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Bond Durability of Adhesives Containing Modified-monomer With/Without-fluoride After Aging in Artificial Saliva and Under Intrapulpal Pressure Simulation

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Clinical Relevance

Adhesives with hydrolytically stable modified monomers could be a promising approach to enhance bonding durability.

SUMMARY

Objective: To evaluate the dentin bond strength durability of adhesives containing modified-monomer with/without-fluoride after storage in artificial saliva and under intrapulpal pressure simulation (IPPS).

Materials and Methods: The occlusal enamel of 48 freshly extracted teeth was trimmed to expose midcoronal dentin. Roots were sectioned to expose the pulp chamber and to connect the specimens to the pulpal-pressure assembly. Specimens were assigned into four groups (n=12) according to adhesive system utilized: a two-step etch-and-rinse adhesive system (SB, Adper Single Bond 2, 3M ESPE), a two-step self-etch adhesive system (CSE, Clearfil SE Bond, Kuraray Medical Inc), and two single-step self-etch adhesives with the same modified monomer (bis-acrylamide)—one with fluoride (AOF, AdheSE One F, Ivoclar-Vivadent) and the other without (AO, AdheSE One,

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Ivoclar-Vivadent). Bonding was carried out while the specimens were subjected to 15-mm Hg IPPS. Resin composite (Valux Plus, 3M ESPE) buildups were made. After curing, specimens were aged in artificial saliva and under 20-mm Hg IPPS at 37°C in a specially constructed incubator either for 24 hours or six months prior to testing. Bonded specimens (n=6/group) were sectioned into sticks (n=24/group) with a cross section of 0.9 ± 0.01 mm^2 and subjected to microtensile bond strength (μTBS) testing using a universal testing machine. Data were statistically analyzed using two-way analysis of variance (ANOVA) with repeated measures, one-way ANOVA tests, and a *t*-test ($p<0.05$). Failure modes were determined using a scanning electron microscope.

Results: The μTBS values of SB and CSE fell significantly after six-month storage in artificial saliva and under IPPS, yet these values remained significantly higher than those for the other two adhesives with modified monomers. There was no significant difference in the bond strength values between fluoride-containing and fluoride-free self-etch adhesive systems (AOF and AO) after 24 hours or six months. Modes of failure were mainly adhesive and mixed.

Conclusions: Based on the results of this study, 1) Fluoride addition did not affect dentin bond durability; and 2) despite the fact that the single-step adhesive system with modified monomer showed stability, bond strengths associated with these systems remained lower than those of multistep adhesive systems.

INTRODUCTION

Patient demand for esthetic restorations has generated an interest in the advancement of bonded restorations.[1] Several adhesive systems have been used for this purpose, ranging from etch-and-rinse adhesives to the more simplified self-etch adhesives that simultaneously condition and prime the dentin. This approach eliminates the rinsing phase, which not only reduces the clinical application time but also significantly decreases the technique sensitivity or the risk of making errors during application.[2] Current developments have focused on improving several aspects in the formulation of these adhesive systems to enhance their durability.

In order to control secondary caries, which is one of the common factors that limits the bond durability,[3] modifications in the adhesive systems' formulation, such that they were characterized by antibacterial activity, were considered beneficial. From this point of view, versions of adhesive systems containing fluoride in composition have been introduced. Fluoride has been added to the adhesive system as a therapeutic agent that supports the inhibition of secondary caries,[4] dentin demineralization, and inhibition of endogenous enzymes that attack the components of the hybrid layer,[4] thus allowing for the maintenance of bond durability.[5]

It has also been claimed[6] that (di)methacrylates are hydrolytically unstable in the acidic aqueous solutions of simplified self-etch adhesives, which may result in lower bond strengths and compromised bond durability. Therefore, monofunctional and cross-linking modified monomers have been developed, such as acrylamides, that offer increased hydrolytic stability[7] and that are less viscous and soluble than UDMA and bisphenol A glycidyl methacrylate (Bis-GMA).[8] Adjunctive use of the hydrolytically stable monomer bis-acrylamide and fluoride in an adhesive system could be a beneficial approach against various bond challenges in the oral environment.

