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Original Research Article

Effect of Different MMP Inhibitors on the Bond Strength and Durability of an Etch-and-rinse and a Self-etch Adhesive

Farklı MMP İnhibitörlerinin Etch-and-rinse ve Self-etch Adezivin Bağlanma Kuvveti ve Dayanıklılığı Üzerine Etkisi

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ABSTRACT

Aim: To compare the effect of different matrix metalloproteinase (MMP) inhibitors on the microtensile bond strength (μTBS) of an etch-and-rinse adhesive and a self-etch adhesive at baseline and after aging.

Materials and Method: Flat dentin surfaces were prepared from 96 extracted, sound human molars. Teeth were randomly divided into two main groups according to the adhesives (Adper Single Bond 2, and Clearfil 3S Plus) used (n=48). Each group was divided into four subgroups according to the dentin surface treatments; untreated (control), pretreated with 2% chlorhexidine digluconate (CHX) before adhesive application, pretreated with 1% benzalkonium chloride (BAC) before adhesive application, and pretreated with 2% hesperidin (HPN) before adhesive application (n=12). Composite resin was then placed on the prepared surfaces and polymerized. The specimens were cut to obtain dentin beams and randomly divided into two groups for microtensile analysis at baseline and after 6 months of aging. Failure types were observed after microtensile tests and data were analyzed by Two-way ANOVA, Tukey HSD, and Chi-Square tests.

Results: Compared with control, all MMP inhibitors significantly preserved and improved the bond strength of both adhesives at baseline and after 6 months of aging ($p < 0.05$). When considering the comparison of MMP inhibitors, the bond strengths of both adhesives in the HPN and BAC groups were significantly higher than the CHX group after aging ($p < 0.05$). The failure types were mostly adhesive and mixed.

Conclusion: The use of 2% HPN and 1% BAC as a separate step before the adhesive application can be recommended to preserve the longevity of resin-dentin bond strength on etch-and-rinse and self-etch adhesives.

Keywords: Adhesives; Benzalkonium chloride; Chlorhexidine digluconate; Hesperidin.

ÖZET

Amaç: Farklı matriks metalloproteinaz (MMP) inhibitörlerinin, başlangıç ve yaşlandırma sonrasında bir pürüzlendirmeli ve yıkamalı adeziv ile bir kendinden pürüzlendirmeli adezivin mikrogerilim bağlanma dayanımı (μTBS) üzerindeki etkisini karşılaştırmak.

Gereç ve Yöntem: 96 adet çekilmiş sağlam insan azı dişinden düz dentin yüzeyleri hazırlandı. Dişler kullanılan adezivlere (Adper Single Bond 2 ve Clearfil 3S Plus) göre rastgele iki ana gruba ayrıldı (n=48). Her grup dentin yüzey işlemlerine göre dört alt gruba ayrıldı; işlem uygulanmayan (kontrol), adeziv uygulanmadan önce %2 klorheksidin diglukonat uygulaması (CHX), adeziv uygulanmadan önce %1 benzalkonyum klorür (BAC) uygulaması ve adeziv uygulanmadan önce %2 hesperidin (HPN) uygulaması (n=12). Daha sona hazırlanan yüzeylere kompozit rezin yerleştirilip polymerize edildi. Örnekler, dentin çubukları elde etmek için kesildi, başlangıç ve 6 aylık yaşlandırmadan sonra mikrotensil analizi için rastgele iki gruba ayrıldı. Mikrogerilim testleri sonrasında kırık tipleri gözlemlendi ve veriler Two-way ANOVA, Tukey HSD ve Ki-Kare testleri ile analiz edildi.

