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Effect of Dimethyl Sulfoxide wet bonding technique on dentin wettability and resin-dentin bond strength; an In vitro study

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Abstract

Objectives: This study evaluated the effect of dimethyl Sulfoxide wet bonding technique in enhancing the dentin bond strength durability and dentin wetting by the adhesive. This pretreatment was compared to ethanol wet bonding technique and the conventional bonding technique.

Methods: Extracted human permanent molars were bonded using a two-step etch and rinse adhesive (single bond 2: SB2) after being treated with DMSO (0.004%, 5%, 50%) or ethanol. Dentin specimens (n=10) that received treatments were divided into four groups and specimens that remained untreated served as the control group. Bonded teeth (n=10) were stored in distilled water and then sectioned into resin-dentin sticks for microtensile bond strength testing. Specimens were further divided into 2 subgroups for microtensile testing; one group was evaluated after 24 h while the other after 6 months of aging. The wettability of the adhesive by DMSO vs. ethanol saturated dentin was evaluated through contact angle measurement.

Results: The use of 50% DMSO with single bond 2 adhesive showed significantly higher immediate and delayed bond strength than the ethanol and the control groups. DMSO 5% produced similar results as the DMSO 50% in the delayed group. All pretreatments produced significantly lower contact angle compared to the control group. Samples treated with 50% had a significantly lower contact angle value than samples treated with DMSO 0.004%.

Significance: Dentin pretreatment using 5% or 50% DMSO may help preserve long term dentin-adhesive bond strength as well as improve wetting denoting enhanced adhesive penetration.

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Introduction

Adhesive bonding procedures targets to create and maintain a tight adhesive-dentin interface that would remain in place for decades, offering marginal sealing, retentive strength, and durability. Despite the recent and continuous improvements in the adhesive knowledge and technology, limited resin-dentin bond durability still exists in both adhesive systems.[1, 2]

The etch-and-rinse technique (E&R) and the self-etch (SE) or etch-and-dry technique, are currently the two approaches used in resin bonding procedures. Resin dentin bonding depends on the formation of a structure formed of resin reinforced demineralized collagen matrix called the hybrid layer.[3]

Conventional adhesive resins failed to totally substitute water, either free or loosely bound water present inside the collagen matrix. Moreover the phase separation of resin monomers that occur during adhesive procedures facilitates hydrolytic degradation of the adhesive resin. Partial and inadequate infiltration of the adhesive results in collagen fibrils exposure and thus become vulnerable to degradation. Collagen degradation is then initiated by endogenous enzymes as matrix metalloproteinases and cysteine cathepsins. Consequently degradation of either the adhesive resin or collagen matrix became a major difficulty affecting the longevity of resin-dentin bonds.[4, 5]

Several strategies have been suggested to prevent the hybrid layer degradation over time. The ethanol wet bonding technique has been recommended to replace water and support the demineralized dentin collagen. The attempt of replacing water within the collagen matrix with high ethanol concentration may reduce/prevent degradation of both collagen and the resin components of the hybrid layer.[6]

Unfortunately, some authors claim that ethanol wet bonding is clinically unfeasible due to technique sensitivity. This is because, for the ethanol wet bonding technique to be able to replace water; a full dehydration protocol is needed which requires more application steps and increased treatment time.[7, 8]

Recently, a newly introduced polar aprotic solvent named dimethyl Sulfoxide was suggested to prevent the hybrid layer degradation over time. (DMSO: $(\text{CH}_3)_2\text{SO}$), its formula is formed of an S=O which is a highly polar group and two CH_3 hydrophobic groups. It is considered as a powerful organic solvent capable to dissolve polar as well as non-polar compounds.[9] Moreover, it has the ability to dissolve a vast variety of substrates as organic molecules, polymers, carbohydrates, peptides, and inorganic salts as well. Additionally, DMSO is thought to be the best penetration enhancer used in medical purposes due to its capability of penetrating biological surfaces and tissues. [10]

Recently in a study conducted by Tjaderhane, et al, it was suggested that extremely low concentration of dimethyl Sulfoxide showed to reduce the long term loss of bond strength due to improved adhesive penetration into the demineralized collagen matrix.[11] According to the considered effects of DMSO, significant effects in dentin bonding may take place if used in higher concentrations.[12]

Thus, the aim of this study is to evaluate the effect of pretreatment of ethanol and three DMSO concentrations on immediate and long term dentin bond strength and on adhesive penetration depths with the use of 2 steps etch and rinse adhesive. The null hypothesis was that different DMSO concentrations have no effect on either immediate or delayed dentin bond strength or the dentin wetting (SE).