Clinical evaluation of bonding durability under complex oral environments is highly recommended; however, *in vitro* testing is required to elucidate the specific factors that cause bond deterioration over time. In order to diminish the distance between *in vivo* and *in vitro* conditions, challenging the adhesive interface under a simulated oral environment at different time periods should be conducted.[9] The absence of an outward fluid flow through dentinal tubules has represented the most critical difference between clinical and laboratory conditions.[10] As a consequence, it is necessary to employ pulpal pressure simulation when adhesive systems are tested *in vitro*.

Thus, the objective of this study was to evaluate the dentin bond durability of fluoride-free and fluoride-containing adhesive systems that contain a modified monomer formulation in their chemistry, compared to a two-step etch-and-rinse adhesive system and a two-step self-etch adhesive system after storage in artificial saliva at 37°C and under intrapulpal pressure simulation. The null hypothesis was that there was no significant difference among the adhesive systems after six months of storage in artificial saliva with simulated intrapulpal pressure simulation.

MATERIALS AND METHODS

Specimen Preparation

Sound human third-molar teeth, extracted from patients with an age group of 18–20 years, were stored in phosphate buffer solution containing 0.2% sodium azide at 4°C. The teeth were collected from the patients after the protocol was approved by the Faculty of Dentistry's Ethics Committee at Cairo University, Egypt. All teeth were used within one month after extraction. Each tooth was trimmed perpendicular to its long axis, exposing the dentin, using a slow-speed diamond saw sectioning machine (Buehler Isomet Low Speed Saw, Lake Bluff, IL, USA) under water coolant. A second cut was made parallel to the occlusal surface, 2 mm below the cemento-enamel junction, thereby exposing the pulp chamber. Remnants of pulp tissue in the pulp chamber were removed using an excavator (Carl Martin GmbH, Solingen, Germany) without touching the walls of the pulp chamber.[11] Dentin surfaces were then wet-polished with 600-grit silica carbide paper to create a standard surface roughness and smear layer. The specimens (n=48) were connected to the intrapulpal pressure assembly during bonding and storage following the same procedures described by Mobarak.[12]

Restorative Procedures

Prepared specimens were divided into four groups (n=12) according to the adhesive systems utilized: Adper Single Bond 2 (SB; two-step etch-and-rinse adhesive system, 3M ESPE Dental Products, St Paul, MN, USA), Clearfil SE Bond (CSE; two-step self-etch adhesive system, Kuraray, Tokyo, Japan), AdheSE One (AO; single-step non–fluoride containing self-etch adhesive system, Ivoclar Vivadent, Schaan, Liechtenstein), and AdheSE One F (AOF; single-step fluoride-containing self-etch adhesive system, Ivoclar Vivadent). Material specifications, manufacturers, compositions (batch numbers), and application procedures are listed in Table 1. Resin composite (Valux Plus, 3M ESPE) of shade A1 was applied in two increments of 2 mm each. Each increment was polymerized for 40 seconds using Bluephase C5 (Ivoclar Vivadent) with an intensity $\geq$500 mW/cm^2. Light intensity was checked using a LED radiometer (Kerr Dental Specialties, West Collins Orange, CA, USA). Specimens were then immersed in artificial saliva[13] for 24 hours or six months at 37°C in a specially constructed large incubator to accommodate the intrapulpal pressure assembly.

Microtensile Bond Strength (μTBS) Testing

After storage of the bonded teeth, each tooth was longitudinally sectioned in both mesio-to-distal and buccal-to-lingual directions across the bonded interface to obtain multiple sticks of approximately 0.9 ± 0.01 mm^2 for the μTBS test. From each tooth, the central sticks were collected. A digital caliber was used to check the cross-sectional area and length of the sticks. Sticks of similar length and remaining dentin thickness were tested, which resulted in a total of 24 sticks for each subgroup. Each stick was fixed to the attachment with a cyanoacrylate adhesive (Rocket Heavy, Corona, CA, USA) and stressed in tension using a universal testing machine (Lloyd Instruments Ltd, Ametek Company, West Sussex, UK) at a cross-head speed of 0.5 mm/min until failure. The tensile force at failure was recorded and converted to tensile stress in MPa units using computer software (Nexygen-MT, Lloyd Instruments). Sticks that failed before testing were counted as 0 Mpa,[14,15] while those with premature cohesive failure were discarded and were not included in the calculations.[16] Two-way analysis of variance (ANOVA) with repeated measure was used to compare the effects of adhesive system, storage time, and their interaction. One-way ANOVA was used to test the effect of differences in adhesive systems on the bond strength values at each storage time. This was followed by the Bonferroni post hoc test for pairwise comparison. A *t*-test was used to compare between 24-hour and six-month μTBS mean values for each adhesive system. The significance level was set at $p \leq 0.05$. Data were analyzed using the SPSS Program for Windows (Release 15 for MS Windows,SPSS Inc, Chicago, IL, USA).

