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ORIGINAL ARTICLE

Effects of ethylenediaminetetraacetic acid and sodium hypochlorite on the bond strength of bonding agents to pulp chamber lateral walls

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Abstract *Background/purpose:* The purposes of this *in vitro* study were to determine the microtensile bond strengths of four different dentin adhesive materials placed in pulp chamber walls, and to test the effects of 5% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA) pretreatments on resin dentin bond strengths.
Materials and methods: Recently extracted human third molars were selected. The teeth were divided into four groups. Specimens in each group were treated as follows: irrigated with distilled water; irrigated with EDTA for 5 minutes; irrigated with sodium hypochlorite for 5 minutes; and irrigated with EDTA for 5 minutes followed by NaOCl for 5 minutes. Treated specimens were dried, bonded with a total-etching adhesive, two self-etching adhesives, or a one-bottle self-etching adhesive system. After the bonding procedure and composite restoration, teeth were sectioned, and 15 dentin sticks were obtained. Microtensile testing was performed, and scanning electron micrographs were taken of each irrigated group.
Results: In the control group, the one-bottle self-etching adhesive system showed statistically higher bond strength values. EDTA irrigation did not affect the bond strength except for the total-etching adhesive. NaOCl significantly reduced the bond strengths of all adhesives. The EDTA and NaOCl combination did not show a statistically significant reduction in bond strengths of the adhesives to pulpal dentin.

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Conclusion: There was a reduction in bond strengths of all adhesive systems used to test pulp chamber lateral walls after endodontic irrigation solutions were used.

Introduction

In the past few years, increasing attention has been focused on the effectiveness of coronal sealing.[1] The success of coronal restorations has a significant effect on the outcome of root canal treatments.[2] Microorganism penetration from the coronal direction potentially contributes to failure of root canal treatment.[3] An ideal adhesive system should keep the restoration in place for a significant amount of time and must completely seal the restoration margins against the ingress of oral fluids and microorganisms.[4]

Adhesion to dentin is a challenge.[4] Dentin is a hydrated, complex, biological structure, and its properties may vary with location. The structure of dentin of pulp chamber walls differs from those of other dentinal regions of the teeth, as it includes predentin, and regular and irregular secondary dentin. The density and diameters of dentin tubules are also greater in pulp chamber walls.[5] Another distinctive point in adhesion to pulp chamber walls is the absence of a smear layer. During endodontic access, because no contemporary cavity preparation techniques are used and no cutting instruments have contacted the walls of the pulp chamber, a smear layer typically has not formed.[6,7]

The use of endodontic irrigants during root canal treatments may also cause some histological changes in pulp-chamber dentin. Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are two of the most common irrigants employed in endodontic treatments. NaOCl is a nonspecific proteolytic agent capable of dissolving necrotic tissue remnants during irrigation,[8] whereas EDTA is generally accepted as the most effective chelating agent with prominent lubricant properties and is widely used in endodontic therapy.[9]

Another factor affecting the quality of bonding is the approach of the adhesive system. According to interactions with the smear layer and the etching technique, dentin adhesives can be grouped into two categories: total-etching and self-etching techniques. Total-etching systems aim to remove the smear layer to provide a predictable substrate for bonding, whereas self-etching systems penetrate the demineralized dentin to modify a hybrid layer that includes the dissolved smear layer.[10,11]

Additionally, the occurrence of shrinkage during polymerization creates stresses at the tooth-composite interface that may exceed the strength of any bond between the composite and enamel or dentin. Bond failure at the interface allows an influx of oral fluids.[12] In order to reduce polymerization shrinkage, a low-shrinking composite, Filtek Silorane, was introduced. So-called siloranes replaced the methacrylates in the resin matrix of dental composites.[13] The ring-opening chemistry of the resin reduces shrinkage of the composite below 1 vol%.[14] Filtek Silorane comes with a two-step self-etching adhesive, which is marketed as 'Silorane System Adhesive'. A hydrophilic self-etching primer is applied and separately light-cured prior to application of the hydrophobic adhesive resin.

