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Original research

The effects of different cavity disinfectants on fracture resistance of tooth fragment reattachments

Purpose
This study was conducted to investigate the fracture strength of reattached tooth fragments after different cavity disinfection protocols.

Materials and Methods
Incisal edges of 144 bovine incisors were sectioned and then randomly divided into 4 different groups as follows: Group C: no disinfectant; Group NaOCl: 2.5% sodium hypochlorite solution; Group NaOCl+Asc: 2.5% sodium hypochlorite followed by 10% ascorbic acid solution; Group CHX: 2% chlorhexidine solution. Teeth were further divided into 3 subgroups according to universal adhesive (G-Premio Bond, Scotchbond Universal, Prime and Bond Universal). Fracture resistance was evaluated using a universal testing machine. Data was analyzed using 2-way ANOVA with Bonferroni tests.

Results
Cavity disinfectant had a statistically significant effect on bond strength ($p<0.05$), with the highest bond strength detected in the NaOCl+Asc Group (148.22 ± 51.64) and the lowest in the NaOCl Group (112.84 ± 43.12). Scotchbond Universal exhibited the highest bond strength values (163.59 ± 49.94); however, there were no significant differences between the other adhesive systems ($p> 0.05$). .

Conclusion
Application of ascorbic acid following sodium hypochlorite treatment can improve dentin bond strength.

Keywords: *Cavity disinfectants, crown fracture, reattachment, universal adhesives*

Nihan Gonulol[1], Emine Sen Tunc[2], Elif Kalyoncuoglu[3], Şukru Ozcelik[4], Hakan Gokturk[5]

Introduction

Crown fracture is a relatively common event that affects mainly children and adolescents (1). Reattachment of the original tooth fragment, if available, appears to be the most conservative approach to restorative treatment, providing conditions are feasible (2). Reattachment of a fractured fragment not only represents a simple and low-cost alternative to other restorative techniques such as composite restorations and laminate veneers, the preservation of the natural tooth also provides better esthetics and wear-resistance (3).

An uncomplicated crown fracture results in the exposure of a considerable number of dentin tubules in the oral cavity. Depending on the location of the fracture line, this number varies from 15,000-45000 per mm^2 (4). Long-term exposure of dentin tubules and inadequate cleaning of fractured surfaces can create a potential pathway of invasion for bacteria that can lead to pulpal disease (5). In order to minimize bacterial invasion and ensure pulpal healing, prompt treatment of dentin is essential; however, this cannot be achieved in all cases (6). According to Diaz et al. (7),

ORCID IDs of the authors: N.G. 0000-0002-7046-7154; E.Ş.T. 0000-0002-5373-8987; E.K. 0000-0003-2784-3975; Ş.Ö. 0000-0002-4435-288X; H.G. 0000-0003-3824-2569

[1]Department of Restorative Dentistry, Faculty of Dentistry, Ondokuz Mayis University, Samsun, Turkiye

[2]Department of Pedodontics, Faculty of Dentistry, Ondokuz Mayis University, Samsun, Turkiye

[3]Department of Endodontics, Faculty of Dentistry, Ondokuz Mayis University, Samsun, Turkiye

[4]Pediatric Dentistry, Private Practice Samsun, Turkiye

[5]Department of Endodontics, Faculty of Dentistry, Dokuz Eylül University, İzmir, Turkiye

Corresponding Author: Elif Kalyoncuoglu

E-mail: elif_kalyoncu@hotmail.com

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whereas 45.7% of patients apply for dental treatment within 2-24 h after traumatic injury, 32.6% of them do not present until after 24h.

Disinfection of fractured surfaces could help to alleviate the problems associated with delayed treatment. Various chemical solutions, including chlorhexidine digluconate (CHX), sodium hypochlorite (NaOCl), and iodine have been tested and proven to be effective as cavity disinfectants. However, the use of a disinfectant could be problematic if it inhibits the wettability and micromechanical bonding of resin to dentin (8).