Fractured sides of all specimens were inspected under a scanning electron microscope (SEM) (515; Philips, Einhoven, The Netherlands) at different magnifications. Failure modes were evaluated at 100× and were classified into six types, as follows: type 1: Adhesive failure at the dentin side; type 2: Cohesive failure in the adhesive layer; type 3: Mixed failure (adhesive failure at the dentin side and cohesive failure in the adhesive layer); type 4: Mixed all (adhesive at the dentin side, cohesive failure in the adhesive and cohesive in resin composite); type 5: Cohesive failure in resin composite; and type 6: Cohesive failure in dentin. The frequency of each mode of failure was calculated for each subgroup.[17]

RESULTS

Two-way ANOVA with repeated measures revealed that there were statistically significant effects for

Table 1: *Material Specifications, Manufacturers, Compositions, Application Procedures, and Batch Numbers*

Material	Specification	Manufacturer	Composition (Batch Number)	Application Procedures
Adper SingleBond 2	Two-step etch-and-rinse adhesive system	3M ESPE Dental Products, St Paul, MN, USA	Etchant: 35% phosphoric acid, colloidal silica (7523)	Etching: apply for 15 s, water rinsing for 10 s, then blot excess water with minisponge (visibly moist surface)
			Adhesive: Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymers of polyacrylic and polyitaconic acids, silica nanofillers (51202)	Adhesive: apply with gentle agitation for 15 s, gently air-thin for 5 s, and light-cure for 10 s
Clearfil SE Bond	Two-step self-etch adhesive system	Kuraray Medical Inc, Sakazu, Kurashiki, Okayama, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylate, DL-camphorquinone, *N,N*-diethanol-*p*-toluidine, and water (00999A)	Priming: Apply onto the visibly moist dentin surface, then leave undisturbed for 20 s and dry with oil-free mild air flow for 5 s
			Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, DL-camphorquinone, *N,N*-diethanol-*p*-toluidine, and silanated colloidal silica (01486A)	Bonding: Apply one coat of Clearfil SE bond and gently oil-free air-thin for 2 s, then light-cure for 10 s.
AdheSE One	Single-step non–fluoride containing self-etch adhesive system	Ivoclar Vivadent AG, Schaan, Liechtenstein	Derivatives of bis-acrylamide, water, bis-methacrylamide dihydrogen phosphate, amino acid acrylamide, hydroxyl alkyl methacrylamide, silicon dioxide, catalysts, and stabilizer (K41349)	Apply onto the visibly moist prepared tooth and leave for 30 s. Disperse excess amount with a strong stream of air until there is no longer any movement of the material, then light-cure for 10 s.
AdheSE One F	Single-step fluoride -ntaining self-etch adhesive system	Ivoclar Vivadent AG, Schaan, Liechtenstein	Derivatives of bis-acrylamide, water, alcohol, bis-metacrylamide dihydrogen phosphate, amino acid acrylamide, hydroxyl alkyl methacrylamide, alkyl sulfonic acid acrylamide, silicon dioxide, initiators, stabilizers, and potassium fluoride (251379)	Follow same application procedures as AdheSE One adhesive system

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate.

adhesive systems ($p<0.001$) and storage time ($p<0.00001$) but not for their interaction ($p=0.33$). The means and standard deviations (SDs) of the µTBS values of all tested groups are presented in Table 2. One-way ANOVA indicated that there was a significant difference among the adhesive systems when tested after 24 hours ($p<0.0001$) and after six-month storage ($p=0.003$). As shown in Table 2, a Bonferroni post hoc test revealed that AO and AOF were significantly lower than SB and CSE at 24 hours as well as at six months. With regard to the effect of storage time, the *t*-test revealed a significant decrease in bond strength values for SB ($p<0.00001$) and CSE ($p=0.0001$) adhesive systems but not for AO ($p=0.89$) and AOF ($p=0.75$) after six-months of storage. In all groups, the major mode of failure was adhesive at the dentin side (type 1), followed by mixed failure (type 3). Cohesive failures in resin composite (type 5) and in dentin (type 6) were very few (Figure 1). Representative SEM micrographs for some modes of failure are presented in Figures 2 and 3.

