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In Vitro Comparison of the Effectiveness of a Resin Infiltration System and a Dental Adhesive System in Dentinal Tubule Penetration

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ABSTRACT

Objective: The aim of this study was to assess and compare the dentin tubule penetration effectiveness of a dental adhesive and a resin infiltration system used with two different surface treatments.

Methods: Dentin specimens were obtained from 42 impacted lower right wisdom tooth, 2 of these specimens were used to detect the effects of surface treatments. Two different surface treatments (37% phosphoric acid and 17% EDTA) were applied to the samples to compare the dentin tubule penetration effectiveness of a dental adhesive –which had been using for treatment of dentin hypersensitivity - and a resin infiltration system. Scanning electron microscopy was used to investigate the tubule penetration effectiveness. For statistical analysis, Kruskal Wallis and Mann Whitney U and One Way Analysis of Variance (ANOVA) and Tukey HSD tests were used.

Results: ICON had shown significantly more resin penetration intensity and more resin penetration depth than Adper Single Bond 2 ($p<0.05$). Phosphoric acid treatment groups had shown significantly more penetration intensity than EDTA treated groups ($p=0.001$).

Conclusion: According to the results of this study, it can be concluded that tubuler penetration effectivenes of ICON resin infiltration system is better than Adper Single Bond 2 adhesive system.

Keywords: Dentin sensitivity, resins, adhesives, microscopy

1. INTRODUCTION

Dentin hypersensitivity is defined as a “short, sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic or chemical stimuli” (1-5). For dentin sensitivity to develop, the dentinal tubules leading from the dentin surface to the pulp must be open (2).

A number of theories have been used to explain dentinal hypersensitivity. The most widely accepted mechanism is described by the “Hydrodynamic theory,” proposed by Branstrom and Astron in 1964 (5-7).

Two basic approaches are used to treat dentin hypersensitivity. The first is to occlude dentinal tubules, preventing the disturbance of hydrodynamic fluid and blocking neural transmission in the pulp (8, 9). This approach involves filling the dentinal tubules or forming a precipitate on their surfaces (10). Because the agents used to treat sensitivity generate a superficial precipitate on the tubules’ surface, no single desensitizing agent is considered ideal for managing dentin hypersensitivity (5, 10-13).

Infiltration resins are generally recently developed materials that are used to treat early enamel lesions (caries) and white spot caries-like lesions. These materials can effectively penetrate the enamel (14, 15). The purpose of this study was to investigate the effectiveness of resin infiltration in occluding tubules and treating dentin hypersensitivity by assessing the penetration of resin into dentinal tubules.

In this study, scanning electron microscopy (SEM) was used to compare the dentinal tubule penetration of a dentin hypersensitivity dental adhesive treatment with that of a resin infiltration system. These treatments were combined with two dentin surface pre-treatments, 37% phosphoric acid and 17% ethylenediaminetetraacetic acid (EDTA).

Two null hypotheses were tested. The first null hypothesis was that the surface pre-treatments would have no effect on resin penetration. The second null hypothesis was that triethyleneglycol dimethacrylate (TEGDMA) containing the resin infiltration system and bisphenylglycidyl dimethacrylate (BisGMA) containing the adhesive system would show similar levels of penetration.

2. METHODS

This study used 42 impacted caries-free human third molar teeth and a protocol approved by the ethics committee with the No. 36290600/03. The teeth were obtained from

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individuals aged 23–30 years. Teeth that had been cracked or damaged during extraction were excluded. Only teeth extracted within 1 month prior to the study were used and these were stored in distilled water. A microtome was used to cut the teeth at their roots 3 mm below the enamel–cement junction. The pulp was removed using an excavator. The buccal enamel layer, cement, and superficial dentin were removed using a drill, and the prepared surfaces were polished using 320, 400, 600, 800, and 1000 grit abrasives, all while cooling the samples with water. The cervical dentin surfaces were examined and the samples stored in distilled water before the treatments were performed. The samples were dried with a gentle stream of air and randomly divided into two groups, each of which was then divided into two subgroups (n = 10). To visualize the tubular openings, a dentin sample from each subgroup was treated with 17% EDTA for 1 min and 37% phosphoric acid for 15 s and examined using SEM. The materials used in the study are listed in Table 1.