Materials and methods

Three distilled water based concentrations of Di-methyl Sulfoxide (0.004%, 5%, and 50%) were prepared in the chemistry lab of Badr University in Cairo. Ethanol 100% was used immediately without any preparation

Selection of teeth:

Thirty extracted human permanent molars were collected from the oral surgery department clinics at Badr University in Cairo. The teeth were completely sound, non-carious, non-restored, non-root canal treated with intact area of bonding

interest. The tooth enamel was cut horizontally to expose flat surface of superficial dentin using medium grit diamond burs (MANI DIA- BURS, 107 μm) with a high speed hand piece under copious water cooling. These teeth were randomly assigned into their groups ($n=10$) according to the type of pretreatment.

A 2 step etch and rinse (Single bond 2, 3M/ESPE, St. Paul, MN, USA) bonding agent was used. The exposed dentin surface was acid etched for 15 seconds using 35% phosphoric acid (3M ESPE) then rinsed for 15 seconds using a three-way air/water syringe. After rinsing, the specimen was gently air-dried for 5 seconds at a distance of 10 cm to remove excess water from rinsing leaving the dentin slightly moist. Pre-treatments were carried on top of acid etched dentine surface. Application was using a solution saturated disposable micro-brush in a circular light pressure rubbing motion for 1 min and then blot drying using filter paper. Control group received no treatment.

Single bond 2 was applied using a different micro-brush by slight rubbing action onto the demineralized dentin surface for 20 seconds. It was then mildly air streamed for 5s. The adhesive was light cured for 20 seconds using an LED device (Woodpecker I-Led Curing Light) that delivers a blue light of intensity of 1200 MW/Cm² output. The LED curing unit was held at a fixed distance of around 2mm according to the manufacturer's instructions perpendicular to the adhesive layer.

Resin composite restoration was built onto the bonded dentin surface in 4 increments, each increment was 1mm in thickness applied using Teflon coated plastic instrument. Each increment was individually polymerized with the LED curing device for 20 seconds.

Micro-tensile bond strength testing:

Teeth were longitudinally sectioned across the bonded interface into 1mm slabs. Composite-dentin slabs were further cut into 1mm sticks producing a beam pursuing a final 1x1 mm in thickness ($\pm 0.1\text{mm}$). Beams were created using a sharp diamond saw (Isomet 4000 linear Precision saw, Buehler) under constant water coolant. The saw was operated at a blade speed of 2500 rpm and a feed rate of 16.7mm/min. Each tooth attained a minimum of 10 sticks. Beams were individually attached to a metal microtensile fixture using a cyanoacrylate adhesive. The beams were fixed so that the bonded interface is perpendicular to the applied load. A universal testing machine (Instron® norwood, IL, USA) was used for the microtensile bond strength testing. Tensile loads were applied at a cross-head speed of 1mm/min until failure. Thickness of the beam was measured using a digital caliper to calculate the precise bond strength (MPa).

Contact angle measurement:

25 extracted intact human third molars were used to obtain dentin discs. Discs were randomly distributed into 5 groups, each group ($n=5$). Teeth were cut parallel to the occlusal surface to obtain a flat dentin disc by removing the occlusal third of the crowns under water coolant. This procedure was carried with a slow speed diamond saw (Buehler Ltd, Lake Bluff, IL, USA). All dentin discs were sanded first with 320 and then 600 grit silicon carbide abrasive papers. Each disc was abraded in a circular manner for 30 seconds to remove any debris from sectioning and obtain clinically similar surface roughness and smear layer on the experimental site. A total of 50 discs were obtained, each one measuring 1mm ($\pm 1\text{mm}$) in thickness and checked using a digital caliper.

