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Effect of Substrate Age and Adhesive Composition on Dentin Bonding

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Clinical Relevance

The bonding efficacy of current dentin adhesives is not affected by the dentin substrate age. Chemical bonding may play a role in the bonding effectiveness of specific adhesives.

SUMMARY

Purpose: To study the effect of dentin age and adhesive composition on the microtensile dentin bond strengths (μTBS) of five dentin adhesives.

Materials and Methods: Sixty extracted caries-free human teeth were assigned to the appropriate age group: less than 21 years of age (<21), 21–40 years of age (21–40), and greater than 40 years of age (>40). For each age group, specimens were randomly divided into five dentin adhesives: (1) Adper Easy Bond (EB, 3M ESPE), a one-step self-etch adhesive; (2) Experimental Adper Easy Bond without the Vitrebond Co-polymer (CP) (EBnoCP, 3M ESPE); (3) Adper Single Bond Plus (SB, 3M ESPE), a two-step etch&rinse adhesive; (4) Experimental Adper Single Bond Plus without CP (SBnoCP, 3M ESPE); and (5) Adper Scotchbond Multi-Purpose (MP, 3M ESPE), a three-step etch&rinse adhesive, as the control group. Specimens were sectioned in X and Y directions and the resulting beams were tested to failure in tension mode at a crosshead speed of 1 mm/min. Statistical analysis was computed using *t*-test and two-way analysis of variance followed by Fisher least significant difference multiple comparison *post hoc* test at $p < 0.05$.

Results: The highest mean μTBS values were obtained in the control group (MP) for all age groups. EB resulted in statistically similar mean μTBS compared to EBnoCP for all age groups: $p = 0.538$ for (<21); $p = 0.974$ for (21–40); and $p = 0.909$ for (>40). SB resulted in statistically higher mean μTBS than SBnoCP for all age groups [$p<0.009$ for (<21); $p<0.028$ for (21–40); and $p<0.041$ for (>40)]. MP, the control group, resulted in statistically lower mean μTBS when applied to the oldest age group (>40) compared to the youngest age group (<21), at $p < 0.04$. When means were pooled for the variable 'age group,' SB resulted in

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significantly higher mean μTBS than SBnoCP at $p < 0.009$, while EB resulted in statistically similar mean μTBS compared to EBnoCP (p=0.9). MP resulted in statistically higher mean μTBS than all other adhesives—SB (p<0.0001), SBnoCP (p<0.0001), EBnoCP (p<0.022), and EB (p<0.046).

Conclusions: **The substrate age influenced the bonding ability of the three-step etch&rinse adhesive. The presence of a carboxylic-based polymer (CP) enhanced the bonding ability of the two-step etch&rinse adhesive.**

INTRODUCTION

Dentin adhesion has not yet achieved the ideal characteristics as a result of dentin tubular structure, organic content, and intrinsic moisture.[1] Physiological changes resulting from dentin aging or changes in response to caries and other aggressive stimuli increase the degree of mineralization of dentin, with a consequent increase in dentin thickness and reduction of dentin permeability.[2–4] Since dentin permeability is an important factor in the adhesion process, reduction of permeability with age may have a direct effect on dentin bond strengths.[1,5] In spite of increased dentin calcification with age, adhesion studies[6–9] have not shown an obvious correlation between dentin age and bonding ability of dentin adhesives.

The interaction of acids or acidic monomers with hydroxyapatite is a fundamental factor in the adhesion process. Acids demineralize dental hard tissues,[10] opening a pathway for the infiltration of resin monomers into the microporosities previously occupied by hydroxyapatite crystals.[4] For dentin, these monomers polymerize *in situ* within the collagen network spaces to create a hybrid layer of collagen and resin.[4,11] While micromechanical dentin-resin entanglement is essential for immediate dentin bond strengths, chemical adhesion would be desirable to improve bonding stability.[4]

