Original Article

Effect of Dimethyl Sulfoxide on Bond Strength of a Self-Etch Primer and an Etch and Rinse Adhesive to Surface and Deep Dentin

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KEY WORDS
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ABSTRACT

Statement of the Problem: Composite bond to dentin is crucial in many clinical conditions particularly in deep cavities without enamel margins due to insufficient penetration of adhesive into demineralized dentin.

Purpose: The aim of this study was to assess the shear bond strength (SBS) of a methacrylate-based and a silorane-based composite resin to surface and deep dentin after pretreatment with dimethyl sulfoxide (DMSO).

Materials and Method: Eighty extracted human premolars were randomly divided into two groups of flat occlusal dentin with different cuts as A: surface group (sections just below the dentinoenamel junction (DEJ) and B: deep group (2 mm below DEJ). Each group was randomly assigned to 4 subgroups and their samples were restored with Adper Single bond (ASB) and Filtek Z350 or Silorane system Adhesive (SA) and Filtek P90 composite resins, using a 3×3mm cylindrical plastic mold. following these steps , the subgroups were assigned as SubgroupA_1: surface dentin+ Silorane System Primer (SSP)+ Silorane System Bonding (SSB)+ P90; Subgroup A_2: surface dentin+ 37% etchant (E37%) + Adper Single Bond (ASB)+ Z350; Subgroup A_3: surface dentin+ DMSO+ SSP+ SSB+ P90; Subgroup A_4: surface dentin+ E37%+ DMSO+ ASB+ Z350; Subgroup B_1: deep dentin+ SSP+ SSB+ P90; Subgroup B_2: deep dentin+ E37%+ ASB+ Z350; Subgroup B_3: deep dentin+ DMSO+ SSP+ SSB+ P90; Subgroup B_4:dentin +E37% +DMSO +ASB +Z350. The specimens were thermocycled at 5± 2/55± 2°C for 1000 cycles and then tested for SBS.

Results: Using DMSO as dentin conditioner increased SBS of ASB to deep dentin (p< 0.001) and SBS of SA to surface dentin (p= 0.003) but had no effect on SBS of SA to deep dentin (p= 1.00).

Conclusion: The ability of DMSO to increase SBS of ASB to deep dentin provides a basis for improving bonding of this composite resin in deep cavities.

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Introduction

In mid-1960s, composite resin restorative materials were introduced to dentistry. [1] These materials have gained high popularity among dentists and patients. They have more conservative cavity designs which basically rely on the effectiveness of current dentin bonding agents. [2] However, composite resins still have some limitations in use due to their physical properties, [3-5] polymerization shrinkage, [6] microleakage, low wear resistance and color stability, [7-8] and lower bond strength to dentin compared with enamel. [9] These properties are related to each other somehow; polymerization shrinkage can cause composite debonding, [6] leading to gap formation, microleakage, secondary caries and so on. [10]

Thus composite resin bond strength to dentin plays an important role in restoration success since the major surfaces of a preparation to be bonded are in dentin, in different depths, sometimes without any enamel margins. Although enamel and dentin are both highly hydrophilic, bonding mechanism seems to be different in each substrate because of basic differences in their organic and inorganic contents. [11] Therefore, bonding to dentin presents a much greater challenge than to enamel, [12] especially in deeper dentin due to a decrease in inter-tubular dentin and an increase in tubular diameter as well as tubular fluid. [13-14]

Efforts to improve clinical performance of methacrylate-based composite resins have led to the development of new monomers such as ring-opening silorane and new filler technology such as nanofillers. [15] Filtek P90 (P90) and Filtek Z350 (Z350) are two dental composite resins on the market that have lower polymerization shrinkage [16] and improved mechanical properties, [17] respectively when compared with other composite resins. Ring-opening polymerization technique in the silorane system, like P90, is a recent important development in dental composite resins. The term silorane comes from "siloxane" and "oxirane", which combines their two advantages, i.e. high hydrophobicity from siloxane and ring-opening monomer from oxirane, to make silorane, with a self-etch and primer adhesive system. [18] The ring-opening process seems to be insensitive to oxygen-inhibiting action unlike methacrylate-based composite resins, which hinders polymerization by converting free radicals to stable species. [19] Generally, the mechanical properties of composite resins are related to their filler load; the more filler load in the composition the higher the mechanical properties such as wear resistance, modulus of elasticity and less polymerization shrinkage. [20] Z350 is a recently introduced nanofilled composite resin with 65–75 wt% of silica and zirconia nanofillers, [21] which has been claimed to have a low shrinkage due to its high filler content. [22]