Bulgular: Kontrol ile karşılaştırıldığında, tüm MMP inhibitörleri başlangıçta ve 6 aylık yaşlandırmadan sonra her iki adezivin bağlanma kuvvetini önemli ölçüde korumuş ve iyileştirmiştir ($p < 0.05$). MMP inhibitörlerinin karşılaştırılması göz önüne alındığında, HPN ve BAC gruplarındaki her iki adezivin bağlanma kuvvetleri, yaşlandırma sonrasında CHX grubundan önemli ölçüde daha yüksekti ($p < 0.05$). Başarısızlık türleri çoğunlukla adeziv ve karışık olarak görüldü.

Sonuç: Pürüzlendirmeli ve yıkamalı, kendinden pürüzlendirmeli adezivlerde rezin-dentin bağlanma kuvvetinin uzun ömürlülüğünü korumak için adeziv uygulamasından önce ayrı bir adım olarak %2 HPN ve %1 BAC kullanımı önerilebilir.

Anahtar Kelimeler: Adezivler; Benzalkonyum klorür; Klorheksidin diglukonat; Hesperidin.

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INTRODUCTION

The purpose of placing an adhesive restoration is to provide a tight adaptation between the restorative material and the dental substrate.[1] When etching with phosphoric acid is used, adhesion to enamel is reliable. However, adhesion to dentin is extremely difficult due to its moist and organic structure. The adhesion between resin and dentin interface can degrade and bond strength values decrease over time.[2] The activation of endogenous collagenolytic enzymes is one of the factors that affects the durability of resin-dentin bonds. These enzymes, called matrix metalloproteinase (MMP) and cysteine cathepsin, are found in odontoblasts, mineralized and demineralized dentin tissue, and pulp.[3]

In previous studies, various enzyme inhibitors and collagen cross-linkers have been used to prevent this degradation over time and stabilize bonding. One of the most known inhibitors is an antimicrobial agent called chlorhexidine digluconate (CHX), which is reported to have the ability to inhibit the activation of MMP-2, 8, 9 and cysteine cathepsins over time.[4,5]

Quaternary ammonium compounds (QAC) are cationic structures with antimicrobial properties such as CHX, thus they have the same anti-MMP properties as CHX. Benzalkonium chloride (BAC), a cationic surface agent of QAC, is used in dentistry as a cavity disinfectant, desensitizer, and endodontic irrigation slotution. Studies have shown that benzalkonium chloride (BAC) significantly inhibits MMP-2, MMP-8, and MMP-9.[6,7]

Flavonoids are polyphenolic compounds found in plant extracts. More than 4,000 structurally different flavonoids have been identified. Hesperidin (HPN) is a flavonoid variety obtained from citrus extracts.[8] Due to its anti-inflammatory, analgesic, antimicrobial and antioxidant effects, its use in the medical field is quite wide. In addition, hesperidin has been shown to inhibit the proteolytic activity of MMPs and prevent collagen degradation.[9]

The literature search yielded no previous study that compared the efficacy of CHX, BAC, and HPN on the microtensile bond strength (μTBS) of adhesives. Thus, the aim of this in-vitro study was to evaluate and compare the immediate and after-aging effects of CHX, BAC, and HPN on the μTBS of an etch-and-rinse and a self-etch adhesive. The first null hypothesis was that the 2% CHX, 1% BAC, and 2% HPN solutions did not affect the immediate bond strength of adhesives. The second null hypothesis was that the solutions used as MMP inhibitors did not affect the μTBS of adhesives after 6 months of aging.