Table 1. *The materials and product details used in the study.*

Material (Manufacturer)	Application Technique	Composition
ICON Dry (DMG, Hamburg, Germany)	Apply the etched enamel surface and set for 30 seconds. Dry with oil-free and water-free air.	%99 Ethanol
ICON Infiltrant (DMG, Hamburg, Germany)	Apply an ample amount of Icon-Infiltrant onto the etched surface by turning the shaft and set for 3 minutes. Remove excess material with a cotton roll and dental floss. Light-cure Icon-Infiltrant for 40 seconds. Repeat the application and set for 1 minute. Remove excess material and light-cure for a minimum of 40 seconds.	TEGDMA-based resin, initiators and stabilizers
Adper Single Bond 2 (3M ESPE, Germany)	3. f or 10 sec onds Apply etchant for 15 seconds. Rinse for 10 seconds. Blot excess water using a cotton pellet or mini-sponge. After blotting, apply 2-3 consecutive coats of adhesive for 15 seconds with gentle agitation using a fully saturated applicator. Gently air thin for 5 seconds to evaporate solvents. Light-cure for 10 seconds.	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly (itaconic) acids, silica particles
Panora 200 Phosphoric Acid (Imıcryl, Konya, Turkey)	Apply dentine surface and set for 15 seconds. Dry with oil-free and water-free air for 10 seconds.	37% Phosphoric Acid
EDTA Solution (Werax, Turkey)		17% Ethylene diamide tetra acetic acid, sodyum hydroxide, distile water

Group 1a samples were treated using 37% phosphoric acid plus Adper Single Bond 2 (3M, Neuss, Germany). The vestibular surfaces of the samples were treated with 37% phosphoric acid to remove the smear layer. Following rinsing and air-drying, Adper Single Bond 2 was applied and the samples were light-cured for 20 s, in accordance with the manufacturer's instructions.

Group 1b samples were treated using 17% EDTA plus Adper Single Bond 2. The vestibular surfaces of the samples were treated with 17% EDTA for 60 s to remove the smear layer. Following rinsing and air drying, Adper Single Bond 2 was applied and the samples were light-cured for 20 s, in accordance with the manufacturer's instructions.

Group 2a samples were treated using 37% phosphoric acid plus Icon (DMG, Hamburg, Germany). The sample surfaces were treated with 37% phosphoric acid for 15 s. The samples were then treated using Icon Dry and allowed to stand for 30 s, prior to air-drying for 5 s. The resin was applied using a circular motion to the sample surfaces for a duration of 3 min. A gentle stream of air was applied for 5 s and the samples were light-cured for 40 s. The resin was applied again for 1 min and the samples were light-cured for 40 s.

Group 2b samples were treated using 17% EDTA plus Icon. The vestibular surfaces of the samples were treated with 17% EDTA for 60 s to remove the smear layer. Icon resin was then applied using the procedure described for group 2a samples.

All of the prepared samples were incubated in distilled water for 24 h at 37°C.

The samples were sectioned longitudinally, and each cross-section surface was treated with 37% phosphoric acid for 5 s to remove the smear layer that had formed during sectioning. Samples were then treated with 5.25% NaOCl for 3 min to remove all organic content. All samples were rinsed with distilled water for 1 min, desiccated for 24 h, and sputter-coated with gold for visualization using SEM.

A total of 80 sample surfaces were initially evaluated under low magnification. For each sample, the cervical region closest to the pulp was photographed at 700× magnification, including the treated surface. The resin density was rated by two observers who had been blinded to the treatments. The scoring system (0-3) used was described by Moradi et al. (16, 17), and the scores are defined below:

0 = Resin was not observed in any of the tubules examined.

1 = Resin was observed in less than half of the tubules examined.