Attempts have been made to enhance composite resin bond strength to dentin. One strategy is to condition the dentin with disinfectants like ozone gas, [23] oxalate desensitizer, [24] NaOCl or EDTA [25] and CHX. [22, 26]

In total etch adhesive systems, the adhesive may not reach the entire depth of demineralized dentin, [11] thus using a penetration enhancer may improve adhesive penetration into demineralized dentin. Recently Tjäderhane *et al.* [27] evaluated the effect of dentin pretreatment with dimethyl sulfoxide (DMSO: $(CH_3)_2SO$) before application of the adhesive. This compound is one of the best known penetration enhancers for medical purposes due to its dipolar amphiphilic nature and small size. [28] The potential for tissue penetration and excellent solvent properties make DMSO an attractive solvent candidate for the dental adhesives. [27] The few studies on DMSO available in dentistry are limited to its cryoprotection ability in preservation of pulpal and periodontal ligament undifferentiated cells. [29]

To the best of our knowledge no studies have been carried out on deep and surface dentin pretreatment with dimethyl sulfoxide for the analysis of the shear bond strength. The aim of this *in vitro* study was to evaluate the effect of surface and deep dentin pretreatment with dimethyl sulfoxide on shear bond strength (SBS) of a silorane-based and a methacrylate-based composite resin.

Materials and Method

Preparation of specimens

In this experimental *in vitro* study, 80 recently extracted human maxillary first premolars, without any caries and cracks, stored in 0.2% sodium azide, were used. The teeth were cleaned and mounted 2 mm below the cementoenamel junction in self-polymerizing acrylic resin (Acropars, Iran) to simulate tooth position in the alveolar bone. The teeth were randomly divided into two groups (n=40), for sections of surface dentin (group A) or deep dentin (group B). The occlusal surfaces of the teeth were sectioned using diamond discs (D&Z; Germany) under water cooling to remove the occlusal enamel and reach the central groove to expose the surface dentin just beneath the dentinoenamel junction in group A and 2 mm below the central groove to meet the deep dentin in group B as in previous studies. [30] The exposed dentin surface of all the specimens was ground with 600-grit abrasive paper to create a standardized smear layer. Then the specimens in each group were randomly assigned to 4 subgroups (n=10), according to the type of composite resins used (two types) and pretreatment (with or without pretreatment) (Figure 1).

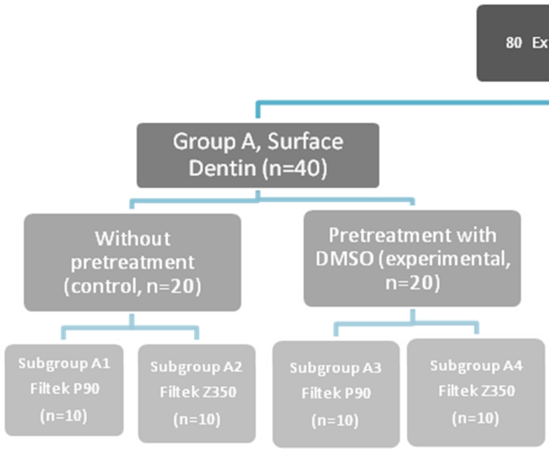

Figure 1: The study design

All the specimens were restored in accordance with the manufacturers' instructions. Composite resin build-up was done by using a 3×3-mm cylindrical translucent plastic mold, placed on the center of each specimen. A light-curing unit (Coltolux II; Coltene, USA) having a light output of 600 mW/cm^2 was used to cure the adhesive systems and composite resins. The light cure unit has been checked frequently after preparation of 5 teeth by using a radiometer (CM 300-1000; Halogen Lightmeter, China). The details of tested materials and their ingredients are summarized in Table 1.

Subgroup A_1: Silorane Adhesive System primer (3M ESPE, USA) was applied with a microbrush on dentin after shaking the bottle well, gently massaged for 15 s, dried with a gentle stream of air and light-cured for 10 s. Then Silorane Adhesive System bond (3M ESPE, USA) was applied, followed by a gentle stream of air and light-cured for 10 s. Filtek P90 composite resin (3M ESPE, USA) was placed in two increments of 1.5-mm thickness and each was light-cured for 40 s.