Polycarboxylic or polyalkenoic acids promote a stable chemical bonding between the carboxylate groups (COO^-) and calcium in hydroxyapatite.[12–14] Glass ionomer cements (GICs) are the only direct restorative material to bond chemically to hard dental tissues as a result of the formation of ionic bonds between carboxylate groups and calcium, enhanced by a shallow interfacial absorption layer into the dentin for the newest resin-modified GIC.[12,14,15] The chemical adhesion provided by GIC has led some manufacturers to introduce carboxylate-based polymers in the composition of dental adhesives. The polyalkenoic acid copolymer first used in Vitrebond (3M ESPE, St Paul, MN, USA) is now known as the Vitrebond copolymer.[16] Mitra and others[16] reported that this specific copolymer bonds chemically to calcium in hydroxyapatite. As several adhesive formulations from the same manufacturer (3M ESPE) now contain this molecule, it is relevant to study whether the copolymer improves the bonding ability of dentin adhesives.

The null hypotheses tested in this study were (1) dentin age does not influence the bonding ability of dentin adhesives; and (2) the inclusion of a polyalkenoic acid copolymer in the composition of two adhesives does not influence dentin microtensile bond strengths (μTBS).

MATERIALS AND METHODS

Sixty extracted sound third molars and maxillary premolars were collected in function of the patient's age (n=20, 15 molars and five premolars per age group): Less than 21 years of age (<21, only teeth with complete root formation); between 21 and 40 years of age (21–40); and greater than 40 years of age (>40). Premolars were used as a result of the difficulty in obtaining extracted molars categorized by age. Teeth were then stored in 0.5% chloramine solution for up to one month and left in distilled water for 24 hours at 4°C prior to use. Middle dentin was exposed by sectioning the crowns parallel to the occlusal surface in a slow-speed diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) under water-cooling. Dentin was polished with wet 600-grit silica-carbide abrasive paper for 60 seconds to create a standardized smear layer.[17] For each age group, specimens were randomly and equally assigned to five dentin adhesives (three molars and one premolar per subgroup), as follows: Adper Easy Bond (EB, 3M ESPE); Adper Easy Bond without the Vitrebond Co-polymer (CP) (EBnoCP, 3M ESPE); Adper Single Bond Plus (SB, 3M ESPE); Adper Single Bond Plus without CP (SBnoCP, 3M ESPE); and Adper Scotchbond Multi-Purpose (SMP, 3M ESPE), a three-step etch&rinse adhesive, as the control group (Table 1). The adhesives were applied with a microbrush (Microbrush International, Grafton, WI, USA) and polymerized according to the manufacturer's instructions (Table 1). The crowns were restored with Filtek Z250, shade A2 (3M ESPE) in three increments of 2 mm each using an Elipar S10 (3M ESPE) curing light with an output of >800 mW/cm^2. Specimens were then automatically sectioned with a slow-speed diamond saw (Accutom 50,