All dentin disks were acid etched for 15 seconds with 35% phosphoric acid etchant, rinsed with water for another 15 seconds and gently dried with a three-way syringe leaving the dentin surface slightly moist. Pretreatments were applied using a saturated micro-brush and left for 1 min. The dentin surface was then gently dried to remove the excess solution using a filter paper. A micro-pipette (Topette Pipettor, Dragon lab) was adjusted/calibrated to dispense a single droplet of 10 μ l of single bond 2 adhesive on the flat dentine surface. For the control group, single bond 2 was applied on the dentin discs immediately using the micro-pipette.

A digital microscope (Dino-Lite microscope, India) was used for the measurement of the adhesive contact angles. The handheld microscope was fixed using the microscope stand for stability during the measurement. The microscope was connected to a laptop for displaying the optical information of the drop, operated using the microscope's own specific software (Dino-Capture 2.0). The droplet's contact angle was recorded for each specimen immediately after the drop deposition and calculated via the software.

1. Results

1.1. Microtensile bond strength evaluation

Multi-factorial mixed model ANOVA was used to study the effect of different tested variables and their interaction. Comparisons of main and simple effects were done utilizing benferroni correction. The significance level was set at $P \leq 0.05$ within all tests. Statistical analysis was performed with IBM® SPSS® Statistics Version 25 for Windows.

In the immediate as well as the delayed groups, there was a significant difference between micro-tensile bond strength of samples with different pretreatment materials ($p < 0.001$). Immediately pairwise comparison showed value recorded with DMSO 50% to have significantly higher value than values found in other samples ($p < 0.001$). In the delayed group, 50% have significantly higher value than values found in other samples except for samples treated with DMSO 5% ($p < 0.001$). Microtensile values for all pretreatments are shown in table (1) and figure (1)

Table (1): Mean \pm standard deviation (SD) of μ TBS (Mpa) for different measurement times, and pretreatment materials

Time of measurement	Pretreatment (mean \pm SD)					P-value
	ontrol	thanol	MSO 0.004 %	MS O 5%	MSO 50%	
After 24 hours	7.64 \pm 2.62 ^B	1.68 \pm 2.55 ^B	6.64 \pm 2.83 ^B	1.55 \pm 2.74 ^B	3.84 \pm 2.13 ^A	0.001*
After 6 months	7.11 \pm 2.11 ^B	0.21 \pm 2.06 ^B	6.58 \pm 2.98 ^B	4.11 \pm 2.58 ^{AB}	2.44 \pm 2.92 ^A	.002*
P-value	.009*	.026*	.001*	.095 ns	.002*	

Different superscript letters indicate a statistically significant difference within the same horizontal row. *, significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$).

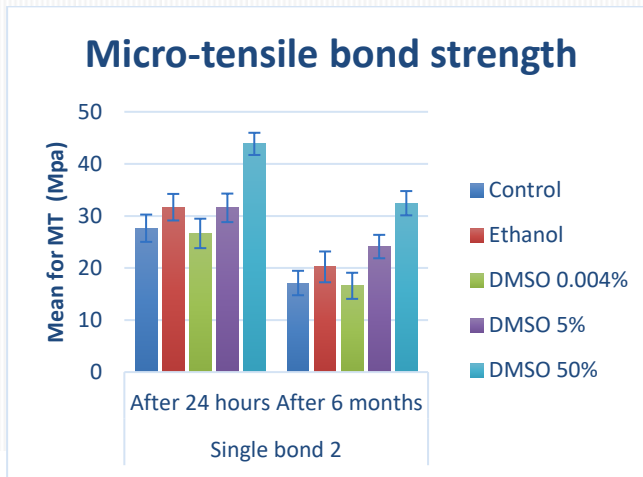


Figure (1): Bar chart showing mean micro-tensile bond strength (Mpa) for different pretreatments in each bonding system inside the two measurement times