DISCUSSION

The µTBS results of both tested adhesive systems, two-step etch-and-rinse (SB) and self-etch (CSE),

Table 2: *Microtensile Bond Strength (μTBS) Values [Mean (standard deviation, SD)] in MPa of Tested Adhesive Systems*[a]

	Adhesive Systems μTBS Values [Mean (SD)]				
Storage Periods	**Adper Single Bond 2**	**Clearfil SE Bond**	**AdheSE One F**	**AdheSE One F**	***P*-Value**
24 h	34.87 (4.9) aA [Ptf/tnt=0/24]	38.11 (5.1) aA [Ptf/tnt=0/24]	16.22 (4.9) bA [Ptf/tnt=5/24]	15.78 (3.9) bA [Ptf/tnt=5/24]	<0.0001
6 mo	20.69 (3.1) aB [Ptf/tnt=0/24]	22.67 (1.8) aB [Ptf/tnt=0/24]	15.60 (1.7) aA [Ptf/tnt=6/24]	14.84 (1.9) aA [Ptf/tnt=6/24]	0.003
p-value	<0.0001	<0.0001	0.89	0.75	

[a] *[ptf/tnt=pretest failure/total number of tested sticks]. Within rows, means with different lowercase letters are statistically significantly different ($p \leq 0.05$, Bonferroni test); Within columns, means with different small capital letters are statistically significantly different ($p \leq 0.05$, t-test).*

revealed significantly higher values compared to the tested single-step self-etch adhesive systems (AO and AOF) after 24-hour and six-month storage times in artificial saliva at 37°C under simulated intrapulpal pressure. This finding correlates with that of Belli and others,[16] who found that SB and CSE were significantly higher than AO under simulated intrapulpal pressure at the 24-hour storage point.

Several reasons could be behind the recorded low bond strength of these single-step self-etch adhesives. One of them is that the combination of acidic hydrophilic and hydrophobic monomers into a single solution may compromise the function of each component.[18,19] It should be noted that AO and AOF have relatively high acidity (pH=1.5).[20] Another reason is that for such types of adhesive systems, a relatively high concentration of solvent is required to keep the monomers blended into the solution. Hence, air-drying of the solvent may not be able to accomplish significant solvent evaporation,[21] which leads to the retention of a greater amount of water in the adhesive layer, even after light-curing. Increased water concentration may indeed dilute the adhesive monomers and lower their inward rate of diffusion as well as limit the degree of polymerization.[22] In the present study, intrapulpal pressure was simulated during bonding and storage, which could intensify the negative effect of residual water retention.

Additionally, Margvelashvili and others[20] reported that the AO adhesive system is an acetone-free

