Original Research

Effect of Dimethyl Sulfoxide Primer Application Before a Two-Step Etch-and-Rinse Adhesive on the Microtensile Bond Strength and Nanoleakage

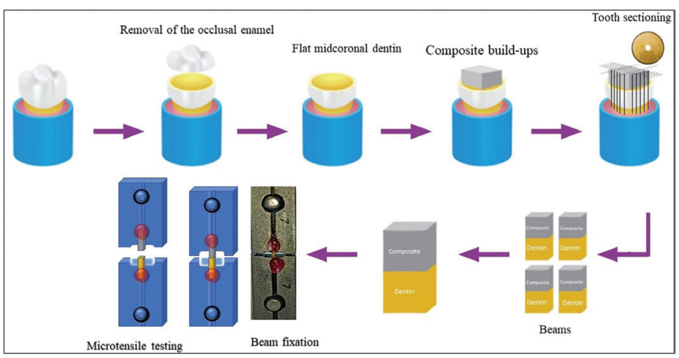
Omar Abdelaziz Ismail[1,2,3], Olfat Elsayed Hassanein[1], Randa Hafez[1,4], Mai Mamdouh[1,5]

[1]Department of Conservative Dentistry, Faculty of Dentistry, Cairo University, Cairo, Egypt, [2]Department of Conservative Dentistry, Horus University, Damietta, Egypt, [3]Department of Restorative Dentistry and Cariology, Institute of Dentistry, University of Turku, Turku, Finland, [4]Department of Conservative Dentistry, Faculty of Dentistry, Sinai University, Sinai, Egypt, [5]Department of Conservative Dentistry, Faculty of Dentistry, Galala University, Al Galala, Egypt

Abstract

Aims: This study investigates the impact of low concentrations of dimethyl sulfoxide (DMSO) on the stability of the dentinal hybrid layer formed using a two-step etch-and-rinse adhesive. **Materials and Methods:** Midcoronal dentine surfaces of 21 extracted molars were etched and randomly recruited into the study groups. This experimental study involved extracting wisdom teeth, preserving them at 4°C in 0.9% sodium chloride with 0.02% sodium azide, sectioning and polishing them, and randomly assigning them to three groups (n = 7/group) for DMSO pretreatment (1% DMSO/water [H_2O], 10% DMSO/H_2O, or no treatment/controls) in preparation for microtensile bond strength assessment and the sectioned beams (0.9 mm^2) where all were subjected to tension until fracture after storage for 24 h after soaking into artificial saliva for 12 months. In addition, nanoleakage assessment was conducted using scanning electron microscopy. The findings were computed statistically using analysis of variance and Tukey's tests (α=0.05). **Results:** The results revealed that immediate bond strength had no significant difference between the three groups ($P > 0.05$). After aging, the control group showed diminished bond strengths less than that of intervention for 1 and 10% DMSO solutions ($P < 0.05$). Regarding nanoleakage, the aged control group showed the highest level of silver infiltration along the hybrid layer. Approximately, 10% of treatment with DMSO in the aged group showed significantly less nanoleakage compared with using a concentration of 1%. **Conclusions:** This study concludes that dentinal pretreatment using DMSO minimizes hybrid layer degradation and improves bond strength in adhesive restorations. It was also inferred that higher concentrations of DMSO may provide a better seal at the tooth–restoration interface within limits.

Graphical Abstract



Address for correspondence: Dr. Omar Abdelaziz Ismail, Department of Conservative Dentistry, Faculty of Dentistry, Cairo University, 11 El-Saraya St. El Manial, Cairo 4240310, Egypt.
E-mails: omar_ismail@dentistry.cu.edu.eg; oaisma@utu.fi

Access this article online

Quick Response Code:

Website: www.jioh.org

DOI: 10.4103/jioh.jioh_11_24

How to cite this article: Ismail OA, Hassanein OE, Hafez R, Mamdouh M. Effect of dimethyl sulfoxide primer application before a two-step etch-and-rinse adhesive on the microtensile bond strength and nanoleakage. J Int Oral Health 2024;16:246-52.

Keywords: Adhesive Dentistry, Dental Bond, Dental Primer, Dimethyl Sulfoxide, DMSO

Received: 13-Jan-2024, **Revised:** 25-May-2024, **Accepted:** 27-May-2024, **Published:** 26-Jun-2024.