Various up-to-date techniques and materials have been tested to improve the clinical success of reattached teeth (2). However, to the best of our knowledge, no studies have investigated how their resistance to fracture is affected by the use of cavity disinfectants on the exposed tooth surfaces. Therefore, this study was conducted to compare the effects of 3 different disinfection protocols on the shear bond strength of 3 different universal adhesives to reattached bovine incisor fragments. The null hypothesis was that differences in cavity disinfectants and adhesive systems would have no effect on the fracture resistance of reattached teeth.

Material and Methods

Study materials

This study was conducted with 3 different cavity-disinfection protocols (2.5% NaOCl solution, 2.5% NaOCl+10% ascorbic acid solution, 2% chlorhexidine solution) and 3 different universal adhesives (G-Premio Bond, Prime & Bond Universal, Scotchbond Universal) (Table 1).

Sample size estimation

Power analysis was performed according to Poubel et al. (9) using the G-power computer program and indicated that a minimum of 12 observations per group were required for a 95% confidence level (1-α), 85% test power (1-β) and effect size of f=0.413.

Specimen preparation

In the present study, bovine incisor teeth were used so there is no need ethical approval. A total of 144 freshly extracted bovine permanent incisors were used in this study. In order to ensure standardization of specimens, teeth were selected according to crown dimensions (26±1 mm inciso-cervical length, 15±1 mm mesio-distal width) and visually examined under x3 loope magnification for any cracks, defects or caries. After cleaning and removal of tissue remnants, teeth were stored in a 0.01% thymol solution until the experiment.

Standardized fragment areas were created by tracing a line perpendicular to the long axis of the root at a distance of one-third away from the anatomical crown and parallel to the incisal edge, and then slicing the tooth along the marked line using a low-speed diamond saw (Isomet, Buehler Ltd, IL, USA) under water-cooling. The separated tooth structures and fragments were submitted to ultrasonic bathing for 2 h to remove the smear layer and then stored individually in distilled water.

Specimens (teeth+fragments) were randomly divided into 4 groups (n=35) according to surface-disinfectant treatment, as follows: Group C: No disinfectant (control group). Group NaOCl: Teeth and fragments were rinsed with 2.5% NaOCl for 20 s, rinsed with distilled water for 10 s, and dried with oil-free air. Group NaOCl+Asc: Teeth and fragments were dis-

Table 1: *The compositions and manufacturer details of the materials used in this study.*

	Composition	Manufacturer	Mode of Application
G-Premio Bond	MDP, 4-MET, MEPS, Methacrylate monomer, Acetone, Water, Initiators, Silica	GC Corp, Tokyo, Japan	Apply to both enamel and dentin surfaces for 20 s and leave undisturbed for 10 s. Then dry for 5 s with oil free air under maximum pressure to allow solvent evaporation and light cure.
Prime&Bond Universal	Mono-, di- and trimethacrylate resins, PENTA, diketone, organic phosphine oxide, stabilizers, cetylamine hydrofluoride, isopropanol, water	Dentsply Caulk, Milford, DE, USA	Apply to both enamel and dentin surfaces and slightly agitate for 20 s. Then dry gently for 5 s with oil free air to allow solvent evaporation and light cure.
Scotchbond Universal	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond Copolymer, Filler, Ethanol, Water, Initiators, Silane	3M ESPE, St Paul, MN, USA	Apply to both enamel and dentin surfaces and rub for 20 s. Then dry gently for 5 s with oil free air to allow solvent evaporation and light cure.
Aeliteflo flowable composite resin	Ethoxylated BİS-GMA triethyleneglycol dimethacrylate, barium glass filler, 72 % (w) filler load	Bisco Inc, Schaumburg IL, USA	Place the flowable composite to the cavity max 1.5 mm depth and light cure for 20 s.
Consepsis	2% chlorhexidine	Ultradent products, South Jordan, UT, USA	Rinse with chlorhexidine solution for 20 s, rinsed with distilled water for 10 s, and dried with oil-free air.
Werax	2.5% NaOCl	Werax, Istanbul, Turkiye	Rinse with NaOCl solution for 20 s, rinsed with distilled water for 10 s, and dried with oil-free air.
Ascorbic acid	10%	Prepared manually in the laboratory	17.61g L ascorbic acid was taken in a 100ml volumetric flask and discolved in 100ml distilled water

infected as above for Group NaOCl, after which they were immersed in a freshly prepared solution of 10% ascorbic acid for 10 minutes and then rinsed with distilled water for 10 s and dried with oil-free air. Group CHX: Teeth and fragments were rinsed with 2% chlorhexidine solution for 20 s, rinsed with distilled water for 10 s, and dried with oil-free air.

