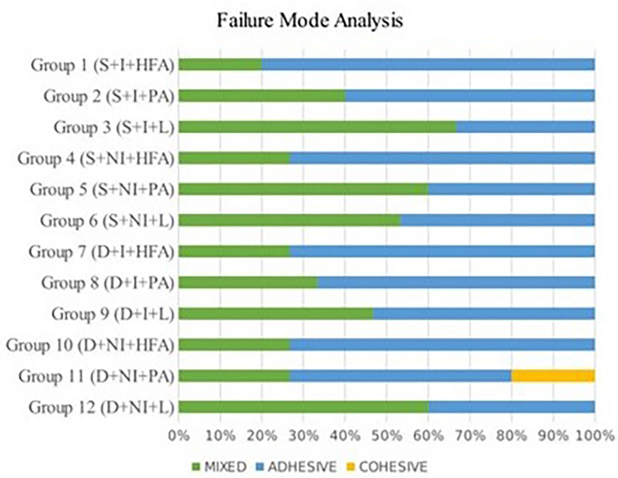

Figure 2. Failure Mode Analysis.

Results

The SBS of sound and demineralized enamel groups and the results of bilateral comparisons with the Bonferroni test are listed in Table 2 and Table 3 . Figure 2 presents the corresponding failure modes observed during the analysis.

In the comparisons of the three etching methods in the resin-infiltrated sound enamel groups, the following statistically significant differences were found ($p<0.05$): group 2 [resin-infiltrated sound enamel & etched with phosphoric acid (S+I+PA)]> group 3 [resin-infiltrated sound enamel & etched with laser (S+I+L)]> group 1 [resin-infiltrated sound enamel & etched with hydrofluoric acid (S+I+HFA)]. In the comparisons of the three etching methods on non-resin-infiltrated sound enamel groups, no statistically significant difference was found between group 4 (S+NI+HFA) and group 6 (S+NI+L) ($p>0.05$). The phosphoric acid etched, no resin-infiltrated sound enamel group 5 (S+NI+PA) showed statistically higher SBS than groups 4 and 6 ($p<0.05$).

The comparisons of the three etching methods on resin-infiltrated demineralized enamel groups results revealed no statistically significant difference between group 7 [resin-infiltrated demineralized enamel & etched with hydrofluoric acid (D+I+HFA)] and

Table 2. Mean values, standard deviations and p values of the tested groups

		SOUND ENAMEL	DEMINERALIZED ENAMEL	
		SBS and Standard Deviation (MPa)	SBS and Standard Deviation (MPa)	p
Resin Infiltrant	HFA	10.73a ± 3.73	19.11d ± 6.57	<0.001
	PA	2922b ± 5.39	30.07e ± 7.12	0.685
	L	22.60c ± 7.53	23.24d ± 8.44	0.765
	p	**<0.001**	**<0.001**	
No Resin Infiltrant	HFA	5.08f ± 2.03	13.93hi ± 4.88	<0.001
	PA	35.58g ±7.61	19.91jk ± 5.08	<0.001
	L	7.43f ±1.65	18.59hj ± 4.35	<0.001
	p	**<0.001**	**0.013**	

Table 3. Mean, standard deviations and p-values of the groups tested

		SOUND	DEMINERALIZED
		SBS and Standard Deviation (MPa)	**SBS and Standard Deviation (MPa)**
HFA	Resin Infiltrant	10.73 ± 3.73	19.11 ± 6.57
	No Infiltrant	5.08 ± 2.03	13,93 ± 4.88
	p	**0.008**	**0.015**
PA	Resin Infiltrant	29.22 ± 5.39	30.07 ± 7.12
	No Infiltrant	35.58 ± 7.61	19.91 ± 5.08
	p	**0.003**	**<0.001**
L	Resin Infiltrant	22.60 ± 7.53	23.24 ± 8.44
	No Infiltrant	7.43 ± 1.65	18.59 ± 4.35
	P	**<0.001**	**0.028**

group 9 [resin-infiltrated demineralized enamel & etched with laser (D+I+L)] (p>0.05). However, group 8 [resin-infiltrated demineralized enamel & etched with phosphoric acid (D+I+PA)] showed statistically higher SBS than group 7 (D+I+HFA) and group 9 (D+I+L) (p<0.05). In the comparisons of the three etching methods on non-resin-infiltrated demineralized enamel groups, no statistically significant difference was found between group 12 (D+NI+L) and group 10 (D+NI+HFA) (p>0.05). Also, there was no statistically significant difference between group 12 (D+NI+L) and group 11 (D+NI+PA) (p>0.05). However, group 11 (D+NI+PA) showed higher SBS than group 10 (D+NI+HFA) (p<0.05). In the comparisons of different resin-infiltrated enamel samples (demineralized/sound) with the same etching method, the results showed that the SBS of group 1 (S+I+HFA) was significantly lower than group 7 (D+I+HFA) (p <0.05). However, there was no statistically significant difference between group 2 (S+I+PA) and group 8 (D+I+PA). Also, no statistically significant difference was noted between group 3 (S+I+L) and group 9 (D+I+L) (p>0.05).

