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Environmental scan electron microscopy of resin/dentin interface after dentin surface pretreatment with dimethyl sulfoxide

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ABSTRACT: Objective: To investigate the capability of a dentin pretreating agent (dimethyl sulfoxide (DMSO)) to affect the resin/dentin interface after bonding with a universal adhesive in etch and rinse mode.

Materials and methods:Flat mid-coronal dentin specimens of12human permanent premolars were exposed and grounded using silicon carbide paper.Each specimen was acid-etched with orthophosphoric acid, rinsed, andblot-dried using filter paper. Specimens were then assigned to pretreatment with 10% DMSO/distilled water containing primer (OT primer) (pretreated group)or to the control group (where no pretreatment was applied) (n =6). A universal adhesive (GLUMA Bond Universal) was applied, and resin composite blocks were created.After 24 h of distilled water storage, specimens were cut in the buccolingual direction and prepared for analysis ofthe resin/dentin interface using an environmental scan electron microscope.

Results: The application of a dentin surface pretreating primer (DMSO) showed morenoticeable and longer resin tags when compared with the control group.

Conclusions:A noticeable improvement in the resin/dentin interface can be observed with dentin surface preparation with DMSO.

Keywords: Micromorphology; Dentin;Surface pretreatment; Dimethyl sulfoxide; Universal adhesive

I. INTRODUCTION

The adhesive technology has been devised to achieve a satisfying cosmetic result with a minimal amount of tooth preparation(1). Despite advancements in the adhesive field, degradation of tooth-bonded interfaces still contributes to the reduced long-term clinical success of adhesive restorations(2).

Bonding to the biologically active, dynamic dentin substrate is still challenging(3).Evidence has proven that adhesive hydrolysis, enzymolysis from cysteine cathepsin and matrix metalloproteinases (MMPs), inadequate resin monomer penetration, and secondary caries are potential threats to hybrid layer degradation(1). Currently, adhesive systems are based on three different strategies: etch-and-rinse, self-etch, and resin-modified glass-ionomer approaches. More recently, a new development was called universal or multi-mode adhesives, which may be used either in etch-and-rinse, self-etch, or selective etch mode(4). This allows the clinician to select the most suitable etching protocol for each clinical situation(5).

Etch and rinse approach (smear layer and smear plug removal) relies on phosphoric acid etching of enamel and dentin(6). Concurrently, dentin etching results in demineralization over a depth of 3-5 **µm**by exposing a scaffold of collagen fibrils that is nearly totally depleted of hydroxyapatite(4). It leaves the collagen fibers soaked in water from rinsing the acid. Furthermore, the preforms of cysteine cathepsin and matrix metalloproteinase (MMPs) bound to collagen are uncovered and activated. These gelatinases (MMP-2-9) and collagenases (MMP-8) slowly begin to degrade the collagen fibrils, inhibiting composite bonding to demineralized dentin in the hybrid layer(7, 8).

Adequate wetting of the dentin surface by adhesive components and subsequent micromechanical interlocking of resin monomers with the demineralized collagen fibrils are two main factors involved in E&Rbonding(3).Therefore, "wet bonding" was introduced to promote higher-quality composite dentin bonds(9).Nonetheless, adequate monomer penetration depends heavily on collagen interfibrillar space management and moisture control.Overwetted dentin after acid etching causes excess water entrapment within the collagen matrix. This activates MMPs and cathepsins and accelerates ester-linked polymer hydrolysis. Also, this causes phase separation of adhesive components during hybridization, limiting cross-linking hydrophobic monomers diffusion deeply in the hybrid layer with blister and globule-like void formation at the resin-dentin interface(2, 10).

Dimethyl sulfoxide (DMSO) has emerged as a polar aprotic solvent capable of improving resin-dentin bonding. A polyfunctional molecule that is fully miscible in most solvents, hydrophilic and hydrophobic monomers(11). Dimethyl sulfoxide is perhaps the best-known penetration enhancer, with the ability tobreakdown the water's self-associative tendency(2).Over ethanol, it has lower volatility and higher permeability, allowing the development of theDMSO-wet bonding concept(12). It allows higher monomer diffusion, better hybrid layer formation, and lower endogenous collagenolytic activity. It improves the wettability of demineralized dentin by dissolving both hydrophilic and hydrophobic monomers and reducing phase separation.