Reattachment procedures

Following surface treatment, the fractured surfaces of the fragments and the remaining tooth structures were selectively etched (enamel only) with 35% phosphoric acid for 15 s, rinsed for 10 s, and dried with oil-free air. Each group of specimens was then randomly divided into 3 subgroups (n=12) according to adhesive system (Table 1). Adhesives were light-cured for 20 s on each surface (10 s on the mesial portion and 10 s on the distal portion) using a LED light-curing unit (Elipar S10, 3M ESPE, St Paul, MN, USA) at 1,000 mw/cm^2. For each specimen, flowable microhybrid composite (Table 1) was applied to the fractured tooth surface, the fragment was positioned in place, and the specimen was light-cured along the fracture line for 40 s (20 s on the buccal surface and 20 s on the lingual surface).

Teeth were stored at 37°C in distilled water for 24 h and then finished and polished using Sof-Lex aluminum oxide discs (3M ESPE, St Paul, MN, USA) for 15 s per disc (coarse, medium, fine, and superfine). Specimens were then subjected to electronic thermocycling (DTS B1, Dentester, Salubris Technica, Istanbul, Turkey) between 5°C and 55°C (1000 cycles, 10 s dwell time, 30 s transfer time between baths).

The root portions of the specimens were embedded in self-curing acrylic resin cylinders 1 mm from the cemento-enamel junction. Fracture resistance was measured by tightly fixing each specimen to a universal testing machine (AGS-X, Schimadzu Corp, Kyoto, Japan) (Figure 1) and applying a 5kN load to the labial surface perpendicular to the long axis of the tooth and incisal to the reattachment line using a stainless-steel tip at a cross-head speed of 1 mm/min. Fracture loads were recorded in *N* and then analyzed under a stereomicroscope (Nikon SMZ 1500, Tokyo, Japan) at x16 magnification. Fracture modes were classified as either cohesive within dentin, adhesive, or mixed, according to the following criteria:

Adhesive failure: less than 25% of the resin composite remaining at the tooth-resin bonding interface; Cohesive failure: more than 75% of the resin composite remaining at the tooth-resin bonding interface; Mixed failure: 25% to 75% of the resin composite remaining at the tooth-resin bonding interface.

Statistical analysis

Statistical analysis was performed using the software program SPSS 23 (SPSS Inc., Chicago, IL, USA). Normality of distribution was verified with a Shapiro Wilk test. Differences among experimental groups were compared using two-way ANOVA followed by Bonferroni correction, with the significance level set at 0.05.

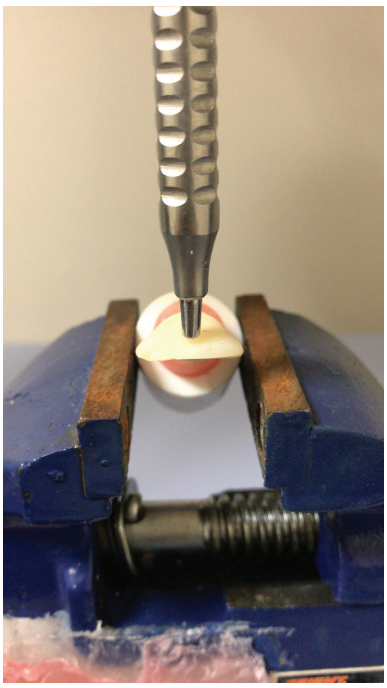

Figure 1. *The view of the test specimen in universal test machine.*

Results

Results of two-way ANOVA are presented in Table 2. As the table shows, mean reattachment strengths were significantly affected by both disinfectant (p=0.003) and adhesive (<0.001); however, the interaction between the two variables was not statistically significant (p=0.927).