2 = Resin was observed in more than half of the tubules examined.

3 = Resin was observed in all of the tubules examined.

The first eight images (10% of a total of 80 images) were evaluated together as part of the calibration process. The remaining images were evaluated independently. Cohen's kappa coefficient was used to assess inter-rater agreement (18). The scores assigned to the images viewed during the calibration process were not included in the kappa analysis. A consensus was reached by discussion for those images that had no inter-rater agreement. The scores determined by

consensus were used in the statistical analyses. The greatest depth of resin penetration was measured in the region closest to the pulp in each image using the SEM device software. The scores and penetration depths were evaluated statistically.

2.1.Statistical analysis

The data were evaluated using SPSS (ver. 21.0; SPSS, Inc., Chicago, IL, USA). Differences in penetration densities among the groups were assessed using a Kruskal–Wallis test, and inter-group comparisons were made using a Mann–Whitney U-test. As Shapiro-Wilk test showed dependent variables were normally distributed (Table 2), one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test were used to compare penetration depths. To analyze inter-group differences, 95% confidence intervals were calculated. A p-value < 0.05 was considered statistically significant.

Table 2. *Tests of normality for penetration depth values*

Tests of Normality[b]							
	Groups	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Penetration depth	Phosphoric Acid +Adhesive	,173	20	,118	,913	20	,072
	Phosphoric Acid +ICON	,124	20	,200*	,950	20	,369
	EDTA+ICON	,128	20	,200*	,907	20	,055

*. This is a lower bound of the true significance, a. Lilliefors Significance Correction, b. Penetration depth is constant when Group = EDTA+Adhesive. It has been omitted.

3. RESULTS

The effects of the surface pre-treatments on the smear layer were evaluated in samples that were not included in the study groups. There were more open dentinal tubules in samples treated with phosphoric acid than in those treated with EDTA, as shown in Figures 1 and 2, respectively.

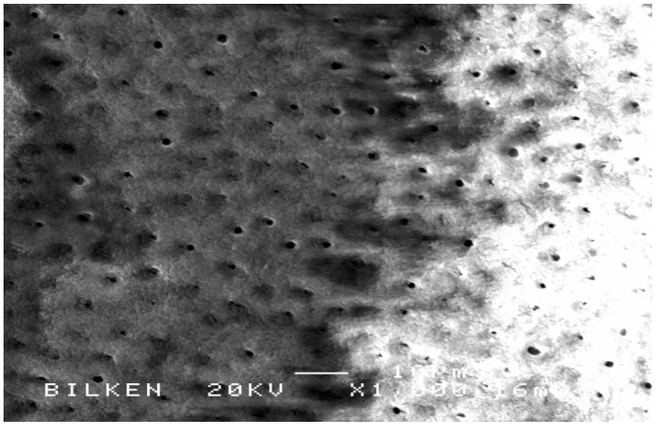

Figure 1. *Image of dentin surface treated with 37% phosphoric acid*

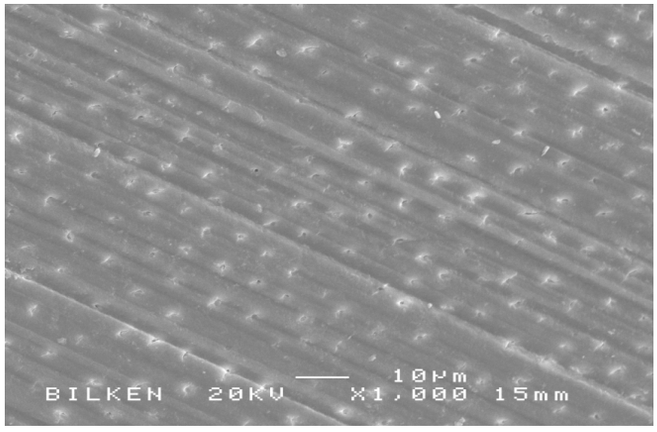

Figure 2. *Image of dentin surface treated with 17% ethylenediaminetetraacetic acid (EDTA)*

The kappa value for inter-rater agreement of 0.79 indicated strong agreement. Surface images at 700× magnification and penetration depth measurements from one sample in each group are shown in Figures 3-9. We did not find resin in any of the EDTA plus adhesive group sample images.