In the comparisons of different no resin-infiltrated enamel samples (demineralized/sound) with the same etching method, the results showed that the SBS of group 4 (S+NI+HFA) was significantly lower than group 10 (D+NI+HFA) (p <0.05). Group 5 (S+NI+PA) showed statistically higher SBS than group 11 (D+NI+PA) (p<0.05). Group 12 (D+NI+L) showed statistically higher SBS than group 6 (S+NI+L) (p<0.05). In the comparisons of resin infiltrant application (resin infiltrated/not resin infiltrated) on sound enamel samples with the same etching method, the results showed that the SBS of group 1 (S+I+HFA) was significantly higher than group 4 (S+NI+HFA) (p <0.05). The SBS of group 5 (S+NI+PA) was higher than group 2 (S+I+PA) (p<0.05). Group 3 (S+I+L) showed higher SBS than group 6 (S+NI+L) (p <0.05). In the comparisons of resin infiltrant application (resin infiltrated/not resin infiltrated) on demineralized enamel samples with the same etching method, the results showed that the SBS of group 7 (D+I+HFA) was statistically higher than group 10 (D+NI+HFA) (p <0.05). Group 8 (D+I+PA) showed higher SBS than group 11 (D+NI+PA) (p <0.05). Group 9 (D+I+L) showed higher SBS than group 12 (D+NI+L) (p <0.05).

Discussion

In this in-vitro study, the effect of three different etching methods on the SBS of composite resin to resin-infiltrated sound and demineralized enamel was evaluated. The results showed that the application of resin infiltrant on demineralized enamel improved the bond strength in all etching methods. However, resin infiltration application on sound enamel impaired the bond strength when a phosphoric acid etching technique was used. Regardless of the application of resin infiltrant, phosphoric acid etching in both sound and demineralized enamel revealed the highest bond strength compared with hydrofluoric acid etching and laser etching groups. Thus, the null hypothesis was partly rejected.

Placement of the composite material on the resin-infiltrated enamel surface might be considered similar to the composite-composite repair procedure. However, the resin infiltrant is different in terms mainly consisting of low-viscosity TEGDMA and no filler particle. Resin infiltrant forms a larger oxygen inhibition layer [18,19]. The oxygen inhibition layer at the appropriate thickness will increase the resin-to-resin bond [20,21]. In this study, however, surfaces were polished to simulate clinical application after resin infiltration, and samples were aged in a thermal cycling device. Thus, it is conceivable that the large oxygen inhibition layer of resin infiltrant may not affect the results of this research.

In this study, three different etching methods were evaluated. In the literature, phosphoric acid, hydrofluoric acid and Er,Cr:YSGG lasers have been tested in various composite-composite repair studies. In this study, three etching methods, 9% HF, 35% PA and Er,Cr:YSGG laser, were applied to different enamel samples (sound/demineralized and resin infiltrated/not resin infiltrated) and the effect on the SBS was evaluated. The results showed that among the resin-infiltrated sound and demineralized enamel groups, the phosphoric acid etched group revealed higher SBS compared with the HF and L groups. There are conflicting results in the literature about the success of phosphoric acid in composite resin repair [13,22–24]. In a study by Lucena-Martín et al., Various combinations of phosphoric acid, air abrasion, hydrofluoric acid and acetone were applied to the surfaces of two microhybrid composites. The study findings indicated that using phosphoric acid for surface treatment led to the lowest SBS [13]. On the other hand, in a recent systematic review conducted by Furtado and colleagues, it was concluded that, strategies aimed at enhancing the bond strength to aged composites may prioritize the utilization of air abrasion or diamond burs, in combination with surface etching using phosphoric acid followed by the application of an adhesive system [24].

Mirzaei et al. conducted a comparison study, evaluating the impact of bur treatment and different powers of Er,Cr:YSGG laser treatment on composite resin surfaces through scanning electron microscope (SEM) evaluation [25]. The group pretreated by bur exhibited a noticeable smear layer, while surfaces pretreated by Er,Cr:YSGG laser displayed irregular and microporous features without a smear layer. The surface prepared by an output power of 4 W, 5 W and 6 W showed more irregularity than that prepared with an output power of 1 W, 2 W and 3 W. In another study of composite surface treatment with an Er,Cr:YSGG laser, laser treatment with an output power of 2 W showed similar effects as conventional acid treatment [26]. In this study, the same laser device and the same etching parameters as that reported by Alagl et al. were used [27]. Alagl et al. showed that the phosphoric acid etching group yielded higher bond strength than the laser etching group [27]. Their result was consistent with our study. Among the resin-infiltrated sound enamel groups, the phosphoric acid etching subgroups revealed superior results compared with the laser and hydrofluoric acid etching groups.