Fracture strengths of the reattached teeth (means and standard deviations) are given in Table 3. When the effect of cav-

Table 2: *Analysis of variance.† Df: Degree of freedom*

	Type III Sum of Squares	df	Mean Square	F	p
Disinfectants	23868,4	3	7956,1	4,9	0,003
Adhesives	91490,3	2	45745,1	28,0	<0,001
Interaction disinfectants * adhesives	3108,1	6	518,0	0,3	0,927

Table 3: *Mean values and standard deviations of fracture strength (kgf)*

Groups	Control	NaOCl	CHX	NaOCl+Asc	Total
G-Premio Bond	107,45 ± 29,51	90,93 ± 28,82	103,57 ± 24,35	125,28 ± 46,59	106,81 ± 34,63^{A}
Scotchbond Universal	160,54 ± 57,98	148,5 ± 48,77	169,89 ± 33,83	175,41 ± 57,59	163,59 ± 49,94^{B}
Prime&Bond Universal	105,15 ± 41,74	99,08 ± 24,30	108,59 ± 32,3	143,95 ± 39,97	114,19 ± 38,44^{A}
Total	123,09 ± 50,12^{ab}	112,84 ± 43,12^{a}	128,64± 42,86^{ab}	148,22 ±51,64^{b}	128,20 ± 48,37

‡Different letters, lowercase in rows and uppercase in columns, indicate statistically significant differences * p < 0.05

Table 4: *The classification of the failure modes.*

Experimental groups	Prime&Bond Universal			Scotchbond Universal			G Premio Bond		
	adhesive	mixed	cohesive	adhesive	mixed	cohesive	adhesive	mixed	cohesive
Control	11	1	0	9	2	1	12	0	0
NaOCl	11	1	0	10	2	0	11	1	0
NaOCl+Asc	11	1	0	9	2	1	11	0	1
Chx	10	2	0	11	1	0	12	0	0

ity disinfectant was examined, Group NaOCl+Asc was found to have the highest bond-strength value (148.22±51.64), and Group NaOCl was found to have the lowest bond-strength value (112.84±43.12), with the difference between the two groups statistically significant ($p<0.05$). No other statistically significant differences were observed among disinfectant protocols ($p>0.05$). When the effect of adhesive system was examined, the mean bond-strength value of the Scotchbond Universal subgroups (163.59±49.94) was significantly higher than that of the other adhesive systems ($p<0.05$). No statistically significant difference was observed between the mean bond strengths of Prime&Bond Universal (114.19±38.44) and G Premio Bond (106.81±34.63).

Failure modes are presented in Table 4. Regardless of adhesive system or cavity disinfectant, the majority of failures were adhesive failures.

Discussion

The simplicity and versality of universal adhesives, which can be used in etch-and-rinse, self-etch, or selective-etch modes, has led to a rapid increase in their use in clinical practice, and further research into these materials is warranted. This study found that a sodium hypochlorite solution decreased the bond strength between tooth fragments reattached using 3 universal adhesives in selective-etch mode. Moreover, an ethanol/water-based universal adhesive exhibited significantly higher bond-strength values than 2 acetone/water-based universal adhesives. Therefore, the null hypothesis that different cavity disinfectants and adhesive systems would have no effect on the fracture resistance of reattached teeth was rejected.

Bovine incisors are considered a valid alternative to human incisors in adhesion tests because their morphology, ultrastructural architecture, microhardness and mineral content are similar to those of human teeth. Moreover, they are more easily available and can be selected and standardized for size and age (9,10). For these reasons, in the present study, bovine incisors were selected and screened for optimum tooth standardization.

In adhesion tests, it is important to standardize the samples before bonding procedures by preparing equivalent enamel and dentin bonding surfaces (10,11). In the present study, in order to obtain exposed areas of equal size, all teeth were cut at a similar distance from the incisal margin. However, the process of creating the standardized fragments that are required to ensure repeatability of this *in vitro* study resulted in the production of a smear layer that does not exist with a naturally occurring fracture (10). In order to better simulate traumatic fracture, after sectioning teeth with a diamond saw, specimens were sonically cleaned to remove the smear layer.