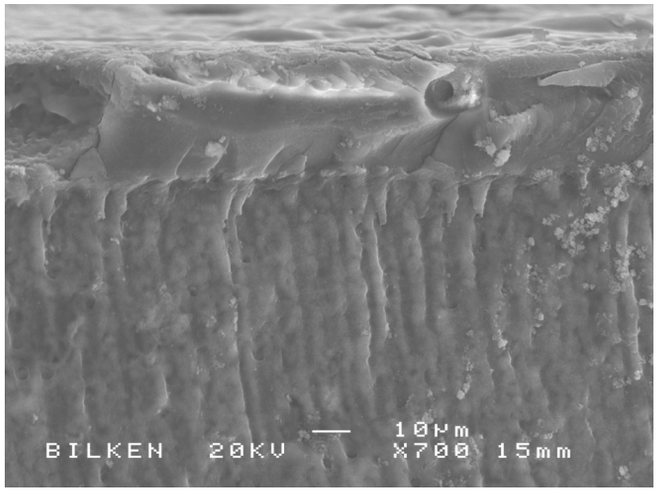

Figure 3. *Scanning electron microscopy (SEM) image of a group 1a (phosphoric acid plus adhesive) sample at 700× magnification*

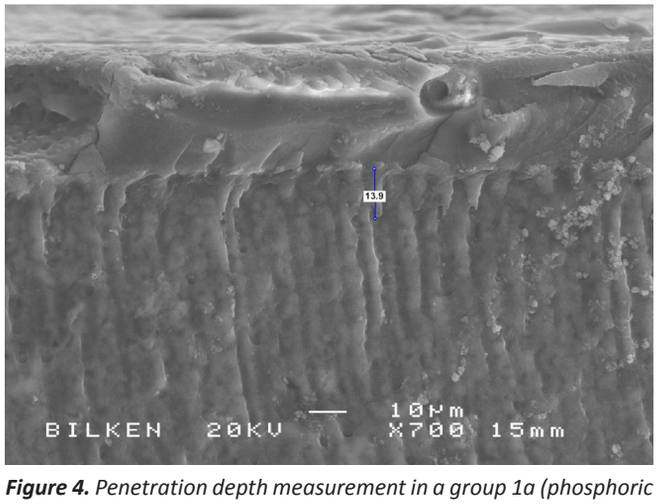

Figure 4. *Penetration depth measurement in a group 1a (phosphoric acid plus adhesive) sample*

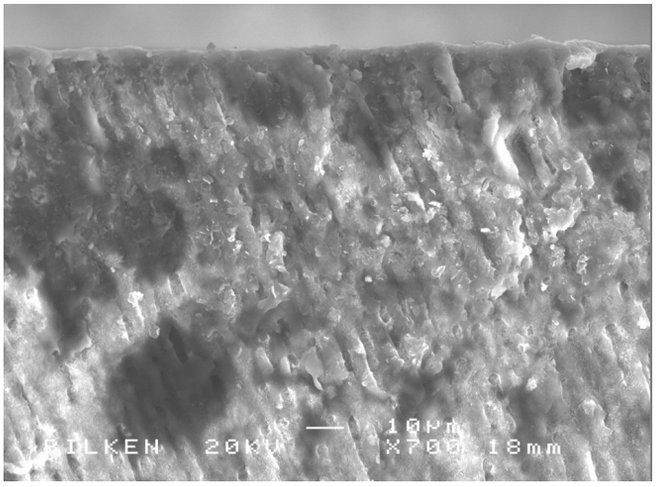

Figure 5. *SEM image of a group 1b (EDTA plus adhesive) sample at 700× magnification*