Introduction

Many developments in dental adhesives' composition and methods have been made to prolong the functionality of dental restorations.[1,2] Particularly, dentine pretreatment piloted several agents to optimize dentine adhesion,[3-5] in search of a durable and reliable bonding agent, given that bonding to dentine is technique-sensitive and liable, which is yet to challenge in bonding to enamel due to the competition with high water content of dentinal tubules, whose morphology is complicated by the endogenous matrix metalloproteinase (MMP) enzymes.[1,2] In this regard, dimethyl sulfoxide (DMSO) has proven to be effective. DMSO is a colorless and odorless organic solvent with superior biocompatible properties, most fitting for dentine pretreatment.[6] DMSO enables the impregnation of adhesive resin into the dentine matrix and facilitates the hybrid layer formation.[7-9]

However, the efficacy of DMSO as a dentine pretreatment agent remains controversial. Some studies have reported significant improvements in bond strength and durability when DMSO is used before adhesive application,[10-12] whereas others have found no significant difference when compared with conventional techniques.[13,14] Hence, to assess the effects of DMSO dentine pretreatment warrants further attention.

The two-step etch-and-rinse adhesive technique involves applying an acid etchant to the dentine surface to create microporosities and expose collagen fibers, enough to maximize the infiltration into the dentine matrix. Thus, bond strength and marginal integrity are improved.[15,16] Still, the application of DMSO as a dentine pretreatment agent may improve its performance.[7,17,18]

This study evaluates the effect of DMSO dentine pretreatment on the bond strength of two-step etch-and-rinse adhesive systems and assesses the nanoleakage at the dentine restoration.

Materials and Methods

Patients gave informed consent that their extracted wisdom teeth can used for academic research approved by the university institutional review board. For this study, the approval code is CU, REC, 34-7-20. The methodical procedures align with the CRIS guidelines (checklist for reporting in-vitro studies). Teeth were utilized within a month of extraction and kept, to stop microbial development, at 4°C in 0.9% sodium chloride with 0.02% sodium azide.

Samples preparation

Following guidelines for specimen preparation,[19] teeth were sectioned at the coronal region, with water rinsing to facilitate the removal of the occlusal enamel. This exposed the flat midcoronal dentine surfaces required for experimenting. Cutting 2 mm below the enamel–dentine level, to establish standardized smear layers, exposed dentine surfaces were polished for 60 s underwater using 320-grit silicon carbide (SiC) paper. Crown sections were randomly assigned to three groups (n = 7/group) as follows (i) "DMSO pretreatment" in three phases: using 1% DMSO/water (H_2O); using 10% DMSO/H_2O (OT Primer S100, OT Dent, Finland); and using no treatment. (ii) "Storage time" in artificial saliva. The composition of the adhesive system and bonding techniques are displayed in Table 1. The wet bonding technique was used in the control group as instructed by the manufacturer. Dentine surfaces were rinsed and blot-dried with absorbent lint-free paper up to dashing out any water uptake by capillarity. The dentine surfaces remained damp but not drenched. Single Bond 2 (3M ESPE, St. Paul, MN,

Table 1 : Adhesive systems, main components, and application mode of bonding agents

Adhesive system	Components	Bonding protocol	Mode of application*
Single Bond 2 (3M ESPE, USA; SB)	Ethyl alcohol; *bis*-GMA; silane-treated silica; HEMA; water (<10%); copolymer of acrylic and itaconic acid; and UDMA	Control 1% DMSO 10% DMSO	a, b, c, f, g, and h a, b, c, d, e, f, g, and h a, b, c, d, e, f, g, and h
Scotchbond Universal Etchant (3M ESPE, USA)	37% phosphoric acid and fumed silica (pH 0.6)		a and b
1% DMSO (Sigma–Aldrich, USA) 10% DMSO (OT Primer, OT Dent, Finland)	1% DMSO/H2O 10% DMSO/H2O		d and e d and e

bis-GMA = *bis*-phenol diglycidylmethacrylate, HEMA = 2-hydroxyethyl methacrylate, UDMA = diurethane dimethacrylate.