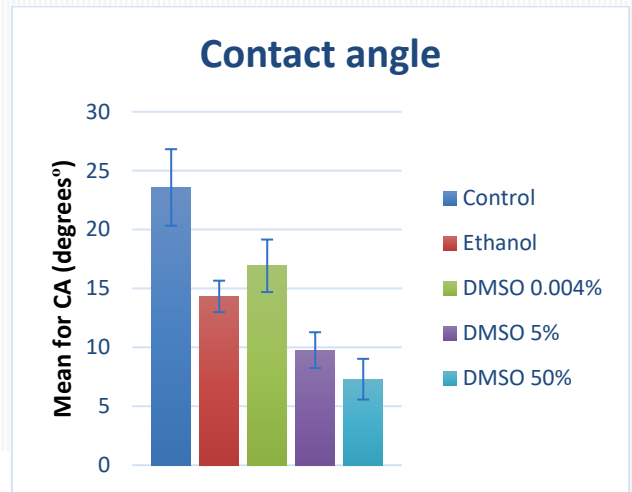


Figure (2): Bar chart showing mean contact angle (degrees) for different pretreatment materials.

Contact angle measurement

Interaction of the pretreatment within SB2: Pairwise comparisons showed that control samples have a significantly higher value than values of samples treated with other materials except for DMSO 0.004% ($p < 0.001$). In addition, samples treated with DMSO 0.004% had a significantly higher value than samples treated with DMSO 50% ($p < 0.001$). Mean and standard deviation (SD) values of contact angle for different bonding and pre-treatment materials were presented in table (2) and figure (2)

Table (2): Mean \pm standard deviation (SD) of contact angle (degrees) for different pretreatment materials

ontrol	Pretreatment (Mean \pm SD)				- value
	thano l	MSO 0.004 %	MSO 5%	MSO 50%	
3.58 ± 3.2 5 ^A	4.33 \pm 1.33 ^{BC}	6.93 \pm 2.23 ^{AB}	.77 \pm 1. 52 ^{BC}	.30 \pm 1 .73 ^C	0.001*

Different superscript letters indicate a statistically significant difference within the same horizontal row *; significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$)

Discussion:

To prevent the collapse of the etched dentin collagen, water is required before the adhesive application to allow infiltration of monomers into the collagen network. However, existing water will as well promote phase separation of the adhesive monomer and create a more hydrophilic co-monomer in the hybrid layer that is more prone to hydrolysis. Moreover, inter-fibrillar water activates the endogenous enzymes triggering proteolytic degradation of the exposed collagen.[13]

DMSO is a potent penetration enhancer that has the ability to replace water through breaking water's self-associative tendency. DMSO molecule is formed of a polar S=O group (hydrophilic) and two hydrophobic CH₃ groups. Cyclic water pentamer is a water structure that is vital for hydrating biomolecules found in biological structures.[11] The negative charge of the oxygen atom found in the DMSO molecule favors forming hydrogen bonds with water. Usually DMSO forms two hydrogen bonds with water molecules thus breaking associative tendency of water. Because DMSO molecule is considered a hydrogen bond acceptor as well as its ability to bond with water more strongly than water bonds to water, it is able to perform these structural effects.[14] DMSO-water hydrogen bonds are considered highly stable. These hydrogen bonds formed are 4 times longer than

hydrogen bonds formed between water-water pairs. Therefore creating more water stabilized smaller complexes than those of the cyclic pentamer.[11, 15]

Inside the immediate SB2 group there was a significant difference found between different pretreatments used, the highest values was found in samples treated with DMSO 50% followed by Ethanol , DMSO5% , control and DMSO 0.004% . It is noticed that the higher the concentration of the DMSO, the greater its immediate bond strength increases. This may be due to the effectiveness of DMSO in replacing water and enhancing the penetration ability with higher concentration. This is in accordance with the findings of Stape et al. and Salim al-Ani et al. [16, 17] who compared different concentrations of DMSO.

The use of 50% DMSO provided increased wetting of collagen by adhesives and better penetration giving higher immediate strength, which correlates with the study of Stape et al. [9, 12].