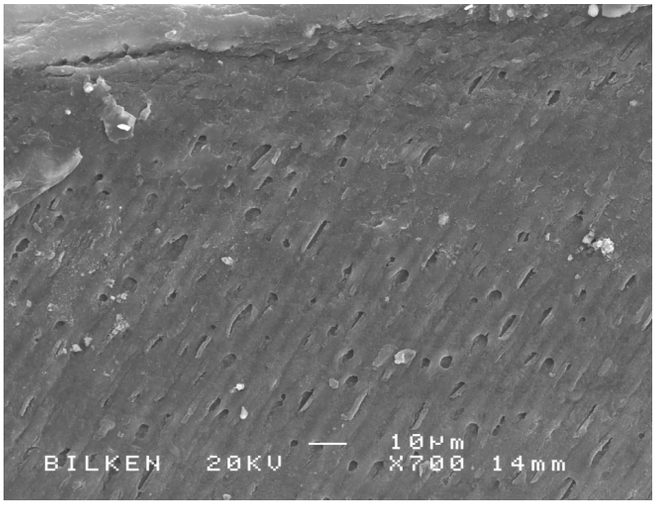

Figure 6. *SEM image of a group 2a (phosphoric acid plus Icon) sample at 700× magnification*

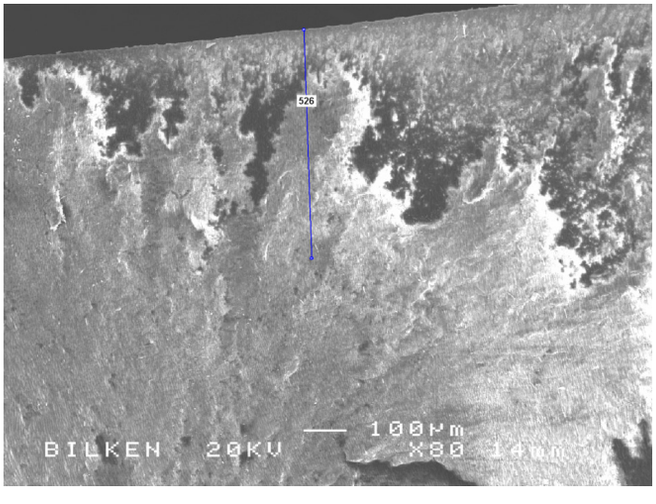

Figure 7. *Penetration depth measurement in a group 2a (phosphoric acid plus Icon) sample*

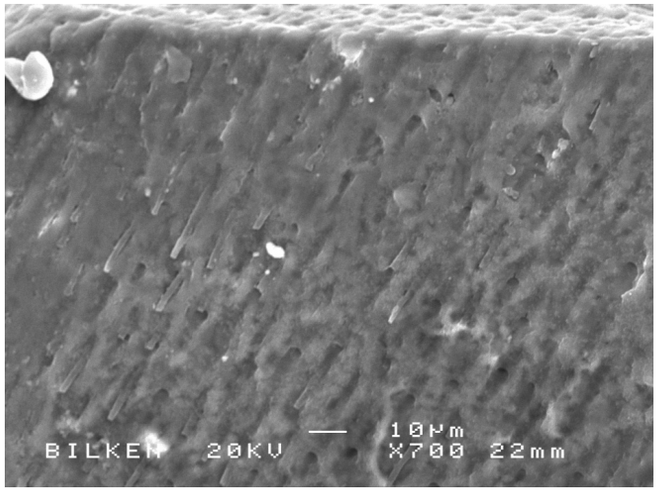

Figure 8. *SEM image of a group 2b (EDTA plus Icon) sample at 700× magnification*