Subgroup A_2: Acid phosphoric etchant gel (Etchant 37; DenFil Vericom Co. LTD, Korea) was applied on dentin for 15 s, rinsed with water thoroughly for 10 s and dried for 3–5 s with a gentle stream of air, leaving the surface slightly moist. Two consecutive coats of Adper Single Bond (3M ESPE, USA) were applied and light-cured for 10 s after gentle drying. Filtek Z350 (3M ES PE, USA) was placed in two increments measuring 1.5 mm in thickness and each was light-cured for 40 s.

Subgroup A_3: Dentin was pretreated with 0.004% dimethyl sulfoxide (Merck, Germany), which was obtained from diluting 3.55 ml DMSO with distilled water to have 100 ml 0.004% DMSO, for 30 s and gently dried, followed by the steps described for subgroup 1.

Subgroup A_4: Etchant gel was applied on dentin for 15 s, rinsed thoroughly for 10 s and dried with a gentle stream

Table 1: Materials, manufacturers and chemical compositions

Material	Manufacturer	Lot number	Type	Composition
Silorane Adhesive System primer	3M ESPE, USA	N469266	Two-step self-etch acid primer	Phosphorylated methacrylates, Vitrebond copolymer, Bis-GMA, HEMA, water/ ethanol solvent, silane treated silica fillers
Silorane Adhesive System bond	3M ESPE, USA	N468024	Two-step self-etch adhesive	Phosphorylated methacrylates, hydrophobic dimethacrylate, TEGDMA, silane treated silica fillers
Filtek P90	3M ESPE, USA	N496908	Silorane- based composite resin	Monomers: 3,4- epoxycyclohexyl- ethyl- cyclo-poly- methylsiloxane (5- 15 % w/w), bis-3,4-epoxycyclohexyl- ethyl- phenyl- methylsilane (5-15 % w/w) Fillers: SiO_2, YtF_3 (55% v, 76% w)
Etchant 37%	DenFil Vericom Co. LTD, Korea	ET426137		H_3PO_4 37%
Adper Single Bond	3M ESPE, USA	N389084	Two-step etch-and-rinse adhesive	Bis- GMA, HEMA
Filtek Z350	3M ESPE, USA	N495372	Methacrylate-based composite resin	Monomers: Bis-GMA, UDMA, TEGDMA, PEGDMA, bis- EMA Fillers: Zirconia/ silica (63.3% v, 78.5% w)
Dimethyl sulfoxide	Merck, Germany			3.55 ml DMSO was diluted with distilled water to have 100 ml 0.004% DMSO

of air, leaving the surface slightly moist. Dentin was pretreated with 0.004% DMSO for 30 s and gently dried, followed by the bonding and restoration steps described for subgroup 2.

Subgroup B_1: The teeth were restored as previously described for subgroup A_1.

Subgroup B_2: The teeth were restored as previously described for subgroup A_2.

Subgroup B_4: The teeth were restored as previously described for subgroup A_3.

Subgroup B_3: The teeth were restored as previously described for subgroup A_4.

The teeth were stored in distilled water for 24 h at 37°C to allow for water sorption and postoperative polymerization. The teeth were then thermocycled (PC300; Vafaei, Iran) in distilled water at 5±2/55±2°C for 1000 cycles with a dwell time of 30 s.

There was a significant interaction effect between composite resin type and dentin depth (p= 0.001), composite resin type and DMSO pretreatment (p= 0.005) but not between dentin depth and DMSO pretreatment (p= 0.82). We conducted a Tamhane post hoc test to compare the subgroups.

Shear bond strength analysis

Each specimen was individually subjected to a shear load applied by a wedge-shaped blade with a thickness of 1 mm in a universal testing machine (ZO20; Zwick/ Roell, Germany) at a crosshead speed of 1 mm/min until failure occurred (Figure 2).