Table 1: *Materials, Batch Numbers, Compositions, and Instructions for Use*

Material	Composition	Instructions for Use
Adper Easy Bond (EB), Lot 380005	Bis-GMA; HEMA; water (10–15 Wt%); ethanol (10–15 Wt%); phosphoric acid-6-methacryloxy-hexylesters; silane-treated silica; 1,6-hehadeniol dimethacrylate; copolymer of acrylic and itaconic acid (Vitrebond copolymer) (1–5 wt%); (dimethylamino)ethyl methacrylate, camphorquinone; 2,4,6-trimethylbenzoyldiphenylphosphine oxide	Dry the cavity with gentle stream of air free of water and oil or by blotting with cotton pellets. Do not overdry. Apply the adhesive with the disposable applicator for 20 s to all surfaces of the cavity. Rewet the disposable applicator as needed during application. Subsequently, air-thin the liquid for approximately 5 s until the film no longer moves, indicating complete vaporization of the solvent. Cure the adhesive with a commonly used curing light for 10 s.
Experimental Adper Easy Bond (EBnoCP), Lot HB2-primer-0427	Same as for Adper Easy Bond, but without the Vitrebond copolymer	Same as for Adper Easy Bond
Adper Single Bond Plus (SB), etchant: Lot N103490; adhesive: Lot 153567-41-3	Etchant: amorphous silica-thickened 35% phosphoric acid gel	Apply Scotchbond Etchant to tooth surface for 15 s. Rinse thoroughly for 10 s. Blot excess water using a cotton pellet or minisponge. Do not air dry! Apply two to three consecutive coats of adhesive for 15 s with gentle agitation using a fully saturated applicator. Gently air thin for 5 s to evaporate solvent. Light cure for 10 s.
	Adhesive: ethyl alcohol (25–35 Wt%); silane-treated silica (nanofiller); Bis-GMA; HEMA glycerol 1,3-dimethacrylate; copolymer of acrylic and itaconic acid (Vitrebond copolymer) (5-10 wt%); diurethane dimethacrylate; water (<5%)	
Experimental Adper Single Bond Plus (SBnoCP), etchant: Lot N103490; adhesive: Lot 153567-41-2	Same as for Adper Single Bond Plus, but without the Vitrebond copolymer	Same as for Adper Single Bond Plus
Adper Scotchbond Multi-Purpose (MP), etchant: Lot N103490; primer: Lot 120768; adhesive: Lot 120161	Etchant: amorphous silica-thickened 35% phosphoric acid gel	Apply Scotchbond etchant to enamel and dentin. Wait 15 s. Rinse for 15 s. Dry for 5 s. Apply Adper Scotchbond Multi-Purpose primer to etched enamel and dentin. Dry gently for 5 s. Apply Adper Scotchbond Multi-Purpose adhesive to the primed enamel and dentin. Light cure for 10 s.
	Primer: water (40–50 Wt%); HEMA (35–45 Wt%); copolymer of acrylic and itaconic acids (Vitrebond copolymer) (10–20 wt%)	
	Adhesive: Bis-GMA (60–70 Wt%); HEMA (30–40 Wt%)	
Filtek Z250, Lot N117387	Bis-EMA, TEGDMA, UDMA, zirconium, silica	

Bis-EMA: bisphenol A-polyethylene glycol dieter dimethacrylate; Bis-GMA: bisphenol A diglycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate, TEGDMA: triethyleneglycol-dimethacrylate; UDMA: urethane dimethacrylate.

Struers A/S, Ballerup, Denmark) into two perpendicular directions to obtain beams with a cross section of 0.7 ± 0.2 mm^2. The beams from the periphery were discarded. The remaining beams were individually attached to a stainless-steel grooved jig with cyanoacrylate glue (Zapit, Dental Ventures of America Inc, Corona, CA, USA) and tested to failure in tension mode (the jig was in line relative to the axis of the applied load) using a universal testing machine (Shimadzu Autograph AG-IS, Tokyo, Japan) at a crosshead speed of 1 mm/min. Failures were analyzed under a stereomicroscope (Leica MZ6, Leica Microsystems AG, Heerbrugg, Switzerland) at 20×. The mode of failure was classified as adhesive (A), mixed (M), and cohesive. Failures were considered A when they occurred at the dentin-adhesive interface; they were of cohesive nature when the failure occurred in dentin (CD) or in composite (CC); and they were of M nature when there was composite and dentin at the interface.

For each tooth, the μTBS values of all beams were averaged for statistical purposes, each tooth serving

Table 2: *Number of Beams per Group, Each Tooth as a Statistical Unit; Mean Microtensile Dentin Bond Strength (μTBS) ± Standard Deviation (SD) (MPa)*[a]