MATERIALS AND METHOD

Specimen and solution preparation

All materials used in this study as well as the manufacturers, and contents of these materials are shown in Table 1. In this study, 96 permanent sound human molars, which were free of cracks and fractures were used. The teeth were used with the approval from the Research Ethical Committee of Ankara University Faculty of Dentistry Clinical Research Ethics Committee (protocol number: 36290600/09). The teeth were cleaned of residual tissue and stored in 0.9% NaCl containing 0.02% NaN_3 to prevent microbial growth until use and coronally sectioned using a slow speed diamond saw (Micracut, Metcon, New York, USA) under water cooling to expose flat mid-coronal dentin surface. The surfaces were examined under a stereomicroscope (Olympus SZ60, Tokyo, Japan) at 40× magnification for any remaining enamel, perforation, and localization of pulp chamber. The exposed dentin surfaces were wet polished with a 600 grit silicon paper for 60 second in order to obtain a standard smear layer. The teeth were randomly divided into two groups (an etch-and-rinse adhesive and a self-etch adhesive) according to the adhesive system used (n=48). Then, each group was divided into 4 subgroups according to the dentin surface treatments: untreated (control), pretreated with 2% CHX solution, 1% BAC solution and 2% HPN solution (n=12). To obtain 2% solution of HPN, 5 gram powdered HPN was dissolved in 100 ml distilled water and the solution was stored at +400C until use. BAC solution at 1% concentration was prepared by dissolving, 10 g powdered BAC in 100 ml distilled water and stored at 37°C until use.

Solution application, bonding procedure, and microtensile test

In the study, 2% CHX, 1% BAC, and 2% HPN solutions were applied with a micro-brush for 60 s with a gentle rubbing onto the dentin surfaces, after etching with 37% orthophosphoric acid for 15 s and rinsing

Table 1. Materials used in the study.

Material	Manufacturer	Composition
Scotchbond™ Etchant	3M ESPE Dental Products, St. Paul, U.S.A.	35% phosphoric acid, water, silica
Adper Single Bond 2, etch-and-rinse adhesive	3M ESPE Dental Products, St. Paul, U.S.A.	Ethanol, Bis-GMA, HEMA, GDMA, polycarboxylic acid copolymer, UDMA, water, CQ, EDMAB, DP1FHP
Clearfil S3 Plus Bond, all-in-one self-etch adhesive	Kuraray Medical Inc, Okayama, Japan	MDP, Bis-GMA, HEMA, water, dl camphorquinone ethanol, colloidal silica
Clearfil Majesty ES-2	Kuraray Medical Inc, Okayama, Japan	Silanated barium glass filler, prepolymerized organic filler, BisGMA, hydrophobic aromatic dimethacrylate, di-camphorquinone
Calasept CHX	Nordiska Dental, Ängelholm, Sweden	Irrigation solution- 2% chlorhexidine digluconate
Benzalkonium Chloride	Sigma-Aldrich,St. Louis,U.S.A.	$C_{21}H_{38}ClN$
Hesperidin	Sigma-Aldrich Co. St. Louis, U.S.A.	$C_{28}H_{34}O_{15}$
Artificial saliva	0.7 mmol / l calcium chloride ($CaCl_2$), 0.2 mmol / l magnesium chloride hexahydrate ($MgCl_2 6H_2O$), 4.0 mmol / l monopotassium phosphate ($KH_2 PO_4$), 30 mmol / l It contains potassium chloride (KCl), 0.3 mmol / l sodium azide (NaN_3) and 20 mmol / l HEPES buffer	

in the etch-and-rinse adhesive groups (Adper Single Bond 2), and before adhesive application in the all-in-one self-etch adhesive groups (Clearfil 3S Plus). The adhesive system was used according to the manufacturer's instructions, then A2 shade composite resin was placed in two increments with a thickness of 2 mm on prepared dentin surface. Each layer was light-cured for 20 s at 1.000 mW/cm^2 (Valo Cordless, Ultradent, Utah, USA). The restored specimens were kept in distilled water at 370C for 24 hours before sectioning for the microtensile test, then rectangular beams of approximately 1 mm^2 were obtained from the specimens with a slow-speed diamond saw under water cooling. A minimum of 18 beams were obtained from each tooth. Half of the beams from each group were tested immediately, while the other half were tested after being stored in artificial saliva for 6 months. The artificial saliva solution was prepared at Ankara University Faculty of Pharmacy according to the formula of Pashley *et al*.[10] The pH of the artificial saliva was adjusted to 7 and it was renewed weekly.