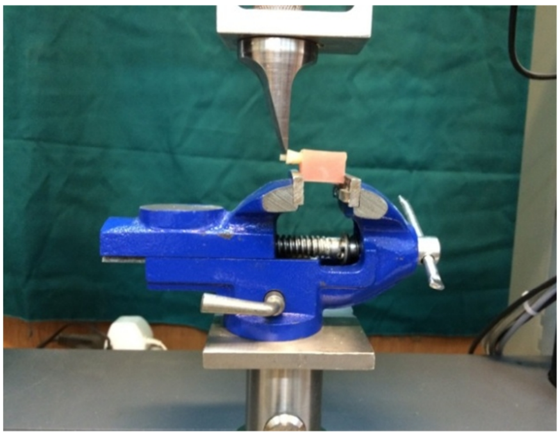

Figure 2: Shear bond strength test

The force (Newton) at failure was recorded and the shear bond strength values (MPa) were calculated from the peak load at failure divided by the bonded surface area in mm^2 ($A=\pi r^2$,3.14×1.5×1.5=7.065mm^2).

Statistical analysis

Three-way analysis of variance (ANOVA) and Tamhane post hoc test were conducted by SPSS 15. Statistical significance was set at 0.05.

Results

The mean values and standard deviations of shear bond strengths are summarized in Table 2.

Table 2: The mean values and standard deviations of SBS

Sub-group	Definition	Mean (MPa)	Std. deviation
A_1	Surface dentin+SA (control)	8.83^{AD}	1.15
A_2	Surface dentin+ASB (control)	14.80^{B}	1.81
A_3	Surface dentin+DMSO+SA	11.04^{C}	0.71
A_4	Surface dentin+DMSO+ASB	16.46^{B}	2.54
B_1	Deep dentin + SA (control)	8.21^{AD}	0.84
B_2	Deep dentin + ASB (control)	9.46^{A}	0.82
B_3	Deep dentin + DMSO + SA	7.87^{D}	1.01
B_4	Deep dentin + DMSO + ASB	13.38^{B}	1.60

* Statistically significant difference (p< 0.05). The mean values followed by at least one similar letter were not significantly different.

Pretreatment with DMSO increased SBS in subgroups A_3, A_4 and B_4 in comparison with subgroups A_1, A_2 and B_2, respectively, and the increase was statistically significant in subgroups A_3 (p= 0.003) and B_4 (p< 0.001) but not significant in subgroup A_4 (p= 0.963). DMSO did not affect SBS of B_3 comparing with B_1 (p= 1.00).

ASB exhibited significantly higher SBS values in subgroups A_2 (p< 0.001), A_4 (p= 0.002) and B_4 (p< 0.001) than in subgroups A_1, A_3 and B_3, respectively. Subgroup B_2 had higher SBS than subgroup B_1, but the difference was not significant (p= 0.09).

SBS was higher in all the surface subgroups compared with deep subgroups; it was significantly different in subgroup A_2 compared with subgroup B_2 (p< 0.001) and in subgroup A_3 compared with subgroup B_3 (p< 0.001) but not statistically significant in subgroup A_1 compared with subgroup B_1 (p= 0.99) and in subgroup A_4 compared with subgroup B_4 (p= 0.14)

Discussion

Dentin pretreatment with DMSO had a significant effect on SBS of ASB to either surface or deep dentin.

Several efforts have been made to improve composite resin bonding to dentin. One is to condition dentin surface with chemicals such as polyacrylic acid, [31] chlorhexidine digluconate [32] and antibacterial agents

[33] such as EDTA and NaOCl. Recently Tjäderhane *et al.* [27] evaluated the effect of 0.004% DMSO on nanoleakage and microtensile bond strength of Filtek Supreme XT to dentin. A methacrylate-based (Z350) and a silorane-based (P90) composite resin were used in this study with their respective adhesive systems: a total-etch system, Adper Single Bond (ASB), and a self-etch system, P90 adhesive bond. We used 0.004% DMSO to condition the surface and deep dentin and evaluate its effect on bond strength of SA and ASB. In this study DMSO increased SBS in the surface DMSO-treated SA subgroup, A_3, and deep DMSO-treated ASB subgroup, B_4, compared with respective control subgroups A_1 and B_2, consistent with a previous study [27] that reported DMSO can improve the resin–dentin bond. In surface DMSO-treated ASB subgroup, A_4, SBS was higher than its control subgroup B_2, and lower in deep DMSO-treated subgroup B_3 than its control subgroup B_1, but the differences were not statistically significant. DMSO is a chemical penetration enhancing [34] amphiphilic solvent, fully miscible in all the solvents used in adhesive dentistry. [27] This agent is able to penetrate biological surfaces [28] and compete with water molecules in inter-peptide hydrogen bonding of the collagen matrix. [35] This may explain why conditioning dentin surface with DMSO before adhesive application improved adhesive penetration into the exposed collagen matrix. The increase in SBS in the surface DMSO-treated ASB subgroup was not as much as it was expected and since this is the first time that the effect of DMSO on surface and deep dentin is studied, we recommend more studies with more specimens and different concentrations of DMSO and SEM microscopy to understand its behavior on different depths of dentin. On the other hand, the adhesive system of silorane is a self-etch one, exclusively used with this composite resin. [36-38]