	<21		21–40		>40		All Ages
	No. of beams[b]	μTBS, Mean ± SD	No. of Beams[b]	μTBS, Mean ± SD	No. of Beams[b]	Mean μTBS, Mean ± SD	μTBS Pooled for Age Group, Mean ± SD
EB	64	60.7 ± 4.4[A,a]	58	56.0 ± 4.0[A,e,f]	68	59.0 ± 9.3[A,g]	58.6 ± 6.1[k]
EBnoCP	58	57.2 ± 5.8[B,a]	64	56.1 ± 7.7[B,e,f]	65	58.3 ± 7.7[B,g]	57.2 ± 6.5[k]
SB	76	44.0 ± 14.4[C,b]	98	47.0 ± 12.6[C,e]	98	42.9 ± 7.3[C,h]	44.7 ± 10.8[l]
SBnoCP	70	27.4 ± 5.3[D,c]	89	32.5 ± 4.7[D,g]	54	30.9 ± 4.4[D,i]	30.3 ± 4.9[m]
MP	89	71.4 ± 4.6[E,d]	113	65.3 ± 10.1[E,F,f]	95	59.6 ± 10.2[F,g]	65.4 ± 9.5[j]

Abbreviations: EB, Adper Easy Bond; EBnoCP, EB without Vitrebond copolymer; MP, Adper Scotchbond Multi-Purpose; SB, Adper Single Bond Plus; SBnoCP, SB without Vitrebond copolymer; <21, less than 21 years of age; 21–40, between 21 and 40 years of age; >40, more than 40 years of age.
[a] *Within each row, means with the same superscript uppercase letter are not significantly different at p > 0.05; within each column, means with the same superscript lowercase letter are not significantly different at p > 0.05.*
[b] *The statistical software automatically compensates for the different number of beams.*

as a statistical unit. Once a normal distribution and equality of the variances were confirmed, statistical analyses included two-way analysis of variance to determine whether a significant difference existed between dentin ages using different adhesives, followed by Fisher least significant difference multiple comparison *post hoc* tests at $p < 0.05$. The fracture types were compared using Spearman correlation coefficient (SPSS 14.0, SPSS Inc, Chicago, IL, USA).

RESULTS

The mean bond strengths, respective standard deviations, and the number of beams tested per group are displayed in Table 2. EB resulted in statistically similar mean μTBS compared to EBnoCP for all age groups: $p = 0.538$ for (<21); $p = 0.974$ for (21–40); and $p = 0.909$ for (>40). SB resulted in statistically higher mean μTBS than SBnoCP for all age groups [$p<0.009$ for (<21); $p<0.028$ for (21–40); and $p<0.041$ for (>40)]. MP, the control group, resulted in statistically lower mean μTBS when applied to the oldest age group (>40) compared to the youngest age group (<21) at $p < 0.04$. All the other pairwise comparisons within the same adhesive resulted in no statistical significance. There were no significant interactions between the independent variables ($p>0.123$).

When means were pooled for the variable 'age group' (Table 2, far right column), MP resulted in statistically higher mean μTBS compared to all other adhesives—SB ($p<0.0001$), SBnoCP ($p<0.0001$), EBnoCP ($p<0.022$), and EB ($p<0.046$). SB resulted in significantly higher mean μTBS than SBnoCP at $p < 0.009$, while EB resulted in statistically similar mean μTBS compared to EBnoCP ($p=0.9$).

There were no pretesting failures in any of the groups. The distribution of the type of failures per group is displayed in Table 3. Most failures (72%) were of the A type. There was a significant correlation between fracture type and adhesive/age group (Spearman rho, $p<0.01$), with MP resulting in a greater number of cohesive failures in dentin.

DISCUSSION

The amount of secondary dentin is directly correlated with dentin age.[18] As teeth age, dentin continues to be secreted, resulting in the dentinal tubules becoming narrower.[2] This increased calcification is part of physiological aging (or physiological sclerosis), as well as a response to external stimuli such as attrition and caries.[2,3] Adhesion studies have not shown a direct correlation between dentin age and dentin bonding. A study compared the second-generation adhesive Scotchbond DC with the third-generation adhesive Scotchbond 2, both of which were applied in teeth with a mean age of 22.5 vs 65.6 years.[9] Scotchbond 2 contains maleic acid and 2-hydroxyethyl methacrylate (HEMA) in the primer; therefore, it may be considered a predecessor of self-