The cross-sectional area of the beams was measured with a digital caliper. The beams were attached to a universal testing machine (Bisco Micro Tensile Tester, Illinois, USA) by a cyanoacrylate glue (Zapit, Dental Ventures of America Inc., USA), and tested under tensile force in a top-bottom manner with a 200 N load cell at a crosshead speed of 0.15 mm/min. The bond strength values (MPa) were measured by dividing the failure load (N) at the time of fracture by the diameter of the cross-sectional area (mm^2). Each beam was observed under a stereomicroscope at ×40 magnification to examine the mode of failure, and the failures were classified as adhesive (A), cohesive in dentin (CD), cohesive in composite (CC), or mixed (M). All procedures were carried out by a single operator.

Statistical analysis

The data were analyzed using IBM SPSS 20. Two-way analysis of variance (ANOVA) test was used to determine and evaluate the difference between the groups and subgroups. Multiple comparisons of the groups were performed with Tukey HSD test. The comparison between the percentage of the failure types was statistically analyzed by Chi-Square test. The level of statistical significance was set at 0.05.

RESULTS

The immediate and after-aging μTBS mean values and standard deviations of all groups are shown in Table 2. The immediate μTBS values of the self-etch adhesive applied groups were significantly lower than those of the etch-and-rinse adhesive applied groups (p= 0.014).

The control subgroup showed significantly lower immediate μTBS values than the CHX, BAC, and HPN subgroups in the etch-and-rinse adhesive (p< 0.001), but there was no significant difference between the immediate μTBS values of the subgroups in the self-etch adhesive (p= 0.344). The after-aging μTBS values of the control subgroup were significantly lower than those of the CHX, BAC, and HPN subgroups in both adhesives (p< 0.05). The after-aging μTBS values of the control subgroup were significantly lower than the immediate μTBS values in both adhesives (p< 0.05).

There was no difference between the immediate and after-aging μTBS values of the CHX subgroup in both adhesives (p> 0.05). No significant difference was found between the immediate and after-aging μTBS values of the BAC and HPN subgroups in the etch-and-rinse adhesive (p> 0.05). However, in the self-etch group, the after-aging μTBS values of the BAC (p= 0.038) and HPN (p= 0.01) subgroups were significantly higher than the immediate values.

The distribution of failure types in all groups is shown in Table 3. The adhesive failure was the most common failure type, and no significant difference was found in the immediate and after-aging failure types of etch-and-rinse adhesive groups (p> 0.05). In the self-etch adhesive groups, there was no significant difference between the after-aging failure types (p< 0.05), but the immediate failure types of the BAC subgroup were significantly different (p= 0.026). The failure type of this group was mostly mixed.

Table 2. Comparison of μTBS values (Mean ± SD) between the MMP inhibitors and between storage times.*

		Immediate	After-aging	Total	Factor	F	p	Effect size (partial eta squared)
Adper Single Bond 2 (etch-and-rinse adhesive)	Control	29.47 ± 2.99 ^{C}	25.34 ± 3.96 ^{D}	27.4 ± 4.06 ^{a}	Solution	29.179	<0.001	.274
	CHX	29.4 ± 2.82 ^{C}	29.25 ± 4.13 ^{C}	29.32 ± 3.51 ^{b}	Time	6.185	.014	.026
	BAC	32.13 ± 3.4 ^{ABC}	29.99 ± 4.19 ^{BC}	31.06 ± 3.93 ^{b}	Solution x time	6.678	<0.001	.079
	HPN	32.7 ± 4.27 ^{AB}	34.33 ± 3.78 ^{A}	33.52 ± 4.08 ^{c}				
	Total	30.92 ± 3.7 ^{b}	29.73 ± 5.1 ^{b}	30.33 ± 4.49				
		Immediate	**After-aging**	**Total**	**Factor**	**F**	**p**	**Effect size (partial eta squared)**
Clearfil S3 Plus Bond (self-etch adhesive)	Control	23.78 ± 3.53 ^{B}	20.3 ± 4 ^{A}	22.04 ± 4.13 ^{a}	Solution	19.516	<0.001	.202
	CHX	24.25 ± 3.64 ^{B}	24.13 ± 3.35 ^{B}	24.19 ± 3.47 ^{b}	Time	.899	.344	.004
	BAC	24.53 ± 3.21 ^{B}	27.53 ± 4.73 ^{C}	26.03 ± 4.29 ^{c}	Solution x time	9.607	<0.001	.111
	HPN	25.53 ± 3.65 ^{BC}	27.93 ± 3.08 ^{C}	26.73 ± 3.56 ^{c}				
	Total	24.52 ± 3.53 ^{b}	24.97 ± 4.9 ^{b}	24.75 ± 4.26				