The purpose of the present study was to evaluate the microtensile bond strength of total-etching [Adper Scotchbond Multi-purpose (ASB); Adper, St Paul, MN, USA] and self-etching [Adper SE Plus (ASA); Adper; Clerafil S^3 Bond (CS3); Kuraray Medical, Okayama, Japan; and Silorane Bond (SSA); Adper] adhesive systems (Table 1) to pulpal dentin surfaces treated with 5% NaOCl and 17% EDTA.

Materials and methods

Recently extracted, sound, human third molars were selected for this study. Impacted teeth were obtained from patients who visited the Department of Maxillofacial Surgery, Faculty of Dentistry, Ataturk University, Erzurum, Turkey. The patients had no systemic or oral diseases. The teeth were collected after informed consent was obtained under a protocol approved by the Ethics Committee of the Faculty of Dentistry, Ataturk University. The teeth were stored in a 0.5% thymol solution at room temperature for no longer than 2 months prior to use and were sterilized in ethylene oxide for 12 hours before sample preparation. The teeth were sectioned through the pulp chamber roof using an Isomet saw under water lubrication (Buehler, Lake Bluff, IL, USA). Pulp tissue was carefully removed without touching the inner surfaces of the pulpal wall.

Teeth were divided into four main groups: (1) non-irrigated control group (immersed in distilled water); (2) irrigated with 5% NaOCl for 5 minutes; (3) irrigated with 17% EDTA for 5 minutes; and (4) irrigated with 17% EDTA for 5 minutes followed by 5% NaOCl for 5 minutes. After irrigation, all teeth were cleaned with distilled water for 2 minutes. Specimens from these four groups were divided into four adhesive subgroups. After the cutting or sectioning process, approximately five to eight dentin sticks were obtained from each tooth. Finally, for microtensile testing, the sample size for each subgroup was 15.

The adhesive systems were applied to pulp-chamber walls according to the manufacturers' directions. Resin composites were condensed into the pulp chamber and cured in 2-mm layers on the bonded surface (Elipar FreeLight II LED; 3M ESPE, Seefeld, Germany). The output of the curing light was checked with a radiometer (Hilux UltraPlus Curing Units; Benlioglu Dental, Istanbul, Turkey). Information on the adhesive systems and composite resins used in this study is given in Table 1.

All restored specimens were immersed in distilled water at 37°C. After 24 hours, the teeth were vertically separated using the Isomet saw, and samples were fixed to a

Table 1 Materials used in this study.

	Material	Manufacturer	Composition	Batch No.
Adhesive	Adper Scotchbond Multi-purpose (ASB)	3M ESPE, St Paul, MN, USA	**Primer:** water, HEMA, copolymer of acrylic and itaconic acid **Adhesive:** BisGMA, HEMA	9CC/ 9RK
	Adper SE Plus (ASE)	3M ESPE	**Liquid A:** water, HEMA **Liquid B:** zirkonia, TEGDMA, di-HEMA phosphate, phosphoric acid, diurethane dimethacrylate, TMPTMA, ethyl 4-dimethyl amino benzoate, dl-camphorquinone	7KL/ 7CF
	Clerafil S^3 Bond (CS3)	Kuraray Medical, Okayama, Japan	Water, BisGMA, HEMA, 10-methacryloyloxydecyl dehydrogenate phosphate, colloidal silica, dl-camphorquinone	00118A
	Silorane Bond (SSA)	3M ESPE	**Primer:** HEMA, BisGMA, water, ethanol, phosphoric acid, 1,6-hexanediol dimethacrylate, dl-camphorquinone **Adhesive:** TEGDMA, substituted dimethacrylate, silica, phosphoric acid, 1,6-hexanediol dimethacrylate, dl-camphorquinone	8BF/ 8BB
Composite	Filtek Z250	3M ESPE	BisGMA, UDMA, Bis-EMA -zirconia/silica	6FA
	Filtek Silorane	3M ESPE	Siloranes - quartz, yttrium fluoride	8BF

sectioning block. Three thin rectangular sticks (1 × 1 × 4 mm) were prepared using the Isomet saw, with resin composite on one side and pulpal wall dentin on the other side (Fig. 1). A digital slide caliper was used to check the thickness (1 ± 0.02 mm) and width of the bonded area (Digital Slide Caliper; Tchibo, Hamburg, Germany).

