ORIGINAL ARTICLE
DOI:10.5152/EssentDent.2023.23023

ISTANBUL UNIVERSITY
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Optimization of Dentin Bond Strength Using the Dimethylsulfoxide-Wet Bonding Strategy: A 6-Month Preliminary Study

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Cite this article as: Sismanoglu S. Optimization of dentin bond strength using the dimethylsulfoxide-wet bonding strategy: A 6-month preliminary study. *Essent Dent.* 2023;2(3):95-100.

Abstract

Background: This study aimed to evaluate the effect of a dimethylsulfoxide (DMSO) wet bonding technique on the dentin bond strength of a multimode adhesive system in both etch-and-rinse and self-etch modes.

Methods: Flat dentin surfaces of 112 bovine incisors were treated with distilled water (controls) or with 50% DMSO (experimental group) before bonding using a multimode adhesive (Single Bond Universal, 3M Dental Products) in etch-and-rinse or self-etch modes. Afterwards, a tygon tube (1 × 1 mm) was placed on each specimen and filled with resin composite (Filtek Flowable; 3M Dental Products). Half of the specimens were subjected to microshear bond strength testing as the immediate bonding (n = 14). The rest of the specimens were stored for 6 months at 37°C in artificial saliva. Failure modes were observed under a stereomicroscope. The data was analyzed by a two-way analysis of varriance followed by a Tukey test ($P = .05$).

Results: The highest bond strength values among the groups were obtained after the application of multimode adhesive in self-etch mode ($P < .05$). The lowest bond strength values were observed in the total-etch groups after 6 months of aging ($P < .05$). The bonding protocol had a significant influence on the bond strength ($P < .05$). Dimethylsulfoxide-wet bonding improved the bond strength of the adhesive interface.

Conclusion: Dimethylsulfoxide-wet bonding was effective to improve the quality of resin-dentin bonds of the tested multimode adhesive by reducing the adhesive failure rates. These findings suggest that 50% DMSO could be a feasible alternative to improve resin-dentin bond quality, however this application requires an additional step.

Keywords: Dimethylsulfoxide, etch-and-rinse adhesives, microshear bond strength, multimode adhesives, self-etch adhesives

INTRODUCTION

Adhesion refers to the condition where 2 distinct surfaces are joined together through physical and chemical interactions. In restorative procedures, adhesion takes place between 2 dissimilar surfaces: the mineralized tooth structures and restorative materials. Bonding to the tooth structure occurs mainly as a result of the replacement of resin monomers with the inorganic compounds of the tooth tissue.[1]

Adhesive systems play a crucial role in ensuring the proper bonding of resin-based materials to the tooth. As a result, manufacturers are actively seeking the ideal adhesive system and technique to achieve optimal adaptation.[2] In parallel with the developments in composite resin and adhesive systems, researchers have focused on the better bonding of restorative materials to tooth structures and old restorations already on the tooth. Different strategies have been developed for dentin adhesion. Adhesion to dentin was first described by Nakabayashi in 1982. Nakabayashi reported that penetration of a hydrophilic resin material into the demineralized surface formed by applying acid to the dentin surface to expose collagen fibers and dentin tubules would increase dentin adhesion.[3]

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Received: October 9, 2023
Accepted: November 20, 2023
Publication Date: December 11, 2023

Since the structure of dentin is complex, adhesion to dentin is more difficult than enamel. While enamel is more homogeneous and its main content is hydroxyapatite crystals, dentin tissue consists of hydroxyapatite crystals as well as collagen fibrils and an extracellular matrix containing biological molecules such as phosphorin, osteocalcin, osteopontin, and osteonectin. In addition, the dentin fluid in the dentin tubules causes the dentin surface to have a constantly moist and hydrophilic structure, which is another factor that makes bonding difficult.[4] Adhesion to dentin occurs micromechanically through the formation of the hybrid layer and resin rods. With the polymerization of the bonding agent, the hybrid layer forms a stable structure. The durability and integrity of the hybrid layer on the bonding surface are more important than its thickness. Any gap in the hybrid layer will disrupt the sealing and ultimately reduce the quality of adhesion.[5]