In the etch and rinse technique, hydrophobic BisGMA monomers generally are able to just partially diffuse into the demineralized dentin matrix.[18] This is due to phase separation of BisGMA/HEMA monomers where hydrophilic HEMA initially floods the demineralized moist dentin substrate while BisGMA monomers cannot diffuse into areas where water residues are present. As a result, HEMA predominantly infiltrated and polymerized producing poly-HEMA chains. This produces a bio-polymer of resin-collagen with low mechanical properties and a poor hybrid layer quality.[19] In DMSO treated samples, it was able to dissolve hydrophilic as well as hydrophobic monomers including BisGMA. DMSO saturated collagen prevented phase separation allowing better diffusion of BisGMA monomers and collagen encapsulation thus creating enhanced hybridized adhesive interface.[9, 20]

It was found that DMSO may be used as optical clearing agent. OCAs are used in the biomedical field, permitting more photons to reach deeper tissues. Thus it allows good

analysis of cells and tissues through attaining significantly higher contrast images at increased depths than untreated tissues. It acts by suppressing the inter-peptide hydrogen bonds within the collagen fibrils changing its organized structure.[21] This leaves the collagen matrix in a condition to be readily and easily infiltrated by resin monomers reducing the exposed collagen at the base of the hybrid layer. It was recently claimed that the optical clearing effect may be somewhat weaker using low DMSO concentrations and may not be enough to demonstrate **improved long term bond strength**. High concentrations are more capable of dissociating the highly cross-linked collagen network, changing its inter-fibrillar spaces into a sparser network of apparent fibrils. [11].

Concerning the ethanol group, it provided higher values than the control group. This may be due to enhancing the penetration of the hydrophobic monomer, yet the values were not statistically significant maybe due to the insufficient treatment time protocol.

Regarding the **aged** group, there was a significant difference between values of samples with different pretreatments after 6 months. The highest was in DMSO 50% followed by DMSO5%, Ethanol, control and DMSO 0.004%. DMSO50% showed significantly higher values than all other pretreatments except for the DMSO 5%.

These results shows that pretreating dentin with higher DMSO concentrations as (50%, 5%) , improved bond strength for SB2 after 6 months of storage occurs, thus providing better bond durability. This is attributed to both, better encapsulation of the adhesive and its anti-MMP effect, as well as breaking down the self -associative tendency of water which allowed the reduction of entrapped water molecules between the polymeric chains leading to increase in post-operative polymerization. Additionally, the relative decrease in free water may result in decrease in the hydrolytic degradation within or above the hybrid layer.

DMSO still showed superiorly significant bond strength and this is in accordance to a

study conducted by L.tjaderhane, et al, in which he explained the improved durability of DMSO pretreated samples was mainly due to the effect of DMSO on the decrease of water content[11].

High bond strength provided by 5% was previously illustrated in a study by Tjaderhane et. al.[11]. Using zymography in their study, they found that above 5% concentration, DMSO demonstrated an inhibitory activity on human gelatinases MMP-2 and MMP-9 which are the most common proteinases present in human dentin. Their findings supported previous studies that indicate that DMSO disrupts interactions between the gelatinase binding site and substrates [22, 23]. In this study, the inhibitory effect lead to reducing the degradation of collagen by endogenous proteases and providing longer term stability to the collagen structure even though the encapsulation by the adhesive resin was incomplete.

Although ethanol provided better delayed bond strength than control groups yet it was significantly lower than the effects of DMSO 50% and 5%. This can be explained by the fact that its application was not sufficient enough to reach the full depth of the demineralized collagen and therefore some water content was left in the deeper layers causing release and activation of endogenous enzymes[24]. The process of ethanol replacing water inside collagen is a lengthy process that requires sufficient amount of time. However, increasing the concentration of ethanol to 100% may have improved the bond strength in some studies [24, 25]. The complete and proper protocol still remains to be the most efficient in replacing water. And therefore 1 minute pretreatment is not enough to replace all water.