Gupta et al. tested different etching methods on water-aged nanofilled composite resin (Filtek Z350) [12]. The repair procedure was completed with the same composite after a total etch adhesive application. Their results showed that the highest bond strength was obtained in the 10% hydrofluoric acid group, while the 37% phosphoric acid group showed the lowest bond strength. On the other hand, the results of our study agree with the study of Ahmadizenouz et al. Their findings showed the superiority of 35% phosphoric acid over Er:YAG laser or 10% hydrofluoric acid in the repair of thermocycled composite resins [28].

The relatively hypermineralized surface zone of WSLs hampers the diffusion of remineralization agents and resin bonding agents through the lesion body. Therefore, removal of the hypermineralized surface by acid etching is recommended [29]. In the literature, various etching methods have been studied prior to resin infiltration of WSLs [30,31]. Meyer-Lueckel et al. evaluated the effect of 37% phosphoric acid and 5% and 15% hydrochloric acid (30-120 s) on removing the hypermineralized surface layer of WSLs [32]. The results of their study demonstrated that 90-120 s application of 15% hydrochloric acid revealed significantly higher effectiveness than 30-120 s application of 37% phosphoric acid. Askar et al. claimed that after etching 5 s with 37% PA, the resin infiltrant revealed 75-88% penetration into the artificially demineralized bovine enamel [33]. However, the authors explained that their findings might be related to the notion that artificially demineralized bovine teeth have a thinner surface layer than natural WSLs of human enamel. In this study, chemical demineralization with acidic buffer solution was used to create WSLs, and this is a frequently used method for resin-infiltration treatment studies to form standard demineralized WSLs [9,34].

Veli et al. evaluated the effect of CPP-ACP, fluoride varnish, microabrasion and resin infiltration treatments of artificial WSLs on the SBS of composite resin [10]. Their results showed that the resin-infiltrated WSL group revealed similar bond strength values with a control group of sound enamel and higher SBS compared with other treatments and the untreated control group. In another study by Attin et al., the sound bovine enamel group showed the highest bond strength followed by the resin-infiltrated demineralized enamel group [35]. In our study, the phosphoric acid group results are in accordance with the results of Veli et al. and Attin et al. [10,35]. Our results showed that PA etched sound enamel revealed significantly higher bond strength than the PA etched demineralized enamel group. Resin infiltration treatment of demineralized enamel before PA etching increased the bond strength compared with the no resin infiltrant (NI) demineralized enamel group. On the other hand, among the no resin infiltrant groups, the HF or L etched demineralized groups revealed higher bond strength than the sound enamel groups. These results can be explained by the assumption that laser and hydrofluoric acid etching are not sufficiently effective on sound enamel but might partially remove the hypermineralized superficial layer of WSLs and allow for adhesive penetrations. In previous studies on laser etching of WSLs, it was found that laser etching before fluoride application increased the fluoride uptake of demineralized enamel [36,37]. As far as the authors are aware, no prior study in the literature has explored the impact of laser etching or hydrofluoric acid etching prior to adhesive application on WSLs.

Wiegand et al. demonstrated that resin infiltration treatment on sound enamel prior to adhesive application did not result in significant differences but increased the bond strength of demineralized enamel [9]. In a study by Jia et al., sound and artificially demineralized bovine enamel groups were divided into 7 subgroups and treated with resin infiltrant, three different commercial adhesives or a combination of resin infiltrant and the adhesives [8]. After subjecting the nanohybrid composite to thermocycling, the SBS was assessed. The study revealed that the SBS of the resin infiltrant did not show significant differences compared to etch&rinse adhesives on both sound and demineralized surfaces. Furthermore, cohesive failures were more commonly observed on demineralized surfaces, similar to the outcomes of our study. According to Sideridou et al., TEGDMA shows the highest water absorption followed by Bis-GMA and Urethan dimethacrylate (UDMA) [38]. ICON is composed mainly of TEGDMA [7]. Resin-infiltrated lesions might reveal heterogeneity in terms of oxygen inhibition and polymerization shrinkage [6]. Therefore, bond strength might decrease with resin infiltrant aging.

Conclusion

Considering the limitations of this in vitro study, the following conclusions may be inferred: -Phosphoric acid, which is conventionally used in enamel etching procedures, showed superior results in both sound and demineralized enamel regardless of resin infiltration treatment. However, in some groups, the difference was not statistically significant. -Regardless of the etching method used, resin infiltrant application in demineralized enamel increased the bond strength of composite resin.

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Author Contributions

Conceptualization and Design, D.T., Z.C.Ö.; Literature Review, D.T., Z.C.Ö., Methodology and Validation, D.T., Z.C.Ö., Formal Analysis, D.T., Z.C.Ö., Investigation and Data Collection: Z.C.Ö.; Resources, D.T., Z.C.Ö.; Data Analysis and Interpretation, D.T., Z.C.Ö.; Writing – Original Draft Preparation, D.T., Z.C.Ö., Writing – Review & Editing, D.T., Z.C.Ö.; Supervision, D.T.; Project Administration, D.T.; Funding Acquisition, D.T.

Conflict of Interest

The authors do not have any financial interest in the companies whose materials are included in this article. The authors confirm that there are no conflicts of interest and there has been no financial support for this work that could have influenced its outcome.

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