The acidic hydrophilic primer with a pH of 2.7 is considered a relatively mild primer. [29] In the SA subgroups DMSO was applied before priming. It seems that the DMSO entrapped in large intra-tubular spaces of deep dentin (subgroup B_3) has a tendency to dilute the primer and weaken its already mild demineralization capacity, leading to lower SBS in deep DMSO-conditioned P90 specimens (subgroup B_3) than non-conditioned one (subgroup B_1). In contrast, comparison of subgroup B_4 and B_2 did not show this compromising effect since DMSO was applied after acid-etching in ASB subgroups, as described in a previous study [27] and this can be the reason why SBS was higher in the ASB subgroups compared to the SA subgroups. Changes in the steps in which DMSO is applied, may lead to different results.

ASB exhibited higher shear bond strength values regardless of pretreatment or dentin depth. Different bond strength results have been reported with the use of total-etch and self-etch adhesives, with higher bond strength for either total-etch [39] or self-etch [40] adhesives. In the present study, ASB subgroups were restored using a total-etch adhesive system with 37% phosphoric acid, a strong acid (pH=0.03–0.05) [41] and the application of an adhesive, ASB. This technique leads to a complete removal of the smear layer, demineralization of the dentin surface and exposure of collagen fibers, [31] creating a demineralized zone measuring 5 to 8μm in thickness [42] but SA system's acidic hydrophilic primer with a pH of 2.7 leads to dentin decalcification limited to a few hundredths of nanometers. [38] After the primer is light-cured, a more viscous and hydrophobic adhesive resin, must be applied and light-activated independently. [43-45] The larger quantity and depth of tags obtained with total-etch systems may promote deeper micromechanical interlocking and greater SBS compared to self-etch systems like silorane. [44-46]

All the deep dentin subgroups presented lower SBS than the surface dentin subgroups, consistent with previous studies. [30] However, the difference was not significant in two pairs; first in the surface and deep SA subgroups without DMSO which might be because of lower etching potential in silorane primer that cannot demineralize the highly mineralized components of the surface dentin in comparison with deep dentin; and second in surface and deep DMSO-treated ASB subgroups due to high demineralizing potential of phosphoric acid utilized in ASB subgroups. Therefore, it seems that DMSO pretreatment is not as much effective on surface dentin as that on deep dentin when using ASB. Self-etch adhesives have a mild acid primer that partially demineralizes the dentin, despite of a total etch system that demineralizes the dentin to a higher extent and this can be the reason why we had higher SBS values in B_2 than in B_1 although the difference was not significant.

Due to insufficient data base on the mechanism by which DMSO affects different depths of dentin and dentin bond strength, further studies are required to evaluate its behavior in contact with acetone-, ethanol- or water-based dentin bonding agents and in different steps of a bonded restorative procedure. Furthermore, SEM and TEM analysis are recommended for better investigation of the depth of resin penetration with or without DMSO.

Conclusion

Within the limitations of this *in vitro* study, pretreatment of dentin with DMSO significantly improved SBS of ASB in comparison with SA. DMSO appeared to enhance SBS of ASB to either surface or deep dentin and the increase was much higher in deep dentin than in surface dentin. Therefore, when using ASB, DMSO might improve SBS in deep cavities with no enamel margins if the results of this study are confirmed by *in vivo* studies. In addition, DMSO could not affect SBS values in deep dentin restored with SA compared with surface dentin.

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Conflict of Interest

The authors of this manuscript certify no financial or other competing interest regarding this article.

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