Uncomplicated crown fractures should never be left untreated. Given the excellent quality of currently available adhesive materials, reattachment of a fractured fragment is the recommended technique when a fragment has been saved following trauma (2,9). A systematic review study concluded that the reattachment of a tooth fragment using an adhesive system with an intermediate composite and with no additional preparation allows fractured teeth to recover an adequate amount of lost strength (12). According to Pamir et al. (13), the use of a material with a low modulus of elasticity at the tooth-composite interface can reduce the effects of compressive and tensile stresses that can cause debonding during functional mobility. Therefore, in the present study, a flowable composite with a low elastic modulus was selected as an intermediate agent for the reattachment procedure.

The proposed technique for fragment reattachment is selective etching of enamel, followed by application of a universal adhesive for self-etching of dentin. Many clinicians prefer to use 30%-40% phosphoric acid for enamel etching because it increases wettability, surface energy, and surface porosity, thereby improving adhesive penetration and enabling the formation of a uniform hybrid layer (9). In contrast, self-etching systems are preferred in dentin because they generate better hybridization, as their resinous monomers are capable of simultaneously demineralizing and infiltrating dentin (9,14). Moreover, because trauma-induced fractures usually involve deep dentin, the use of a self-etching adhesive system has been recommended in such cases in order to avoid any pulpal damage (9,15). For these reasons, the present study examined the use of 3 different universal adhesives in selective-etch mode for the reattachment of tooth fragments.

The findings showed the bond strength of reattached teeth to be material-dependent. The fact that the Scotchbond Universal specimens exhibited the highest bond strength regardless of disinfectant protocol can be attributed to the material composition of this adhesive, which differs from the other tested adhesives in several ways. First, unlike the other adhesives, Scotchbond Universal contains a polyalkenoic acid copolymer (Vitrebond copolymer, 3M Oral Care) derived from resin-modified glass ionomer technology that has been reported to prevent surface wetness from negatively affecting the durability of the adhesive bond to ensure long-term bond strength (16). Moreover, the water content by volume of Scotchbond Universal, G-Premio Bond, and Prime & Bond Universal are 10%, 25%, and 5%-24.5%, respectively, and, according to Choi et al. (17), the ideal range for universal adhesives is 10%-15%, where-

as adhesives containing more than 25% water by volume are reported to undergo phase separation, resulting in less chemical interaction with the substrate. Finally, Scotchbond Universal is water/ethanol-based, whereas both G-Premio Bond and Prime & Bond Universal are water/acetone-based, and studies have reported that the faster evaporation of acetone-based adhesives results in a relatively thin layer of adhesive that is subject to oxygen inhibition and may eventually lead to bond failure (18).

According to the literature, the prognosis of uncomplicated crown fractures may differ due to a variety of concurrent problems, such as bacterial contamination of dentin and pulp (13). Compounds such as chlorhexidine gluconate-based solutions, sodium hypochlorite, hydrogen peroxide, sodiumethylene tetraacidic acid, and iodine have been reported to possess antibacterial properties that make them suitable for use as cavity disinfectants; however, some authors have expressed concern that their application to dentin might have negative effects on the sealing capacity of adhesive bonding resins (19,20,21). Several mechanisms have been put forward to explain NaOCl's adverse effect on dentin bond strength, including removal of the organic matrix from treated dentin, which leaves a less receptive bonding surface, and dissolution of collagen fibrils, which leads to a breakdown of the bonds between carbon atoms and disorganization of the primary collagen structure, thereby preventing the formation of a consistent hybrid layer (22-24). Another problem that has been reported is associated with NaOCl's oxidizing activity, which prematurely terminates chain formation, resulting in incomplete polymerization of the adhesive resin (25). The significant reduction in bond strength values of all the tested adhesive systems found in this study following NaOCl treatment is in line with these earlier findings.

A number of natural antioxidants – e.g. ascorbic acid, sodium ascorbate, rosmarinic acid, green tea extracts, and proanthocyanidin – have been reported to reverse the negative effects of NaOCl on dentin bond strength (22). According to Prasansuttiporn et al. (26), these antioxidants remove the remnants of NaOCl through oxidation-reduction, thereby stabilizing the resin-dentin interface and improving bond strength to NaOCl-treated dentin. Vongphan et al. (27) reported that application of 10% sodium ascorbate to NaOCl-treated dentin for 10 min enhanced the bond strength of a total-etch system, and Morris et al. (28) reported a reversal in the decrease in bonding of resin cements with the application of 10% ascorbic acid. Similarly, the present study found 10% ascorbic acid application to NaOCl-treated dentin for 10 min increased the bond strength values in all the groups tested.