*a = dentine etching for 15 s, b = water rinsing for 15 s, c = blot drying leaving dentine slight moist, d = DMSO-pretreatment application for 40 s according to bonding protocol, e = gentle blow-drying for 10 s, f = active bond application for 10 s, g = air-drying for 5 s, h = light curing for 10 s

USA) was applied for 20 s, with or without 40 s of DMSO pretreatment, and then treated for 10 s. Two-millimeter increments of composite build-ups (Filtek Supreme, 3M ESPE, USA) were carried out, and processed at double-timing conditions (40 s). LED device (light emitting diode, Bluephase 20i, Ivoclare Vivadent, Schaan, Liechtenstein) producing 1100 mW/cm^2 was used to process all resin components.

Microtensile bond strength analysis

Following the non-trimmed microtensile strength testing parameters set forth by the Academy of Dental Materials, CA, USA,[19] each tooth underwent testing on seven beams during each storage period. A blinded operator tested the specimens. Using cyanoacrylate adhesive, each beam was individually glued to a specially designed microtensile testing jig. The beams were then tested under tensile pressures at a crosshead speed of 0.5 mm/min until causing failure. Failures during pretesting were noted and treated as 0 MPa in statistical analyses of microtensile bond strength (μTBS). The average bond strength of the beams evaluated over different periods represented the microtensile strength for each tooth (n = 7).

Fracture pattern analyses

Following microtensile testing, fracture surfaces were examined at a magnification of 50× using a light microscope. Unrecognized surfaces were examined by scanning electron microscope (QUANTA FEG250 Scanning Microscope, Dawson Creek Drive Hillsboro, OR, USA). Failure patterns were categorized based on the broken side into (a) failures that occur before testing are referred to as pretesting failures; (b) cohesive failures, which occur inside the dentine or resin alone; (c) adhesive failures, which occur at the interface between the composite and dentine; and (d) mixed failures, which occur at the hybrid layer and involve cohesive failure of the dentine substrate and the dentine itself.

Interfacial nanoleakage examination

Six beams from all groups were assessed. After preparation, three beams were evaluated 24 h later. Three further beams were kept in artificial saliva for 12 months before the assessment of nanoleakage.[20] After surface-drying, beams were covered in two coats of nail varnish, spaced 1 mm apart from the interfaces. Following a 10-min rehydration in distilled water, samples were submerged into a 50% (w/v) solution of ammoniac silver nitrate (pH 9.5) for 24 h at no source of illumination. After rinsing adequately, they were immersed into an image developer solution (Kodak, UK), and left under fluorescent light for eight hours.

After removing the nail varnish with 1000-grit SiC paper, epoxy glue (Pox Glass resin, Pioneers for Polymers & Chemicals, Alex, Egypt) was applied. Polishing with 1 and 0.05 μm diamond paste (Buehler Ltd, USA) followed. After being ultrasonically cleaned, epoxy blocks were placed on a glass slab and thinly coated with carbon-gold using a scanning microscope (QUANTA FEG250 Microscope) for examination. Two blinded operators assessed the patterns and extensions of silver uptake at magnification ranges between 1000× and 10,000×.

Statistical analysis

The nanoleakage values and μTBS (MPa) of all beams from the same tooth were averaged. A two-way analysis of variance with Tukey's honestly significant difference tests was calculated, with storage duration and DMSO concentrations being the factors and statistical significance set at $\alpha = 0.05$.

Results

Microtensile bond strength analysis

Results for μTBS are shown in Figure 1. There was no significant change between the control group and the DMSO-pretreated groups after 24 h of storage ($P > 0.05$). Following the 12-month storage period in artificial saliva, the control group displayed a 31.5% decrease in microtensile strength, which was significantly less than that of the DMSO-pretreated groups ($P = 0.014$). Immediate results of 10% DMSO (mean = 46.9) were higher than the control group (mean = 38.3) but the difference was not statistically significant ($P = 0.36$). Either with storage for 24 h or 12 months, DMSO pretreatment among groups was not remarkable [Figure 1].

Fracture pattern analyses

Figure 2 shows the fracture pattern of the aged and immediate specimens. Only in the aged control group adhesive failure was the predominant fracture pattern, not mixed failure. After a 24-hour incubation period (i.e., immediate bond strength). Although the control group showed two pretesting failures; eight pretesting failures following a year of storage were detected.