Fifteen dentin sticks were obtained from each group. The ends of each specimen were attached to a microtensile device (Microtensile Tester; Bisco, Schaumburg, IL, USA) using a cyanoacrylate adhesive (Zapit; DVA, Corona, CA, USA) and stressed in tension at a speed of 1 mm/min. The data were analyzed by a one-way analysis of variance and Tukey's honest significant difference multiple *posthoc* test ($P < 0.05$).

After the test, fractured surfaces were examined under a stereomicroscope (SZ-PT; Olympus, Tokyo, Japan). Failures were classified as: adhesive failure; cohesive failure between the resin and dentin; mixed failure in the dentin and adhesive material or composite resin; and adhesive failure and cohesive failure mixed together. In order to observe the samples under a scanning electron microscope (SEM), four, sound, recently extracted, human third molar teeth were used. The pulp-chamber dentinal walls were treated as follows: (1) no treatment; (2) 5% NaOCl for 5 minutes; (3) 17% EDTA for 5 minutes; and (4) 17% EDTA for 5 minutes followed by 5% NaOCl for 5 minutes. Then all disks were coated in a vacuum evaporator (Polaron Sc500 Sputter Coater, VG Microtech, Tokyo, Japan) with a thin film of gold and observed with an SEM (JSM-5600; JEOL, Tokyo, Japan).

Results

Microtensile bond strengths in MPa are shown in Table 2 for all groups. In untreated groups, the self-etching adhesive CS3 showed statistically higher bond strength values than the other adhesive systems ($P < 0.05$), while the microtensile bond strengths of ASB, ASE, and SSA did not differ statistically ($P > 0.05$). Irrigation with 17% EDTA caused a statistically significant decrease in bond strengths of ASB, whereas changes in ASE, SSA, and CS3 were not statistically significant compared to the control group. In the 5% NaOCl group, all dentin adhesive systems showed a statistically significant decrease. In the combined EDTA/NaOCl group, the bond strength of the ASB total-etching system significantly decreased ($P < 0.05$), while changes in the other systems were not statistically significant. All statistical results are shown in Table 2, and failure types are represented in Fig. 2.

In SEM observations of the pulp-chamber lateral walls, there was no smear layer in any group because all procedures were performed without touching the dentinal walls. An irregular dentin surface was observed in control specimens. SEM photographs of the pulp-chamber wall dentin after rinsing with only distilled water revealed smooth, open dentinal tubules with few remnants of pulp tissue. According to Torabinejad et al's classification,[15] there was no erosion of the pulp chamber lateral walls in the EDTA group. In the EDTA and EDTA/NaOCl groups, severe erosion was observed. After NaOCl application, the dentin surface was partially removed, and dentinal tubule orifices were enlarged and showed a "funnel" configuration (Fig. 3).