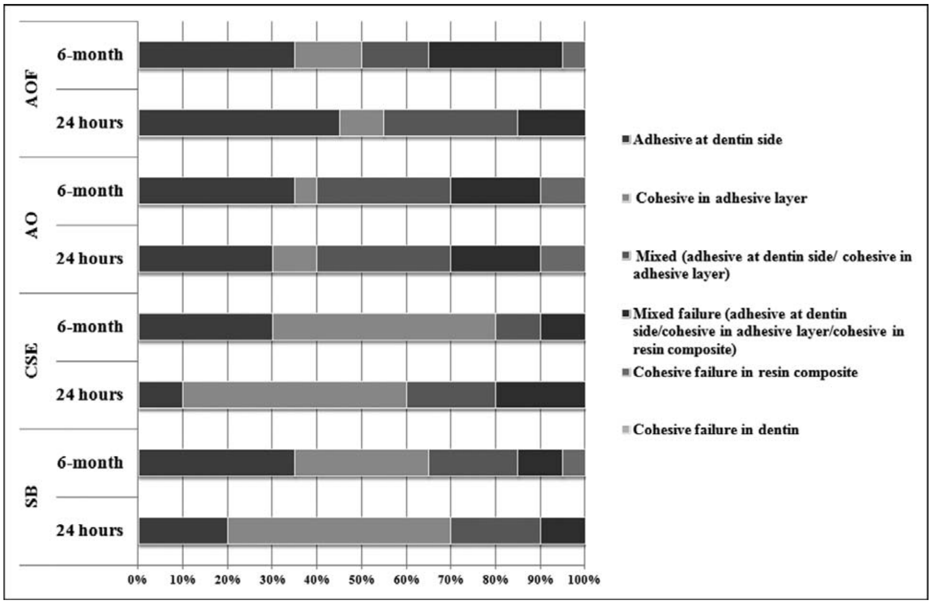

Figure 1. *Modes of failure of tested adhesives.*

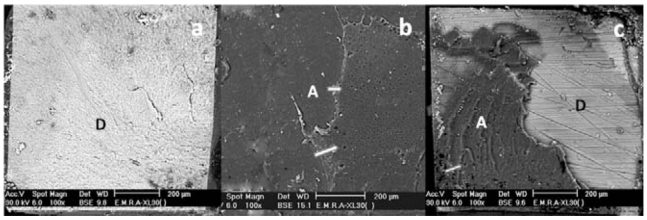

Figure 2. *SEM photomicrography of representative fractured AO specimens after six-month storage showing type 1, adhesive at the dentin side (a); type 2, cohesive failure in the adhesive layer (b); and type 3, mixed failure (adhesive at the dentin side [AD] and cohesive at the adhesive layer [CA]) (c). White arrows denote voids in the adhesive layer.*

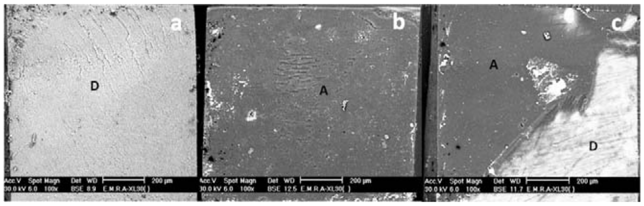

Figure 3. *SEM photomicrography of representative fractured AOF specimens after six-month storage showing type 1, adhesive at the dentin side (a); type 2, cohesive failure in the adhesive layer (b); and type 3, mixed failure (adhesive at the dentin side [AD] and cohesive at the adhesive layer [CA]) (c).*

adhesive system so that, based on the manufacture's claim, the probability of solvent/monomer phase separation was eliminated. In reality, the phase separation that occurred between the hydrophilic and the hydrophobic components of this adhesive cannot be discounted. A varying infiltration gradient can be established as a consequence of phase separation within the adhesive and/or as a result of the difference in molecular weight or affinity to dentin.[19,23] SEM photomicrographs of the AO fractured sticks support the above-mentioned explanations wherein type 2 failure (cohesive failure in the adhesive layer) blisters (droplets) were observed. These droplets were usually detected with single-step self-etch adhesives and were attributed to water sorption from the moist dentin substrate through an osmotic process caused by a gradient imbalance at the interfaces.

The µTBS results of the tested adhesive systems after six-months of storage in artificial saliva and under intrapulpal pressure support the rejection of the null hypothesis. This was clearly shown with SB and CSE, for which µTBS values were significantly decreased after six months of storage. Campos and others[24] agreed with these findings; however, they used thermo-mechanical stressing in addition to the intrapulpal pressure simulation. The decrease in bonding effectiveness of such adhesives may be caused by the degradation of interface components *via* hydrolysis (mainly resin and/or collagen).[2] In etch-and-rinse adhesive systems, there is a discrepancy between the depth of demineralization and resin infiltration, which leads to a zone of hydroxyapatite-depleted collagen fibrils that are left exposed and unsupported.[24,25] The mineralized dentin matrix contains many endogenous enzymes (for example, alkaline phosphatase and metalloproteases) that may be released and activated during etching or more slowly during water storage. These enzymes could attack the denuded collagen,[26] the ester bonds of resins,[27] or both. Thus, the demineralized dentin at the bottom of the hybrid layer would become a weak link in the bonding interface over time.[28] Furthermore, Pashley and others[29] reported that bonding of contemporary etch-and-rinse adhesive systems to water-saturated dentin leaves a very thin layer of water or hydrogel between the infiltrated adhesive and the collagen fibrils of the matrix. This layer seems to provide a fluid-filled continuum from resin tags into the base of the hybrid layer through lateral branches of dentinal tubules throughout the full thickness of the hybrid layer. In addition, permitting the diffusion of water molecules from dentin across the adhesive layer reduces the frictional forces between the polymer chains, causing what is known as plasticization.[27] The aforementioned explanation is supported by the detected shift in the mode of failure patterns of the Adper Single Bond 2 toward a more complex interfacial fracture after six months of storage under intrapulpal pressure.