*CHX, chlorhexidine digluconate; BAC, benzalkonium chloride; HPN, hesperidin; SD, standard deviation A-D: There is no difference between groups with the same letter. a- c: There is no difference between time and solution interaction groups with the same letter.

Table 3. The distribution of failure types (%) between subgroups and between storage times.

Main Groups	Subgroups	Immediate Failure Distribution %				After-aging Failure Distribution %			
		A	CC	CD	M	A	CC	CD	M
Adper Single Bond 2 (etch-and-rinse adhesive)	Control	60	3.3	0	36.7	80	3.3	0	16.7
	CHX	70	0	6.7	23.3	53.3	6.7	0	40
	BAC	56.7	10	0	33.3	63.3	10	3.3	23.3
	HPN	50	6.7	6.7	36.7	50	3.3	0	46.7
Chi-Square test		p= 0.407				p= 0.132			
Clearfil S3 Plus Bond (self-etch adhesive)	Control	53.3	10	0	36.7	60	6.7	0	33.3
	CHX	63.3	0	0	36.7	63.3	3.3	0	33.3
	BAC	36.7	0	0	63.3	66.7	6.7	0	26.7
	HPN	66.7	6.7	0	26.7	53.3	6.7	0	40
Chi-Square test		p= 0.026				p= 0.96			

CHX, chlorhexidine digluconate; BAC, benzalkonium chloride; HPN, hesperidin; A, adhesive failure; CC, cohesive in composite failure; CD, cohesive in dentin failure; M, mixed failure SD, standard deviation.

DISCUSSION

The present study evaluated and compared the immediate and after-aging μTBS values between different types of adhesives and dentin which was pretreated with or without different MMP inhibitors. According to the findings of this study, the immediate μTBS of the self-etch adhesive control group was significantly lower than that of the etch-and-rinse adhesive control group. These findings correlate with the result of the study which reported that all-in-one adhesives have weaker bond strength than etch-and-rinse adhesives because of having weak acidity, high water permeability, and forming a weaker hybrid layer.[11]

The chemical properties of MMP inhibitors play an important role in their effectiveness when applied without washing and affect their ability to interact with the target enzyme.[2] Therefore, the MMP inhibitors used in this study were applied to the dentin surface as an additional step. This approach may result in increased working time during adhesive application. However, the application time which was 60 s in this study seems acceptable under clinical conditions. Also, the mechanical properties of adhesives such as the degree of conversion and E-modulus might be affected by adding the MMP inhibitors into adhesives.[12]

According to our findings, the application of MMP inhibitors as a separate step cause significantly lower immediate bond strength in the self-etch adhesive compared to the etch-and-rinse adhesive. These findings are correlated with the study by Collares *et al*.[13], which indicates that the efficacy of MMP inhibitors may depend on the adhesive system used with non-simplified systems being more stable over time than simplified ones. Also, Munoz *et al*.[14] reported that MMP inhibitors may be more effective on etch-and-rinse adhesives which have a separate etching application than on self-etch adhesives because they act on the released collagen. Furthermore, 10-methacryloyloxydecyl-dihydrogen phosphate (MDP) is a bifunctional monomer that binds to the dental substrate, such as CHX.[15] CHX competes with MDP for dentin calcium, impairing bonding properties in MDP-based systems. Thus, it can be thought that the interaction between CHX and MDP may affect the competitive bonding to dentin and contributed to the bond strength reduction.[3]