Over the past few decades, several methods to preserve the hybrid layer in dentin bonding have been developed. These approaches include biomimetic remineralization,[6] the use of cross-linking agents,[7,8] extrafibrillar demineralization,[9] suppression of enzymatic activity,[10] and elimination of residual water.[11] It's worth noting that the moisture or wetness of dentin surfaces has a significant impact on their bonding performance. Proper management of moisture is essential for successful bonding procedures.

Hydrophobic dimethacrylates in a single bottle with water in simplified systems are materials that form highly strong and cross-linked resin polymers and have very low water solubility. Therefore, in single-bottle systems, phase separation may occur between organic solvent and hydrophilic and hydrophobic monomers, and monomer infiltration into dentin may be prevented due to residual water remaining in the environment. Pashley et al[12] modified the wet-bonding technique to prevent this situation and developed the ethanol wet bonding technique. Thus, they stated that the residual water in the environment can be removed more easily with the high evaporation ability of ethanol applied to dentin.[13] With this approach, the presence of residual water at the resin-dentin interface is reduced, and the hydrolytic degradation process is slowed down.[13] The ethanol wet-bonding technique, which involves using ethanol instead of water in hydrophobic adhesives, has shown promising results in laboratory settings.[14] However, it presents challenges when applied clinically due to issues like premature evaporation when repeatedly opening the adhesive bottle. Moreover, the initial steps requiring a series of ethanol concentrations to pretreat the dentin surface can be both time-consuming and less feasible for application in real-world clinical scenarios.

Dimethyl sulfoxide (DMSO) is a versatile organic liquid with strong dipolar and aprotic properties. It has the unique ability to dissolve a wide range of both polar and nonpolar substances, making it valuable in organic synthesis and the chemistry. Dimethyl sulfoxide is often referred to as a universal solvent due to its remarkable solvent capabilities.[15] In the fields of bioengineering and pharmaceuticals, DMSO serves as a valuable solvent in biological research and as a carrier for drug delivery purposes.[16] Dimethyl sulfoxide is particularly noteworthy for its remarkable abilities in enhancing tissue penetration and permeability of cell membranes.[17,18] Moreover, it actively interacts with collagen fibers. Dimethyl sulfoxide serves as a highly effective cryoprotectant for stem cells in cellular biology. This helps protect stem cells from damage when subjected to the freeze-thaw process.[19]

In the context of chemical and biological activities and taking into account the hybrid layer's characteristics, scientists have acknowledged that DMSO serves not only as a viable choice for dissolving dental adhesives but also as a multifunctional adhesion primer in dentin bonding. DMSO has demonstrated its usefulness as a solvent for different monomers and adhesive components during toxicological evaluations of dental adhesives.[20-22] Its appeal lies in its exceptional ability to dissolve both hydrophilic and hydrophobic components.[23] Additionally, DMSO exhibits higher permeability and lower volatility compared to ethanol. This led to the development of the concept of DMSO-wet bonding, prompting numerous researchers to assess its effectiveness.[23,24] However, studies exploring the impact of the DMSO-wet technique on bond strength remain limited, and as of now, no research has been identified regarding its application with multimode adhesives.[25]

This study aimed to investigate the adhesive performance of a multimode adhesive system in different modes on the dentin surface by removing excess water, which cannot be removed from the dentin surface with air, by DMSO application. The null hypothesis of the present study is that there would be no difference between the different modes of multimode adhesive systems, whether DMSO is applied or not.

MATERIAL AND METHODS

Specimen Preparation

In this study, all the methods adhered to the regulations outlined in the Declaration of Helsinki. Since no human-derived material was used in the study protocol and the study was not an animal study, ethics committee approval was not required.