Table 3: *Type of Fractures per Group (%)*

Fracture Type (Total %)	Adhesive/Age														
	EB			EBnoCP			SB			SBnoCP			MP		
	<21	21–40	>40	<21	21–40	>40	<21	21–40	>40	<21	21–40	>40	<21	21–40	>40
A (72.1)	70.3	70.7	85.3	65.5	98.1	83.1	69.7	62.2	79.6	92.9	82.0	85.2	56.2	55.6	56.8
M (7.8)	0	3.4	0	3.4	0	0	21.0	22.4	4.1	2.8	7.9	6	16.8	11.3	0
CD (17.9)	21.9	24.1	13.2	27.6	1.9	15.4	5.3	12.2	14.3	4.3	9.0	2	22.5	30.8	43.2
CC (2.2)	7.8	1.4	1.5	3.4	0	1.5	3.9	3.1	2.0	0	1.1	0	4.5	2.3	0

Abbreviations: A, adhesive; CC, cohesive in composite; CD, cohesive in dentin; EB, Adper Easy Bond; EBnoCP, EB without Vitrebond copolymer; M, mixed; MP, Adper Scotchbond Multi-Purpose; SB, Adper Single Bond Plus; SBnoCP, SB without Vitrebond copolymer; <21, less than 21 years of age; 21–40, bewteen 21 and 40 years of age; >40, more than 40 years of age.

etch adhesives. Authors reported shorter marginal gaps when Scotchbond 2 was used in older teeth. Another study,[7] which included a copolymer-containing dentin adhesive (Scotchbond Multi-Purpose), did not find any correlation between dentin bond strengths and dentin age for teeth younger than 30 years vs. those older than 50 years of age. A different study[6] used an acetone-based etch&rinse two-step adhesive and a resin-modified GIC in young subjects vs. subjects older than 60 years of age. No differences in bond strengths were found for any of the materials for different ages. A more recent study[8] used Single Bond (the unfilled version of SB used in the present study) in dentin from 18 to 22–year-old or 55- to 60–year-old patients. Bonding to older dentin with 30 seconds of etching time resulted in higher bond strength than was achieved when dentin was etched for 15 seconds. However, no statistical differences were found between young and older dentin for the same etching time.[8]

The relatively high bond strengths obtained with the self-etch adhesive EB were somewhat unexpected. A recent research project[19] using the same metholodogy found lower mean bond strengths for EB than were obtained in the present study. The operator-related variability may have, therefore, played an important role. According to the respective manufacturer's literature, the composition of EB is similar to that of SB, except for the presence of methacrylated phosphoric esters in the former.[20] This similarity between the chemical composition of both adhesives may indicate that the bond strengths depend not only on the type of surface treatment prior to applying each material (ie, etching with phosphoric acid with a pH of 0.5 vs the application of an acidic monomer with a pH of 2.4[21]) but also on the way in which the material is applied on the dentin surface. Using transmission electron microscopy (TEM), Mine and others[21] showed a thin dentin hybrid layer as a result of the application of EB, resembling other 'ultra-mild' self-etch adhesives. The same authors suggested that the chemical interaction between the carboxylate groups in EB and the hydroxyapatite crystals available on the mildly decalcified dentin surface might be responsible for the bonding ability of EB.

In the present study, the presence of the copolymer (CP) in EB did not influence mean dentin bond strengths independent of the age group. On the other hand, SB (the commercial version with CP) resulted in significantly higher dentin bond strengths than SBnoCP for all of the three age groups. This apparent paradox may be explained by the concentration of the CP in the two adhesives. While EB contains 1–5%wt of CP, SB contains twice as much CP (Table 1). Therefore, the removal of the CP from SB, as in the SBnoCP group, may be more detrimental to the behavior of this particular adhesive than the removal of CP from the composition of EB, as in the EBnoCP group.