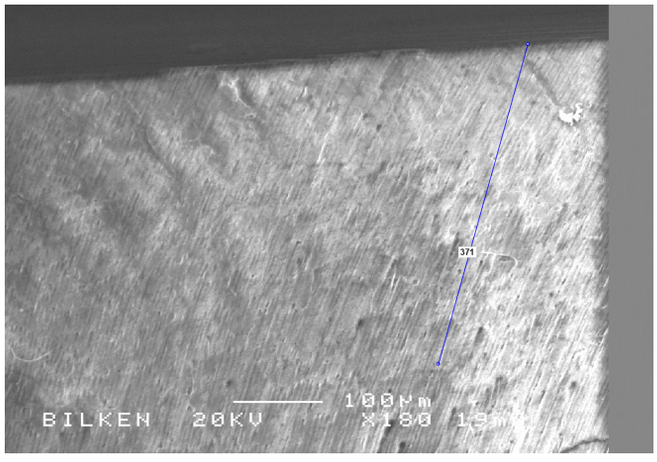

Figure 9. *Penetration depth measurement in a group 2b (EDTA plus Icon) sample*

Comparing the penetration densities using a Kruskal–Wallis test demonstrated significant differences among the study groups (p=0.000). There was a significant difference in penetration density between the phosphoric acid and EDTA surface pre-treatment groups (p=0.001). Additionally, the Icon group demonstrated significantly more penetration density than did the adhesive group (p=0.000). (Table 3, Table 4)

Table 3. *Mean ranks of study groups for penetration density*

Groups	N	Mean Rank
Group 1a (Phosphoric Acid+Adhesive)	20	35,60
Group 1b (EDTA+Adhesive)	20	12,50
Grup 2a (Phosphoric Acid+ICON)	20	61,75
Grup 2b (EDTA+ICON)	20	52,15

Table 4. *Test statistics of Kruskal Wallis and Mann Whitney U for penetration density*

Variable	Penetration Density	
Kruskal Wallis	1a, 1b, 2a, 2b*	Chi-square= 55,325 df=3 Asymp. Sig.= ,000
Mann Whitney U	Phosphoric acid vs EDTA	U=473,000 Z=-3.256 Asymp. Sig.= ,001
Mann Whitney U	Adhesive vs ICON	U=142,000 Z= – 6,551 Asymp. Sig.= ,000
Mann Whitney U	1a vs 1b*	U=40,000 Z=-4,954 Asymp. Sig.= ,000
	1a vs 2a*	U=45,000 Z=-4,377 Asymp. Sig.= ,000
	1a vs 2b*	U=97,000 Z= – 2,947 Asymp. Sig.= ,003
	1b vs 2a*	U= ,000 Z=-5,888 Asymp. Sig.= ,000
	1b vs 2b*	U= ,000 Z= – 5,831 Asymp. Sig.= ,000
	2a vs 2b*	U= 130,000 Z= – 2,063 Asymp. Sig.= ,039

**Statistically significant difference between groups*

Multiple comparisons using the Mann–Whitney U-test demonstrated significant differences between penetration densities of groups 1a and 1b, groups 1a and 2a, groups 1a and 2b, groups 1b and 2a, groups 1b and 2b, and groups 2a and 2b (p=0.000, p=0.000, p=0.003, p=0.000, p=0.000, and p=0.039, respectively). (Table 4)

The mean and standard deviation for penetration depts of each group is listed in Table 5. The results of the multiple inter-group penetration depth comparisons are shown in Table 6. Group 2a samples had the deepest level of resin penetration, followed by group 2b, group 1a, and group 1b, in decreasing order.

Table 5. *Mean and standard deviation values of penetration depths*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Group 1a (Phosphoric Acid+Adhesive)	20	15,685	10,9048	2,4384	,0	42,3
Group 1b (EDTA+Adhesive)	20	,000	,0000	,0000	,0	,0
Grup 2a (Phosphoric Acid+ICON)	20	818,950	396,8596	88,7405	201,0	1461,0
Grup 2b (EDTA+ICON)	20	621,750	294,6923	65,8952	269,0	1384,0
Total	80	364,096	438,4873	49,0244	,0	1461,0

**The mean difference is significant at the 0.05 level.*

Table 6. *Tukey's honest significant difference results for the multiple inter-group comparisons among the penetration depth results*