Interfacial nanoleakage examination

Figure 3 displays representative scanning electron microscope micrographs demonstrating the infiltration of silver over hybrid layers. The hybrid layers with substantial silver deposits strongly impregnated in them (aged control) exhibited the highest levels of silver uptake. Compared with aged control, the immediate control manifested less silver absorption. Before aging, the three groups were comparable. After aging, higher nanoleakage levels were highest in the control group showed the highest nanoleakage level. DMSO groups showed the least silver

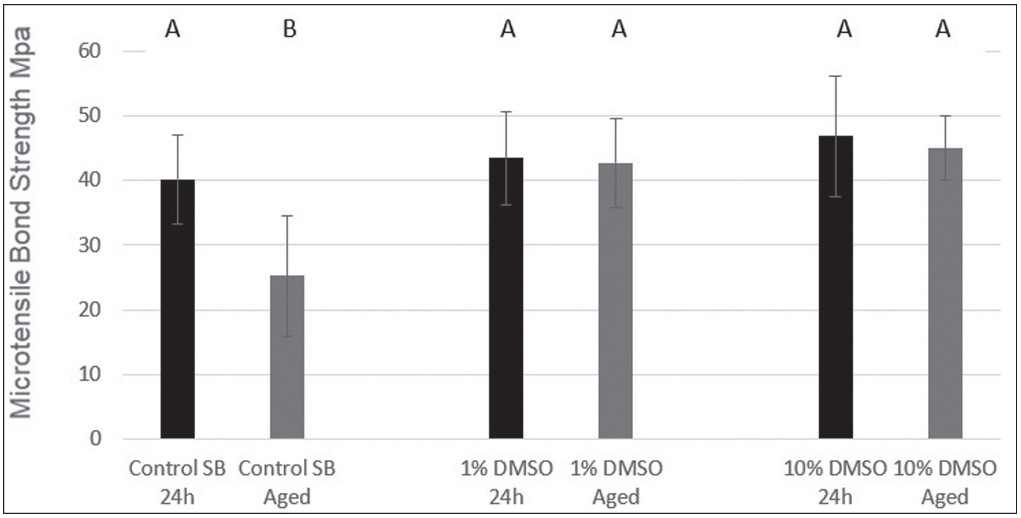


Figure 1: Microtensile bond strength (MPa: mean ± SD) of the control group and different concentrations of DMSO-pretreated groups ($n = 7$ teeth/group). Upper case letters indicate the statistically significant differences between the tested groups ($P < 0.05$)

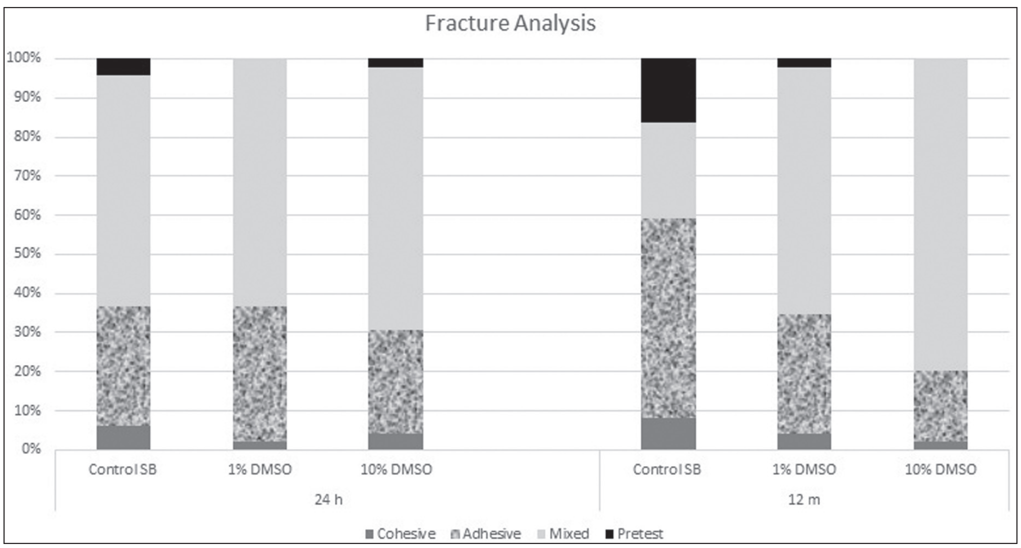


Figure 2: Fracture patterns in percentages (%) of tested specimens after the bond strength test at 24 h or 1 year of aging in artificial saliva at 37°C

uptake with a slight increase after storage. 1% DMSO/H_2O showed higher silver uptake than 10% DMSO/H_2O only after aging.