The sample size for the microshear bond strength (μSBS) testing was determined to be at least 14 samples in each study group based on previous research[26] using G* Power computer software (version 3.1.9.6; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). This calculation considered an alpha level of 0.05, a beta level of 0.2, a standard deviation of 4 MPa, and an effect size of 0.46.

In the present study, 112 bovine incisor teeth without any defects on the surface were used. Teeth with microscopically visible defects were not included in the study. Tissue residues and attachments on the teeth were cleaned under running water with the help of a periodontal curette. After collection, the teeth were disinfected in a 0.5% chloramine-T solution at +4°C. The teeth were embedded in molds fabricated with cold acrylic, leaving the crown part outside. Enamel tissue on the incisal surfaces of the teeth was removed using a precision saw (IsoMet High Speed Pro; Buehler, Lake Buff, Ill, USA) under water cooling. In order to obtain a standardized smear layer, the incisal surface of all specimens were wet-polished using 600-grit silicon carbide paper for 60 seconds and prepared for adhesive bonding.

Dentin Bonding Protocol

The specimens were randomly divided into 2 main groups according to the adhesive mode to be used (n=56). The other half of the specimens were acid-etched with 37% phosphoric acid (Scotchbond Etchant; 3M Dental Products, St. Paul, MN, USA) for 15 seconds, rinsed for 15 seconds, and blot-dried with paper, whereas the other half remained unetched. Subsequently, the specimens were randomly divided into 2 subgroups (n=28).

The experimental groups underwent a 60-second pretreatment with 50% DMSO and were then blot-dried before applying the adhesive (Single Bond Universal; 3M Dental Products, St. Paul, MN, USA), which was photopolymerized by a light-emitting diode curing unit (Valo Grand; 1000 mW/cm^2, Ultradent, South Jordan, UT, USA) for 10 seconds. Afterwards, a tygon tube (diameter of 1 mm and height of 1 mm) was placed on each specimen and filled with resin composite (Filtek Ultimate Flowable; 3M Dental Products, St. Paul, MN, USA), followed by 20 seconds of photopolymerization. The tygon tube was removed by gently cutting with a surgical blade.

The pretreatment was modified from using 50% DMSO to using distilled water in the control groups, while all other procedural steps remained unchanged. After 24-hour storage in artificial saliva (distilled water, 1 mM $CaCl_2$, 50 mM KCl, 2 mM KH_2PO_4, and 0.01% NaN_3; pH adjusted to 7 with 1 M KOH) at 37°C, half of the specimens were subjected to the μSBS testing as the immediate bonding subgroup (n=14). The rest of the specimens were stored for up to 6 months at 37°C in artificial saliva. The artificial saliva was prepared and replaced on a weekly basis.

Microshear Bond Strength Testing

The specimens were de-bonded using a microshear testing machine (MOD Dental, Esetron Smart Robotechnologies, Ankara, Turkey) at a cross-head speed of 1 mm/minute, and the μSBS values (MPa) were calculated by dividing the failure load (N) by the surface area (mm^2).

Failure modes were assessed using a stereomicroscope (SMZ745T, Nikon Co., Tokyo, Japan) at ×40 magnification to identify how the specimens failed. The failure modes were categorized as follows: failure occurring at the dentin-resin composite interface (adhesive failure), failure within either the dentin or resin composite material itself (cohesive failure), or a combination of adhesive and cohesive failures (mixed failure).

Statistical Analysis

Data for μSBS were analyzed using a 2-way ANOVA and the Tukey HSD tests since data passed Kolmogorov–Smirnov and Shapiro–Wilk normality tests ($P > .05$). Statistical analysis was conducted using a statistical software (GraphPad Software, San Diego, CA, USA) with a level of significance as 5%.

RESULTS

Table 1 shows the mean μSBS values and standard deviations of the study groups. The highest bond strength values among the groups were obtained after the application of multimode adhesive in self-etch mode ($P < .05$). The lowest bond strength values were observed in the total-etch groups after 6 months of aging ($P < .05$). All groups behaved similarly to the aging factor. After 6 months of aging, a decrease in bond strength values was observed in all groups ($P < .05$). Although the bond strength values were better preserved with DMSO-wet bonding, a statistically significant decrease was observed after 6 months of aging.