Polycarboxylates used in classical GIC-based materials do not demineralize dentin as deeply as phosphoric acid; however, they result in stable chemical adhesion between COO^- groups and Ca^{2+} groups in hydroxyapatite.[12,13,16] Carboxylate groups replace phosphate ions of the substrate and bond ionically with calcium of hydroxyapatite.[13] This chemical bonding mechanism is explained by the adhesion-decalcification concept.[10] A different reaction occurs with resin-modified GIC. The self-adhesive bonding mechanism of these materials is twofold: (1) the ionic bonding to hydroxyapatite around collagen, as in conventional GIC, and (2) the micromechanical interlocking for those resin-modified GIC that additionally hybridize dentin.[22] For Vitrebond, the first material to contain the copolymer used in EB and SB, there is no evidence of the second mechanism (ie, hybridization or gel phase deposition).[22] Since the bonding associated with Vitrebond is stable and there is an tight contact between this material and dentin, chemical interaction is the primary bonding mechanism for this resin-modified GIC.[22] A five-year clinical study[23] with a CP-containing resin-modified GIC material reported excellent retention rates. As Mitra and others[16] reported, the CP in Vitrebond bonds chemically to calcium in hydroxyapatite, which supports the idea that the CP in dentin adhesives may also bond chemically to hydroxyapatite. The percentage threshold of CP for effective bonding effectiveness remains to be determined, as it may vary depending on the solvents and other components of each adhesive system. Nevertheless, this chemical adhesion may have somehow contributed to the good clinical performance of etch&rinse CP-containing materials.[24–26] On the other hand, there are not many published clinical studies with EB. A recent study[27] in noncarious cervical lesions reported a 100% retention rate at 12 months, which was not statistically different from that of SB (92.86%).

Taking into account that acid etching removes calcium from the superficial 3–4 μm of intertubular dentin, the interaction of the CP with calcium after etching (as in SB and MP) is somewhat difficult to understand. Using TEM, Van Meerbeek and others[28] showed a deposition of a dark electron-dense amorphous phase on the dentin surface, extending into the dentin tubule orifices and lining the tubule walls when dentin was bonded with Scotchbond Multi-Purpose. As the tubules walls are not completely demineralized, it is quite possible that the chemical bonding to calcium on the tubule walls is partially responsible for the bonding efficacy of both SB and MP. Additionally, the deposition of calcium-carboxylate salts on dentin may be responsible for the moist resistance of bonded interfaces.[29]

The decrease in bond strengths for MP in the >40 age group indicates that this particular material may not bond as well to older dentin. One study[30] compared the bond strengths to caries-affected dentin of MP with and without the polyalkenoate component (CP) in the primer. Removal of CP from the primer lowered the bond strength of MP to caries-affected dentin, which indicates that the amount of residual calcium in caries-affected dentin may be crucial to establishing ionic bonding with the polyalkenoate in the primer. In our study, we used ideal dentin without preexisting caries lesions or old restorations, which does not allow for a direct comparison between the results of both studies.

One of the limitations of the present study is that when data are pooled, as in μTBS testing, the variance is averaged out and information about the variation may be lost. Additionally, the number of beams varied among groups, as a function of the dimensions of the teeth used. Regarding the high number of cohesive fractures in dentin for MP, the ultimate bond strength of the bonding resin (the hydrophobic resin in MP) may have played a role in the dentin bonding ability of the respective adhesive, as shown in previous studies,[31,32] leading to a greater number of cohesive failures.

We have to reject both null hypotheses. Although dentin age was not a significant factor when means were pooled for dentin adhesive, age influenced the bonding ability of MP for the older age group, and the presence of a polyalkenoic acid copolymer (CP) in the composition of SB increased the respective dentin μTBS.

CONCLUSIONS

Within the limitations of an *in vitro* study, the substrate age influenced the bonding ability of the three-step etch&rinse adhesive. The presence of a carboxylic-based polymer (CP) enhanced the bonding ability of the two-step etch&rinse adhesive Adper Single Bond Plus (as in the commercial version), resulting in statistically higher mean μTBS compared to the same formulation without the CP.

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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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