Discussion

Our results indicated that the use of 1% and 10% DMSO as a dentine pretreatment can prevent hybrid layer degradation and maintain bond strength even after 1 year of aging. Two different concentrations of DMSO were used, 1% and 10%, which represented the safe and low cytotoxic concentration. According to Al-Ani *et al*.,[21] higher concentrations' cytotoxic effects need more investigations. We utilized Single Bond 2 from 3M ESPE to assess the stability of resin/dentine bonds in the μTBS test. This testing compares various adhesives effectively, although its clinical applicability remains controversial.[19,22]

However, DMSO pretreatment improves the adhesives' penetration and makes the dentine surface wet.[23] Collagen fibrils' potential to form hydrogen bonds with each other and the self-assembly of water molecules within dentine is disrupted by DMSO, which triggers highly crosslinked collagen to separate into a sparse fibril network.[12,9] Greater gaps in the collagen meshwork expose collagen at the base of the hybrid layer and allow exposed collagen fibrils to impregnate the adhesive resin.[24] The ability of DMSO to form hydrogen bonds with oxygen to backbone amide to proteins and stop collagen matrix from collapsing.[25]

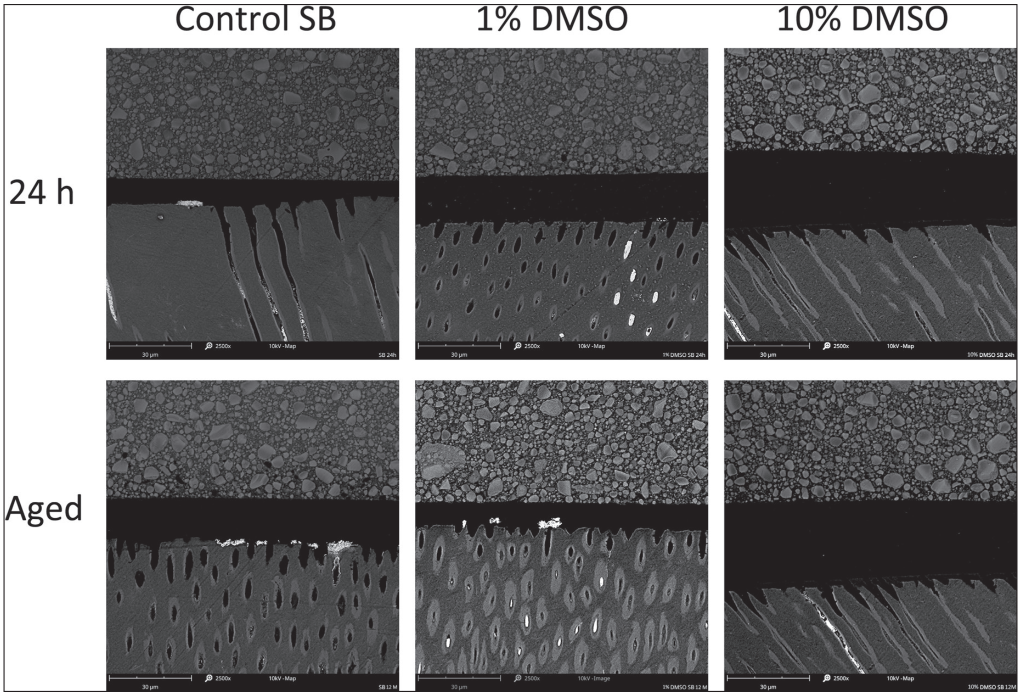


Figure 3: Representative scanning electron microscope micrographs showing nanoleakage extensions of resin-dentine interfaces bonded to dentine using 1% or 10% DMSO/H_2O solutions as pretreatments. Beams were tested at 24 h or after 1 year of aging in artificial saliva at 37°C