	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
Phosphoric Acid+Adhesive	EDTA+Adhesive	15,68500	78,17608	,997
	Phosphoric Acid +ICON	-808,26500*	78,17608	,000
	EDTA+ICON	606,06500*	78,17608	,000
EDTA+Adhesive	Phosphoric Acid +Adhesive	-15,68500	78,17608	,997
	Phosphoric Acid +ICON	818,95000*	78,17608	,000
	EDTA+ICON	-621,75000*	78,17608	,000
Phosphoric acid+ICON	Phosphoric Acid +Adhesive	803,26500*	78,17608	,000
	EDTA+Adhesive	818,95000*	78,17608	,000
	EDTA+ICON	197,20000	78,17608	,064
EDTA+ICON	Phosphoric Acid +Adhesive	606,06500*	78,17608	,000
	EDTA+Adhesive	621,75000*	78,17608	,000
	Phosphoric Acid +ICON	-197,20000	78,17608	,064

**The mean difference is significant at the 0.05 level.*

Tukey's HSD test showed there was no significant difference between the penetration depth values in the phosphoric acid plus adhesive group and the EDTA plus adhesive group samples (p=0.997). Similarly, there was no significant difference between the penetration depth values in the phosphoric acid plus Icon group and the EDTA plus Icon group samples (p=0.064). There were statistically significant differences among all other groups (p<0.05).

When all the surface treatments were compared, the samples in groups pre-treated with phosphoric acid showed deeper penetration, but the differences between these samples and those in the groups pre-treated with EDTA were not statistically significant (p=0.280).

The Icon group samples showed a significantly deeper level of penetration than the adhesive group samples (p=0.000).

4. DISCUSSION

In this study, the penetration of a highly effective enamel infiltration resin and an adhesive system used in sensitivity treatment were compared using different surface pre-treatment procedures (14, 15). The Icon manufacturer's instructions recommend removing the hyper-mineralized layer on the surface of the tooth enamel with HCl. However, due to the differences in the enamel and dentin mineral content, the cellular structure of dentin, the risk of pulpal inflammation, and the lack of reports in the literature describing HCl application to the surface of dentin at different concentrations and for different durations, we did not use HCl. Instead, we used phosphoric acid and EDTA for dentin surface pre-treatments.

EDTA is usually applied clinically at a concentration of 15–17% and can remove the smear layer in less than 1 min (19).

We looked at the smear removal procedures used in similar studies and decided to apply 17% EDTA solution to the dentin surface for 1 min in our study (20).

Ersöz and Özyurt described how 37% phosphoric acid removed tubular plugs and peritubular dentin in addition to removing the smear layer on the surface of dentin; the openings became significantly wider and funnel-shaped after they were emptied (21). In our study, the samples pre-treated with phosphoric acid showed more extensive and deeper resin penetration. SEM images showed that the application of 17% EDTA for 1 min did not widen the tubules sufficiently to allow resin infiltration. These results demonstrate that at the concentrations and durations used in this study, phosphoric acid is more effective than EDTA for removing the superficial smear layer. Therefore, the first null hypothesis, which states that the different surface pre-treatments would not affect resin penetration can be rejected.

Sauro et al. investigated the application of similar experimental adhesives to dentin samples pre-treated with either 5% EDTA or phosphoric acid and found that the resins infiltrated a smaller area in EDTA-treated samples compared with those treated using phosphoric acid (22).

To optimize the penetration of hydrophobic monomers (e.g., BisGMA), the collagen matrix in the demineralized dentin may be treated with ethanol rather than water. This is the basis of the ethanol-wet bonding technique (23). As a result, the acidified collagen matrix may be less hydrophilic and phase separation of hydrophobic monomers may be prevented (24). Following the surface pre-treatment procedures, we applied a primer containing 99% ethanol to the surfaces of samples to be treated with Icon, in accordance with the manufacturer's instructions. It is likely that this ethanol-wet bonding step used in our study enhanced resin penetration in the samples treated with Icon.