As a result of the self-etch application of multimode adhesive, no significant difference was observed between the DMSO-wet bonding technique and control groups, but an increase in bond strength values was observed. This increase is also supported by failure mode observations. When the failure modes of the bond strength test are analyzed, it is noteworthy that less adhesive failure was observed in the groups with the DMSO-wet bonding technique, and even cohesive failure was observed in a few specimens (Figure 1).

DISCUSSION

Adhesion to dentin tissue is more challenging compared to enamel, requiring greater care during adhesive application due to the moist and complex nature of dentin tissue.[27] Moreover, the composition of the smear layer can vary depending on the adhesive system. Multimode adhesive systems are a relatively recent addition to the dental market. They follow an “all-in-one” concept, similar to single-step self-etch adhesives, but they offer the flexibility of being used with phosphoric acid as etch-and-rinse adhesive or

Table 1. Mean ± Standard Deviation Microshear Bond Strength Values (MPa) of Different Groups

Groups		Self-Etch	Total-Etch	*P*
Immediate	Control	26.9 ± 2.2^{A}	23.3 ± 2.2^{AB}	< .01
	Experimental	26.4 ± 1.7^{A}	24.3 ± 2^{A}	.177
6-month aged	Control	21.3 ± 3.3^{B}	19.9 ± 2.4^{C}	.701
	Experimental	23.4 ± 2^{B}	21.2 ± 2^{BC}	.147

Control, water pretreatment; Experimental, 50% dimethyl sulfoxide pretreatment; 6-month aged, 6 months of aging. Different superscript uppercase letters indicate statistically significant differences in each column; *P*-values indicate statistical differences in application mode of the adhesive.

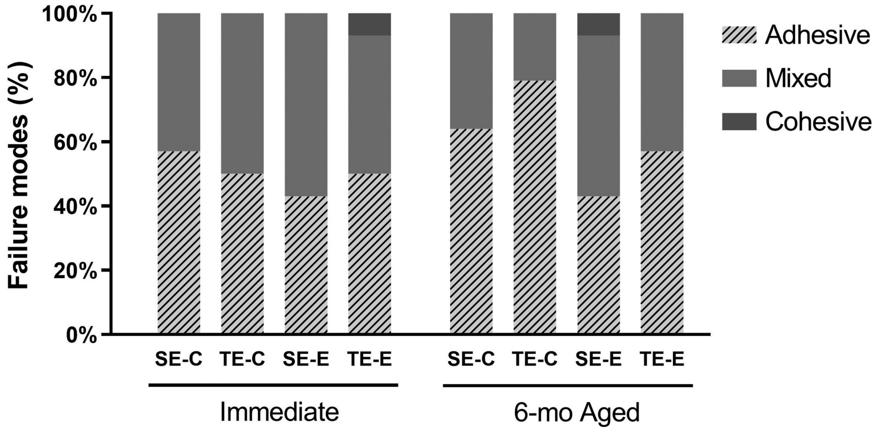

Figure 1. SE-C, self-etch mode with control (water pretreatment); TE-C, total-etch mode with control; SE-E, self-etch with experimental (50% dimethyl sulfoxide pretreatment); TE-E, total-etch with experimental; 6-mo Aged, 6 months of aging.

with no etching as self-etch adhesives, making them versatile for various applications.[28] In the present study, statistically significant differences in the shear bond strength values of the multimode adhesive under the influence of DMSO application were observed. Therefore, the null hypothesis—there would be no difference between the different modes of multimode adhesive systems, whether DMSO is applied or not—was rejected.