Metalloproteinase activities within the hybrid layer were suppressed by DMSO pretreatment.[12,26,27] One interpretation of how DMSO inhibits MMPs is that it obstructs the binding sites that allow MMPs to engage with their substrate, dentine collagen. Unlike other polar solvents, DMSO dissolves the majority of typical proteins by interacting with protein peptides, their solubilizing ability. DMSO may leverage enzymatic stripping to lessen the breakdown of collagenous fibrils.[28] Alternatively, enzymes may have a greater affinity for DMSO compared with pure water. Therefore, denaturation can occur when DMSO forms bonds with hydrophobic moieties in proteins.[25,29]

The post-acid-etching remnant voids were not filled with adhesive resins due to insufficient adhesive infiltration into the etched dentine, allowing the percolation of water and microbes, leaving collagen bundles prone to deterioration over time.[30] We found that nanoleakage was significantly higher in the 1% DMSO-aged group than in the 10% DMSO-aged group, impacting the seal at the tooth–restoration interface. Nevertheless, no statistically significant difference was found between the 1% and 10% DMSO aged groups regarding μTBS. The lower nanoleakage observed in the 10% DMSO group after aging may be attributed to the better sealing ability of the higher concentration of DMSO.[1,31]

Here, the DMSO dry bonding technique was not used for performing a blot drying step. The wet approach ensures that the collagen network is fully saturated with the DMSO solution.[32] In contrast, the DMSO dry bonding technique involves utilizing a 30-s heavy blow of air to significantly dry the DMSO layer after its application before the adhesive is applied.[12] This drying step removes a significant proportion of the solvent, causing residual water to be trapped within the collagen network. Although techniques utilize DMSO as a solvent that can enhance the bonding properties of dental adhesives by improving collagen fiber preservation and resin infiltration into the tooth structure, how the DMSO is handled before applying the adhesive is a major concern. It is important to note that the selection of either technique may depend on various factors, such as the adhesive system used, manufacturer instructions, operator preference, and desired clinical outcome.

The use of DMSO as a dentine pretreatment is not without potential concerns. One potential concern is the cytotoxicity of DMSO. Although concentration proportions are relatively low, the biocompatibility of DMSO and its long-term effects on the dental pulp and surrounding tissues should be further examined. Hebling *et al*.[33] assessed the cytotoxicity of very low concentrations <0.01% DMSO, reporting an activity of odontoblast-like cells contacted pulp tissue repair was slightly affected by DMSO. Al-Ani *et al*.[21] assessed the cytotoxicity of resins-based methacrylate after incorporation of DMSO, at weight concentrations of 0, 0.01, 0.1, 1, 5, or 10% w/w%, endorsing cell viability is unaffected by the addition of DMSO to hydrophobic resins. Nevertheless, adding DMSO to self-etch hydrophilic resins at a concentration of 1% w/w aggravates cytotoxicity.

Other confounders include the specific adhesive system used, the methodology limitations, and the aging conditions. The use of different concentrations of DMSO may induce diverse proportions of nanoleakage at the dentine–restoration interface, which minimizes the generalizability of the suggested conclusions. Sample variability, including differences in wisdom tooth structure and morphology, can also confound observations. The use of extracted wisdom molars, not actively contributing to mastication, does not represent the harsh oral environment. Timing of storing dentine beams in artificial saliva composition and dentine permeability may interact with the efficacy of bonding agents, their durability, and hybrid layer quality.

Conclusion

Within the limitations of this investigation, using DMSO as a dentine pretreatment aids in preventing hybrid layer degradation and maintaining bond strength in adhesive restorations. As low concentrations as of 10% of DMSO amenable to inducing minimal cytotoxic effect, improved the durability of etch-and-rinse adhesive should further testing and clinical trials attest to such a validity.

Acknowledgment

Not applicable.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Author contributions

Conceptualization: OAI and OEH; Methodology: OAI and MM; Investigation: OAI and MM; Data Curation: OAI and RH; Writing—Original Draft: OAI and MM; Writing—Review & Editing: OAI, OEH, and RH; Visualization: OAI; Project Administration: OAI; and Supervision: OEH, MM, and RH.

Ethical policy and institutional review board statement

Faculty of Oral and Dental Medicine, Cairo University, Ethical committee approved and gave it code: CU, REC, 34-7-20.

Patient declaration of consent

Not applicable.

Data availability statement

Not applicable.

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