In their sensitivity treatment study, Ünlü and Bala concluded that the inclusion of both water and ethanol as solvents in Single Bond enhanced the material's properties (25). They also said that the presence of hydroxyethyl methacrylate (HEMA) may have meant that the dentinal tubules were blocked more effectively by the Single Bond reagent. However, Ünlü and Bala also described how many of their patients' tooth-sensitivity problems recurred (25). This suggests that adhesives may not be ideal for long-term sensitivity treatment. It is likely that this long-term failure in sensitivity treatment is due to superficial blocking of the dentinal tubules that is subsequently reversed by brushing or dietary acid (5, 11-13). Therefore, although ethanol and HEMA may be effective in treating short-term dentin sensitivity, they are not sufficient for successful treatment in the long term. The depth of resin penetration may be insufficient.

The BisGMA monomer is one of the main monomers used in adhesive dentistry and is highly viscous (26). The low-viscosity reagent TEGDMA is added to dilute viscous resins, enhancing their infiltration capacity (27, 28). In this study, we compared the penetration of an adhesive containing BisGMA with Icon containing TEGDMA. Statistical analysis showed there were significant differences among all groups ($p<0.05$). Resin penetration was most effective in the phosphoric acid plus Icon group samples, probably due to the highly effective penetration properties of TEGDMA. This was followed by the EDTA plus Icon group samples, the phosphoric acid plus adhesive group, and the EDTA plus adhesive group samples, all in decreasing order of penetration. We found that the Icon resin infiltrated samples in both surface pre-treatment groups more effectively than did the Adper Single Bond 2 reagent. This is probably because Icon contains TEGDMA, which has a higher penetration coefficient than the combination of HEMA and ethanol present in Adper Single Bond 2. Penetration depth of resin may also be affected by viscosity of the materials used. Adper Single Bond 2, is a filled adhesive resin with low viscosity. The size of the fillers are approximately 5 nm (29) but it was proved that these small nanofillers could not penetrate into the interfibrillar space of 20 nm to form the hybrid layer (30, 31). In an in vitro study, Araújo et al. revealed that addition of hydrophobic monomers and solvents (mainly ethanol) into TEGMA blends resulted in decreased penetration depth (32). Altohugh all materials used in this study are manufacturing as low viscosity materials, our results could be attributed to their different monomer and solvent compositions.

The resin penetration depth measurements demonstrated that the phosphoric acid plus Icon group samples showed the deepest penetration, followed by the EDTA plus Icon, and phosphoric acid plus adhesive group samples, in decreasing order. No resin penetration was found in the EDTA plus adhesive group samples. Therefore, the second null hypothesis, which states that Icon and Adper Single Bond 2 would show similar levels of dentin penetration was also rejected.

Griffiths et al. investigated adhesives containing different monomers on dentin pre-treated with phosphoric acid and found that the resin penetrated deeper when the adhesive contained TEGDMA compared with other adhesives (33). We demonstrated that Icon containing TEGDMA penetrated dentin deeper and more effectively than did Adper Single Bond 2 containing BisGMA and HEMA, in samples that had been pre-treated with phosphoric acid.

TEGDMA has a low degree of monomer conversion and when it penetrates parts of the tooth close to the pulp there is a risk of adverse outcomes, including pulpal inflammation and necrosis (28). TEGDMA is typically applied to the enamel surface; toxicity studies will be required to evaluate its safety for use in the cervical region or near the pulp. In addition to assessing the depths of penetration investigated here, more comprehensive studies should also be performed to investigate how effectively these reagents block dentinal tubules. The limitation of this study was to evaluate the penetration with a 2D SEM image. It could be insufficiant to give precise results and further studies are needed to evalute the penetration in 3D manner.

5. CONCLUSION

Within the limitations of this *in vitro* study we concluded the following. For removing the superficial smear layer, treatment with 37% phosphoric acid for 15 s is more effective than treatment with 17% EDTA for 1 min. The Icon resin infiltration system penetrates dentinal tubules more effectively than does the adhesive system tested. Treatment of dentinal hypersensitivity is an area of active research and more *in vitro*, *in vivo*, and clinical follow-up studies will be required to determine the ideal treatment materials and methods.

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