In addition to the insufficient application time, the full protocol required several applications of ethanol with different concentrations to the etched dentin, a part of that was due to the fact that ethanol has a very high vapour pressure, and therefore a single application is extremely volatile reducing its time inside the dentin and decreasing its effect, and thus came the idea

of multiple applications. In the current study, ethanol was applied only once for only one minute for the sake of clinical efficiency which is clearly not enough to replace enough water inside dentinal collagen.

Contact angle measurement test was chosen to evaluate the adhesive wettability. It is the most common method for evaluating micro/nanostructured surface wettability. Concerning the pretreatment type, it was shown that the pretreatment type significantly affected the contact angle. The control groups presented significantly less wetting ability than the DMSO and ethanol pretreatments

The theory of Hoy's solubility parameters has been commonly used to assess the miscibility of two different solutions. This is done through comparing the values of their total cohesive energy (δt) that allows holding polymers together. According to this theory, if the value of δt between the two solutions compared or a solution/ substrate is less than $5(J/cm^3)^{1/2}$, at that time, the solution will be able to wet the substrate. Consequently, the substrate will swell facilitating entry of the solution.

It was previously reported that water saturated collagen Hoy's δt value is $30.15(J/cm^3)^{1/2}$ while for ethanol is $26.1(J/cm^3)^{1/2}$ [13]. Obtained from the computer chemistry consultancy, DMSO's solubility parameter is $26.06(J/cm^3)^{1/2}$. [26] This clarifies that the difference between either ethanol or DMSO and the substrate is less than $5(J/cm^3)^{1/2}$. Meaning that both ethanol and water were able to wet and go into the dentin collagen matrix. This explains the significantly higher wetting obtained by Ethanol and DMSO wet bonding techniques.

In the DMSO group, the decrease in contact angle was more evident than the ethanol denoting that DMSO offers better dentin wettability. This may be related to ethanol's high vapor pressure when compared to DMSO. Vapor pressure is that property which determines the time that solvents needs to evaporate.[27]. Solvents having high vapor pressure evaporate faster. Thus the limited wetting ability of ethanol may be attributed to its rapid evaporation after its application.

This result was in agreement with Jingmei Guo, et al.[26]

DMSO lowers the cohesive forces and surface tension of water by breaking the self-associative tendency of water. It is regarded as a good wetting agent due to its low surface tension and high dielectric constant making it capable to solvate polymers and adhesives[28]. Surface tension of pure water is 73mN/m^3 while pure DMSO is 42.0973mN/m^3 [29]. It is categorized as a polar aprotic solvent having the polarity needed to breakdown water's self-associative tendency, forming stable complexes with water resulting in lower viscosity. This could explain the higher wettability induced by higher DMSO percentage, as the higher the DMSO content the more the reduction in surface tension. This correlates with a study by Mehtala P, et al that examined the effect of ascending DMSO percentages on dentin collagen wettability, concluding that contact angle values decreased with increasing DMSO vol%. [28]

As for the effect of the pretreatments within SB2, the control group showed the highest contact angle value. As previously mentioned water has a very high δh value of $40(\text{Jcm}^3)^{1/2}$ achieving sufficient dentin collagen expansion. Due to the fact that monomers as Dimethacrylates (ex.BisGMA) exist, it undergoes phase separation when applied to water wet dentin as these monomers are not soluble in water. Considering the ethanol wet bonding, application of ethanol 100% for only 1 minute was not enough to remove water and allow the entrance of the adhesive.[7]

DMSO 50% showed significantly better wettability compared to the 0.004%. This may be due to the increased concentration ability to better perform its effects as already mentioned. Reducing the amount of water through the dissociating effect of DMSO reduced phase separation of more hydrophobic monomers and prevented its repulsion from the base of the hybrid layer.

2. Conclusion:

Within the limitations of this study, the following could be concluded:

1. Using DMSO as a pretreatment to acid etched dentin increases the bond strength in concentrations of 50% when using Single bond 2 adhesive and improves durability of the bond in 5% and 50%
2. Using ethanol in its simplified application form is insufficient to provide better bond strength and durability compared to the DMSO for 1 minutes pretreatment time
3. Ethanol and DMSO improve the contact angle of Single bond 2 adhesive but the 1 minute application time of Ethanol is still insufficient.

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