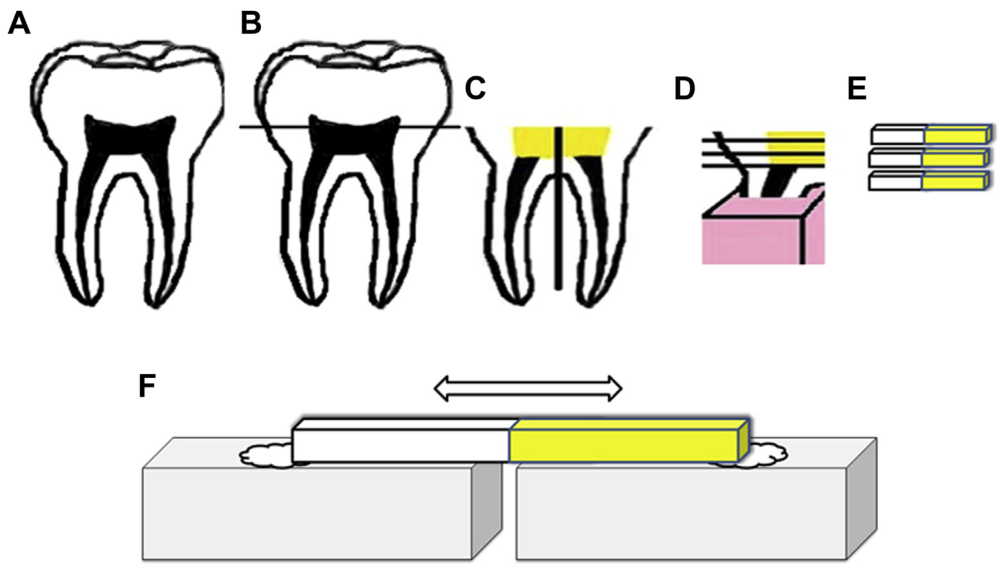

Figure 1 Schematic diagram of specimen preparation for microtensile bond strength testing.

Discussion

Ideally, an adhesive system should achieve high bond strengths and prevent coronal microleakage. Coronal leakage was extensively presented as having a negative effect on the success of root canal treatment.[16,17] Bonding to deep dentin, as is found on pulp chamber walls, can occasionally be more difficult to achieve than bonding to superficial dentin. Pulpal dentin contains predentin, irregular secondary dentin, and a high tubule density with large diameters; these variations make pulp-chamber walls a more-challenging bonding surface.[18] In addition, cutting instruments generally do not contact pulpal dentin during endodontic access, and so the dentin should largely be free of a smear layer,[7] and during root canal treatment, this dentinal section is subjected to a series of irrigants with different wettability characteristics, surface tensions, and chelating effects that tend to affect its mineral and organic contents and surface energy.[19]

The selected irrigants used in this study are commonly employed for root canal preparation. EDTA is widely used as a chelator in endodontic therapy. A chelator reacts with calcium ions in hydroxyapatite crystals and removes calcium ions from the dentin.[20] According to our study results, application of 17% EDTA for 5 minutes to pulpal wall dentin did not influence the adhesive bond strength compared to the un-irrigated group, except for the ASB group. It was reported that EDTA solutions have a strong demineralizing effect, causing enlargement of the dentinal tubules, softening of the dentin, and denaturation of collagen fibers.[21] In our study, SEM microphotographs revealed that irrigation with EDTA alone did not cause marked erosion and did not influence the bonding strengths of the adhesives. There is no research investigating the effect of the EDTA on dentin adhesive bond strengths on pulpal dentin free of a smear layer. Santos et al[1] investigated the effect of chlorhexidine and EDTA combinations on bond strengths of self-etching adhesives on bovine incisors and found that neither chlorhexidine nor EDTA affected the bond strengths. The effect of EDTA on the bond strength of adhesive materials is an area requiring additional study.

Results of this study indicated that the 5% NaOCl-treated group had significantly lower bond strengths for all adhesives. The mean bond strengths of the four adhesive systems to pulpal wall dentin in this study were 11.63 $\pm$ 7.84 MPa for ASB, 8.78 $\pm$ 3.59 MPa for ASE, 11.81 $\pm$ 3.81 MPa for CS3, and 9.95 $\pm$ 4.03 MPa for SSA in the NaOCl group. These results agree with other reports that evaluated the effect of NaOCl on pulpal dentin. Ozturk

Table 2 Microtensile bond strengths (mean $\pm$ SD MPa) of adhesives to pulp-chamber lateral walls with different irrigant preparations.

	Nonirrigated	EDTA	NaOCl	NaOCl + EDTA
ASB	16.99 $\pm$ 7.09^{B,x}	10.78 $\pm$ 5.97^{B,y}	11.63 $\pm$ 7.84^{A,xy}	9.51 $\pm$ 3.67^{C,y}
ASE	13.33 $\pm$ 7.47^{B,xy}	16.33 $\pm$ 6.09^{AB,x}	8.78 $\pm$ 3.59^{A,y}	11.75 $\pm$ 3.46^{BC,xy}
CS3	26.85 $\pm$ 10.89^{A,xy}	21.57 $\pm$ 10.33^{A,y}	11.81 $\pm$ 3.81^{A,z}	30.87 $\pm$ 7.07^{A,x}
SSA	13.83 $\pm$ 4.93^{B,x}	12.91 $\pm$ 5.55^{B,x}	9.95 $\pm$ 4.03^{A,x}	14.33 $\pm$ 3.90^{B,x}

In the same column, subgroups identified by different superscript uppercase letters differ statistically; in the same line, subgroups identified by different superscript lowercase letters differ statistically ($P \leq 0.05$).
See Table 1 for definitions of abbreviations.