The current study concluded that 2% CHX application did not negatively affect the immediate bond strength of both adhesives. These findings are in accordance with previous meta-analyses studies. Kiuru *et al*.[4] and Montagner *et al*.[5] examined various studies on CHX efficiency and indicated that CHX had no effect on the immediate bond strength.

The efficacy of 1% BAC on the immediate μTBS of etch-and-rinse and self-etch adhesives correlates with the studies that indicated 1% BAC has no negative effects on the immediate bond strength.[16,17] In contrast to these findings, Comba *et al*.[18] reported that 1% and 0.5% BAC concentrations decrease the immediate bond strength of adhesives when incorporated into adhesives. These different results can be explained by the use of different forms of BAC and the way BAC is incorporated into adhesives.

According to the findings, the application of 2% HPN enhanced the immediate bond strength of the etch-and-rinse adhesive. Thus, the first null hypothesis was rejected. HPN is a phenolic flavonoid similar to natural flavonoids, and is reported to have collagen crosslinking properties that enable them to strengthen the resin-dentin bond.[7] Therefore, the interaction between HPN and collagen might be similar to the cross-linking effect of natural flavonoids. Islam *et al*.[19] stated that HPN application improves the mechanical properties of collagen, and this significantly affects the tensile strength of dentin. Therefore, the increase in the immediate μTBS between the etch-and-rinse adhesive and dentin pretreated with HPN can be attributed to the improvement in the mechanical properties of the hybrid layer.[19]

In a study on hesperidin, Islam *et al*[9] evaluated the effect of different concentrations of Hesperidin on microtensile bond strength and stated that 2% hesperidin showed the best results. Thus, a hesperidin solution at 2% concentration was used for this study.

There are few studies examining the effect of hesperidin application on the bond strength of an etch-and-rinse adhesive system in the literature. Dávila-Sánchez *et al*.[8] investigated the effect of a 6.5% HPN solution on a universal adhesive with a total-etch mode and, unlike our findings, found no significant difference between the immediate bond strengths of the control and HPN group.

In this study, the application of 2% HPN did not affect the immediate bond strength of the self-etch adhesive. Unlike our findings, Islam *et al*.[9] reported that HPN increased the immediate bond strength of self-etch adhesives. The difference between the findings might be due to the methodology used, as HPN was not applied as a separate step in their study, but was used by added to the the primer solution. Beck *et al*.[7] examined the effects of HPN at a 5% concentration on bond strength, both by adding it to the primer of the self-etch adhesive and by using it as a separate step. Consistent with our findings, they stated that HPN did not affect the bond strength of the self-etch adhesive when used as an additional step. Also, they stated that HPN reduced the immediate bond strength when added to the primer.

The after-aging μTBS values of both adhesives without MMP inhibitor application were found to be significantly lower than the immediate μTBS values. Thus, the second null hypothesis that 'pretreatment with different MMP inhibitors had no effect on the prevention of enzymatic degradation after 6 months of storage in artificial saliva' was rejected. These findings are in line with the previous studies that indicated adhesives initially had sufficient bond strength with dentin structure, and that the bond degrades over time.[5,20] The hydrophilicity and water absorption properties of adhesives along with the destruction of the collagen fibers in the hybrid layer by collagenolytic enzymes, are related to bond degradation.[21] These enzymes, called matrix metalloproteinases (MMPs), are considered one of the main factors of resin-dentin degradation and found in odontoblasts, mineralized and demineralized dentin tissue and pulp. The use of MMP inhibitors is recommended to improve the durability of resin-dentin bonds.[22]

In this study, the after-aging μTBS of both adhesives pretreated with 2% CHX was significantly higher than that of the untreated group. This finding is in accordance with the meta-analysis by Kiuru *et al*.[4] indicating that the use of 2% CHX as an MMP inhibitor can preserve collagen integrity after aging. Similarly, Zheng *et al*.[23] reported that the application of 2% CHX stabilizes the collagen fibers after aging and prevents the degradation of resin-dentin bonds.