Chlorhexidine digluconate (CHX) is the most popular antimicrobial solution and matrix metalloproteinase (MMP) inhibitor. The high bactericidal effect of 2% CHX has been attributed to its ability to precipitate cytoplasmic contents, which leads to cell death. Numerous studies have reported on the effects of CHX application on dentin bond strength (19,29,30). For example, de Castro (29) reported that CHX treatment prior to dentin acid-etching did not adversely affect the μTBS of composite resin to dentin. Mohammed Hassan (30) similarly reported that the CHX application prior to etching did not affect dentin bond strength; however, dentin bond strength was significantly reduced when CHX was applied after etching. These findings are consistent with the results of the present study, which found CHX application had no negative effect on the dentin bond strength of the reattached tooth fragments.

Conclusion

The findings of this *in-vitro* study showed sodium hypochlorite followed by 10 min ascorbic acid application resulted in the highest bond strengths of the different adhesive protocols tested. However, this may not be the preferred choice in clinical practice because it requires longer chair time. Chlorhexidine application, which was found to have no negative effect on the bond strength of reattached teeth, may be a more practical, time-saving solution, especially for anxious trauma patients. In addition, this study found that the ethanol/water-based universal adhesive Scotchbond Universal performed better than the acetone/water-based adhesives tested in terms of bond strength.

Türkçe özet: *Diş fragmanlarının reataşmanlarının kırılma direnci üzerine farklı kavite dezenfektanlarının etkileri. Amaç: Bu çalışma, farklı kavite dezenfeksiyon protokollerinden sonra diş fragmanlarının reataşmanının kırılma dayanımını araştırmak amacıyla yapılmıştır. Gereç ve Yöntemler: 144 dana kesici dişinin kesici kenarları kesilerek rastgele 4 farklı gruba ayrıldı: Grup C: dezenfektan yok; Grup NaOCl: %2,5 sodyum hipoklorit solüsyonu; Grup NaOCl+Asc: %2,5 sodyum hipoklorit ve ardından %10 askorbik asit solüsyonu; Grup CHX: %2 klorheksidin solüsyonu. Dişler ayrıca uygulanan üniversal adezive göre (G-Premio Bond, Scotchbond Universal, Prime ve Bond Universal) 3 alt gruba ayrıldı. Kırılma dayanımı Universal test cihazı kullanılarak değerlendirildi. Veriler, Bonferroni ve 2-way ANOVA testleri kullanılarak analiz edildi. Bulgular: Kavite dezenfektanının bağlanma dayanımı üzerine istatistiksel olarak anlamlı bir etkiye sahip olduğu bulundu($p<0.05$). En yüksek bağlanma dayanımı NaOCl+Asc Grubunda (148.22 ± 51.64) ve en düşük ise NaOCl Grubunda (112.84 ± 43.12) tespit edildi. Scotchbond Universal, en yüksek bağlanma dayanımı değerlerini gösterirken (163,59 ± 49,94); diğer adeziv sistemler arasında anlamlı bir fark yoktu. Sonuç: Sodyum hipoklorit tedavisini takiben askorbik asit uygulaması dentin bağlanma dayanımını arttırabilir. Anahtar kelimeler: askorbik asit, kavite dezenfektanları, kron fraktürü, reataşman, universal adeziv*

Ethics Committee Approval: All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and based on welfare of animals.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: NG, EST, EK participated in designing the study. NG, EST, EK, SO, HG participated in generating the data for the study. NG, EST, EK, SO, HG participated in gathering the data for the study. NG, EST, EK participated in the analysis of the data. NG, EST wrote the majority of the original draft of the paper. NG, EST participated in writing the paper. EST, HG have had access to all of the raw data of the study. NG, EST have reviewed the pertinent raw data on which the results and conclusions of this study are based. NG, EST, EK, SO, HG have approved the final version of this paper. EK guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The author had no conflict of interest to declare.

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