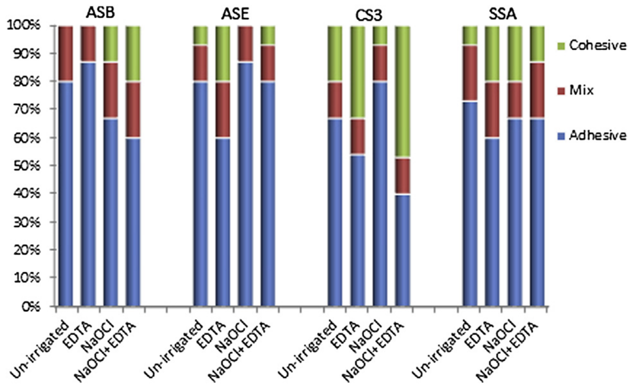

Figure 2 Failure type of bonding agents after different irrigations.

and Ozer[6] reported that bond strengths of adhesive systems were statistically significantly reduced after 5% NaOCl application. In addition, adverse effects of NaOCl on bond strengths were proven.[1,22] These effects might have been caused by damage to the organic matrix, mainly collagen, leaving mineralized surfaces of dentin after application of NaOCl.[23] In addition, NaOCl breaks down to sodium chloride and oxygen. Through chemical reactions, oxygen can cause strong inhibition of the interfacial polymerization of adhesive materials.[24] Otherwise, there might be some reactive residual free-radicals in NaOCl-treated dentin that might compete with the propagating free radicals generated during light activation of the adhesive system, resulting in premature chain termination and incomplete polymerization.[25]

In our study, 17% EDTA followed by 5% NaOCl irrigation did not statistically reduce the microtensile bond strengths of the adhesive systems tested except for the ASB total-etching adhesive. However, it was reported that the combination of EDTA and NaOCl reduced the bond strengths of a self-etching adhesive.[26] This discrepancy might be related to the absence or presence of a smear layer: Yürdagaven et al created a smear layer in their study.[26?] Additionally, in contrast to results of our study, Santos et al.[1] used bovine incisors in their study and demonstrated that application of 5.25% NaOCl + 17% EDTA caused statistically significant reduced bond strengths with a self-etching adhesive. We preferred to use sound, human, third molars in our study, and this aspect might explain the discrepancy. The use of NaOCl as an endodontic irrigant, even if associated with EDTA, should be carefully evaluated when subsequent coronal sealing is performed.

Results of our study showed that one type of self-etching adhesive, CS3 bond, had a higher bond strength than the other self-etching adhesives and the total-etching adhesive in the nonirrigated control group. The CS3 bond adhesive is a mild self-etching adhesive with a pH of 2.7. It depends on an MDP monomer to decalcify, penetrate, and create a chemical bond with calcium ions and hydroxyapatite, simultaneously allowing for a two-fold bonding mechanism (micromechanics and chemical bonding).[27] In contrast, in the etch and rinse system, the bonding mechanism is mainly based on micromechanical interlocking of resin tags as a result of a reduction in the solid intertubular dentin area. In