The decrease in bond strength of CSE after six-months of storage under simulated intrapulpal pressure simulation supports the findings of Abdalla and others.[30] This reduction in bond strength may be attributed to slow water sorption of the adhesive, which decreases its mechanical properties and its bond strength. Hosaka and others[31] suggested that if the acidity of the self-etch adhesives is unable to etch through both smear layer and smear plugs then hybridized smear plug material could prevent water from seeping from the dentinal tubules into the adhesive during bonding. However, the hybridized smear plugs may be porous enough to permit sufficient seepage of water to elute unreacted monomers or oligomers from the bonded interface over the next 90 days of pulpal perfusion, replacing their volume with water that would weaken the adhesion.[32] In addition to 2-hydroxyethyl methacrylate (HEMA), CSE primer contains 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer, which is believed to increase its wetting to moist dentin on one side. Nevertheless, it is hydrophilic and, hence, more prone to hydrolysis.[33]

Interestingly, there was no significant difference in the bond strength values between fluoride-containing and fluoride-free self-etch adhesive systems (AOF and AO) after 24 hours or six months of storage. This finding opposes those of previous researchers,[5,34] who reported stability of fluoride-containing adhesive. This might be explained by the fact that in our study, the AOF we used contains potassium fluoride fillers rather than sodium fluoride fillers, in which case the higher molecular weight of potassium (58.0967 g/mol), compared to sodium (41.89 g/mol), could have played a role. In particular, those that reported bond stability used an adhesive system with sodium fluoride fillers that are characterized by their easy solubility.[35] Therefore, it might be expected that using an adhesive system containing fillers with higher molecular weight might decelerate the fluoride release and, consequently, might not exceptionally stabilize the bond strength over the tested period. It should also be noted that the release of fluoride from the adhesive is time dependent. Thus, further investigation is required to determine whether the possible positive effect of fluoride-containing adhesives on the bond durability can be elucidated over a longer duration than that tested in the present study. Interfacial compositional analysis is also required to detect the release of fluoride and its reaction with the hybrid layer. Moreover, there is still a possibility that the amount of fluoride present in the tested adhesive system was not adequate to reveal a positive effect. The quantity of fluoride that should be included in the adhesive system in order to obtain a significant effect on the bond durability is not yet known.

In the present study, AO and AOF adhesives maintained their stability from 24 hours to six months under intrapulpal pressure storage. The bond stability of these adhesives can be attributed to their altered chemical composition, as they contained acrylamido alkylsulfonic acid that would contribute to a chemical interaction with the tooth substance through the phosphoric ester group. In addition, they contained bifunctional acrylic amides, which are responsible for the formation of the polymer network. Such hydrolytic stability may possibly protect the bond from long-term water degradation.[36] Our AO results corroborate with those of Belli and others,[16] who tested this adhesive under simulated intrapulpal pressure for one year and reported its low but stable bond strength. Meanwhile, the results of this study raised some points of concern that require further investigation. One point is that despite the recorded stability of the adhesives with modified monomer (AO and AOF), they revealed significantly lower μTBS values than did the other multistep adhesives, even after six months. Thus, further investigation is required to determine whether the recorded low bond strength of adhesives with modified monomer (AO and AOF) would remain stable while that of the other adhesives (CSE and SB) will continue to degrade over time, compensating for the large gap that was recorded initially in favor of CSE and SB bond strength. Another negative aspect recorded for these adhesives in both the present study and that of Belli and others[16] was the large number of pre-test failures. The μTBS test procedure is difficult to perform, time consuming, and extremely technique-sensitive, especially in regard to stick preparation, which can result in pre-test failures. However, the presence of the pre-test failure with these two adhesives in particular may point out that these adhesives produce nonuniform hybridization with dentin. This calls for additional research to enhance the homogeneous bonding of these adhesive systems to dentin.

CONCLUSIONS

Based on the results of this study, the following conclusions were drawn:

1. Fluoride addition did not affect dentin bond durability.
2. Despite the fact that the single-step adhesive system with modified monomer showed stability, bond strength remained lower than that associated with the multistep adhesive systems.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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