Most of MMPs in the dentin are insoluble and bind to the collagen matrix. BAC disrupts the intermolecular interactions between collagen and MMPs by connecting its cationic part to the negatively charged part of the collagen.[6] According to these findings, pretreatment with a 1% BAC solution as an MMP inhibitor was as effective as CHX in preserving the bond strength of both adhesives. This finding is consistent with a previous study that evaluated the stability of bond strength of resin-dentin interfaces pretreated with a 1% BAC solution, after 6 months of aging.[24] Also, XU *et al*.[25] reported that MDP-containing primer in combination with BAC significantly resisted degradation of the resin-dentin interfaces, and greatly improved the durability of dentin bonding. In contrast to these findings, it has been reported that the application of BAC in phosphoric acid could not prevent bond strength loss over time.[26,27] In these studies, BAC was applied in phosphoric acid, and the surfaces were washed after application, whereas we applied BAC as a separate step after the etching step for the etch-and-rinse adhesive, and before the application of the self-etch adhesive. Sabatini and Pashley[24] reported that although BAC incorporated in phosphoric acid etchants binds well to demineralized dentin after rinsing, concerns remain regarding whether its partial displacement after rinsing reduces its MMP inhibitory effect. Thus, the differences in these results can be due to differences in the methodology.

In the present study, the μTBS of both adhesives pretreated with 2% HPN after 6 months of storage in artificial saliva was significantly higher than that of the untreated group. Hiraishi *et al*.[28] determined that HPN can deactivate the catalytic sites of MMPs, and cross-link amino acids. Thus, the positive effects of HPN obtained in the present study might be attributed to these properties. This finding supports the study by Beck *et al*.[7] which indicated that pre-adhesive application of 5% HPN stabilizes the bond strength of adhesives, whereas incorporating HPN into the primer is unable to prevent the bond strength deterioration over time.

When considering most of the HPN studies in the literature, it is reported that HPN has been applied by adding it to a primer. In contrast, in this study, a 2% HPN solution was used as an additional application step to prevent the degradation of resin-dentin bonds. In line with our findings, Islam *et al*.[9] reported that the after-aging μTBS of the adhesive to which 2% HPN was added to its primer was significantly higher than that of the control group. Also, Davila-Sanchez *et al*.[8] reported that 6.5% HPN incorporated to a primer stabilizes the bond strength after aging with 25,000 thermocycles.

The comparison between the effects of 2% CHX, 1% BAC, and 2% HPN on the μTBS of adhesive systems after 6 months of artificial saliva storage revealed that the application of 2% HPN demonstrated better improvement in the durability of the bond between adhesives and dentin compared to the application of 2% CHX and 1% BAC.

Scherrer *et al*.[29] stated that failures resulting from micro-tensile bond strength tests are predominantly adhesive and mixed types. Similarly, in the present study, the failures occurred after the μTBS test were determined to be mostly adhesive and mixed types. These findings were in accordance with the study of Campos *et al*.[30] which stated that it is desired to have adhesive-type failures that can more accurately reflect the bond strength.

A limitation of this study was the storage time of the specimens for aging. It is necessary to examine the effects of HPN at longer aging times. Also, further in-vivo studies are needed to evaluate the effect of HPN on the bond strength of adhesives.

CONCLUSIONS

Within the limitations of this in-vitro study, it can be stated that the use of 2% HPN as a separate step before the adhesive application is as an effective as applying CHX and BAC to preserve the longevity of resin-dentin bond strength.

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