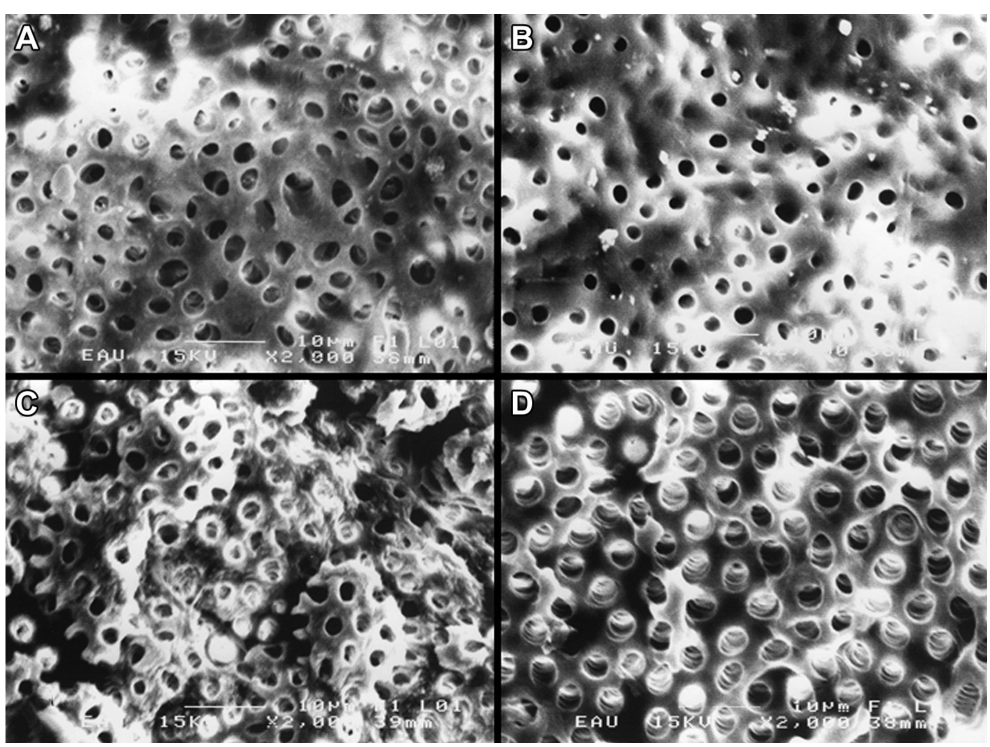

Figure 3 (A) SEM photograph of pulp-chamber wall dentin after rinsing with only distilled water revealing smooth, opened dentinal tubules with few remnants of pulp tissues. (B) Application of 17% EDTA did not affect the dentin. There was no erosion of the dentinal surface. (C) After NaOCl application, the dentin surface was partially removed, and dentinal tubule orifices were enlarged and had a "funnel" configuration. (D) A combination of EDTA and NaOCl caused severe erosion of the dentin surface.

agreement with our findings, Kijsmanmith et al[7] found that microtensile bond strengths of self-etching adhesives were significantly greater than those of etching adhesive systems. According to their results, the bond strength of the self-etching adhesive was 22.49 MPa, and that of the etch and adhesive system was 15.58 MPa. Previous studies[28,29] investigating microtensile bond strengths of different adhesives on coronal dentin showed higher strengths (MPa) than did the Kijsmanmith et al[7] study, which supports our results. Kijsmanmith et al[7] claimed that because of the irregular dentin surface and the absence of a smear layer, etching conditions may need to be adapted for bonding to pulp-chamber dentin.

In this study, bond strengths of the ASB adhesive were significantly reduced after irrigation, but especially after NaOCl treatment. It was reported that NaOCl used in endodontic treatment can adversely affect adhesion of a total-etching adhesive system but least affected self-etching adhesives.[23] However, our findings revealed that there was no meaningful difference between total-etching and self-etching adhesives, except for CS3, which resulted in notably higher microtensile bond strengths. The Silorane System Adhesive showed similar results to another self-etching adhesive, Adper SE Plus. This similarity can possibly be explained by the similar chemistry between these two adhesive systems. Despite the fact that Silorane has a different chemistry, the SSA-Bond is methacrylate-based and is, therefore, compatible with conventional methacrylate composites as well.[30]

This study was conducted in an *in vitro* environment, and several factors such as oral fluids, occlusal forces, thermal changes were not taken into account, and only human impacted third molars were tested. Within the limitations of this study, it was concluded that there was a significant reduction in bond strengths of all adhesive systems used to bond to pulp chamber lateral walls after endodontic irrigation solutions *in vitro*.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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