In real-life situations, shear stresses are believed to be a primary factor contributing to the breakdown of adhesion between restorative materials and the development of bonding issues. For evaluating dental materials and techniques in laboratory settings, shear bond strength tests are among the most commonly used methods.[29-31] In the current study, the μSBS test was performed due to its practicality and widespread use. It's worth noting that the results we obtained are considered as "immediate bond strength" since the specimens were tested after a 24-hour period. Furthermore, half of the specimens underwent aging through immersion in artificial saliva.

According to one of the comprehensive studies examining the effects of pretreatment of the dentin surface with DMSO, it was reported that 5% DMSO inhibited matrix metalloproteinase activity and the 6-month and 12-month microtensile bond strength values were statistically significantly higher compared to the control group.[20] Additionally, it was reported that DMSO application also prevented the increase in nanoleakage. In another study, it was found that immediate and 6-month bond strengths were higher as a result of DMSO-wet bonding technique application compared to the water-wet bonding (control) group.[32] Although similar results were obtained with our study, this increase was not statistically significant in the present study due to the preference for different generation adhesives. It has been reported that dentin pretreatment with DMSO at 50% concentration effectively reduces collagen exposure, reduces nanoleakage and collagen degradation and improves bonding durability.[33] The number of studies examining the effect of DMSO-wet bonding technique on the use of self-etch adhesives is very limited. In a study examining this effect, it was emphasized that the DMSO-wet bonding technique reduced hydrolytic degradation and produced promising results in terms of bond strength of a self-etch adhesive as a result of a 2-year long-term investigation.[24]

In a previous study, the effect of a 2-step etch-and-rinse adhesive system with water-wet bonding (control), ethanol-wet bonding and DMSO-wet bonding techniques on microtensile bond strength was investigated.[34] They reported that DMSO-wet bonding exhibited significantly elevated bond strength values compared to control and ethanol-wet bonding techniques at both immediate and 6-month aging. Similar to the present study, they also reported a decrease in adhesive failure pattern. Although an increase in bond strength values was observed with DMSO-wet bonding technique in the present study, this increase was not statistically significant. This difference may be due to the dilution of DMSO with water at a concentration of 50% in the present study, while in the other study, the dilution process was carried out with 50% ethanol. Another reason may be the generation difference between the adhesive systems used.

A recent systematic review concluded that the DMSO-wet bonding technique inhibits matrix metalloproteinases and increases the penetration depth of adhesives, which significantly improves bond strength, according to data from included studies.[25] In addition, researchers working on 2-step total-etch adhesive systems concluded that the DMSO-wet bonding technique requires less technical precision and fewer/simpler clinical steps.[34] However, in the case of multimode adhesives, it should be kept in mind that the DMSO-wet bonding technique would require more technical precision by requiring an additional application step.

Considering both the present study and previous research, it is evident that DMSO has the potential to stabilize collagen fibrils,[35] increase the surface moisture of dentin, and inhibit the matrix metalloproteinase activity.[20] These effects play a role in maintaining the integrity of the hybrid layer and improving the long-term bonding between resin and dentin. However, there are substantial concerns related to the application duration, techniques, and toxicity associated with DMSO, which have restricted its widespread use in adhesive dentistry.[25] These promising results, especially in laboratory studies where the dynamic structure of the oral environment cannot be mimicked, need to be supported by clinical studies.

CONCLUSION

Within the limitations of this laboratory study, the DMSO-wet bonding technique showed effectiveness in enhancing the bond strength of the multimode adhesive, whether used in self-etch or etch-and-rinse modes, and this improvement was observed to last for up to 6 months. These results indicate that 50% DMSO could be a practical option for enhancing the quality and durability of resin-dentin bonds. However, it's important to note that implementing this technique does involve an additional step in the bonding process. Further research is needed to assess potential interactions between multimode adhesives and DMSO, especially in the context of long-term aging of the adhesive interface.

Ethics Committee Approval: No ethical approval was required for this article since it does not contain any studies with human participants or animals performed by the author.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Declaration of Interests: The author has no conflict of interest to declare.

Funding: The author declared that this study has received no financial support.

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