Scan electron microscopic analysis has been used a lot in dentistry to assess qualitative aspects. Research into dental structures and their interactions with adhesive systems, resin cement systems, and restorative materials are some of its uses.In this investigation, an environmental scan electron microscope was used to assess the ultra-morphology of HL. Environmental scan electron microscopy allows for the imaging of wet, live, and insulating samples without the need for prior specimen preparation or coating.During imaging, a gaseous (low vacuum) atmosphere is kept around the sample. Electron beam-induced abnormalities, like shrinkage and cracking, are less common in samples treated by environmental scan electron microscopy, allowing for the use of the same specimen for several analyses(13).

A recent systematic review showed the effect of DMSO as a dentin pretreatment on the hybrid layer integrity of different adhesive formulas(12). However, there is still alack of knowledge about its effect on the performanceofuniversal adhesives.Thus, the purpose of this current investigation was to assess how DMSO affected the universal adhesive applied by E&R in terms of themicromorphology atthe resin/dentin interface.

II. MATERIALS AND METHODS

Materials used in the study are described in **Table 1**. All involved materials were applied according tothe different manufacturers' instructions.

Material& Manufacturer	**Chemical Composition**	**Batch Number**
GLUMA Etch 35 Gel (Kulzer GmbH, Hanau, Germany)	35% phosphoric acid, pigments, thickening agents, water	K010205
OT Primer S100 (OT Oy Dent, Turku, Finland)	10%wt DMSO, 90%wt H_2O	S1877m
GLUMA Bond Universal (Kulzer GmbH, Hanau, Germany)	UDMA, acetone, 4META, 10MDP, water	KB10052
CHARISMA diamond Universal nanohybrid resin composite **(Kulzer GmbH, Hanau, Germany)**	TCD-urethaneacrylate, UDMA, TEGDMA, Aminobenzoic acid ester, barium, aluminum, boron, fluoride silicate glass and colloidal silica, BHT,titanium dioxide, pigments,Camphorquinone	K010078

UDMA; urethane dimethacrylate, 4-META; 4-methacryloyloxyethyl trimelliate anhydride, 10-MDP; 10-methacryloyloxydecyl dihydrogen phosphate, TCD; tricyclodecane, TEGDMA; triethylene glycol dimethacrylate, BHT; butylated hydroxytoluene

Teeth selection and preparation

Twelve freshly extracted intact human permanent premolars were collected from patients who came to extract their teeth due to periodontal diseaseat theFaculty of Dentistry at Mansoura University. An approved consentfrom those patients and an approved ethical committee protocol (No. A14020822)were obtained to collect

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and use the collected teeth in this research.Teeth were cleaned, disinfectedwith 0.5% chloramine T, and stored in a refrigeratorin distilled wateruntil usage. Each tooth was centralized in a cylindrical container.The occlusal enamel and superficial dentin of each tooth were cut using a diamond disc until the mid-coronal dentin level was exposed. A standard smear layer was enhanced by using silicon carbide paper for 30 s to grind the exposed dentin surface.

Dental bonding/ restorative procedure

The dentin surface of each specimen underwent acid atching using 35% phosphoric acid (Gluma Etch 35 Gel, Kulzer, Hanau, Germany) for 15 s, then was water-rinsed for 30 s. The drying of the surface was done by blotting using filter paper.

For the control group, the universal adhesive (GLUMA Bond Universal,Kulzer, Hanau, Germany) was applied with no dentin pretreatment. One coat of the adhesive was applied and actively rubbed for 20 s. The surface was gently air-drieduntil the adhesive film no longer moved,then light-cured for 10 s.

In the DMSO-pretreated groups,10% DMSO/water primer(OT Primer S100, OT Oy Dent, Turku, Finland) was applied, actively rubbed for 60 s, and then air dried for 30. After that, the UA was applied.

Resin composite blocks(CHARISMA diamond,Kulzer, Hanau, Germany)in two increments were built up, packed well and light-cured for 20 s.Specimens were kept in distilled water for 24 h at 37 °C. Then, specimens of each group were prepared for resin/dentin interface micromorphological analysis.

Micromorphology of the resin/dentin interface

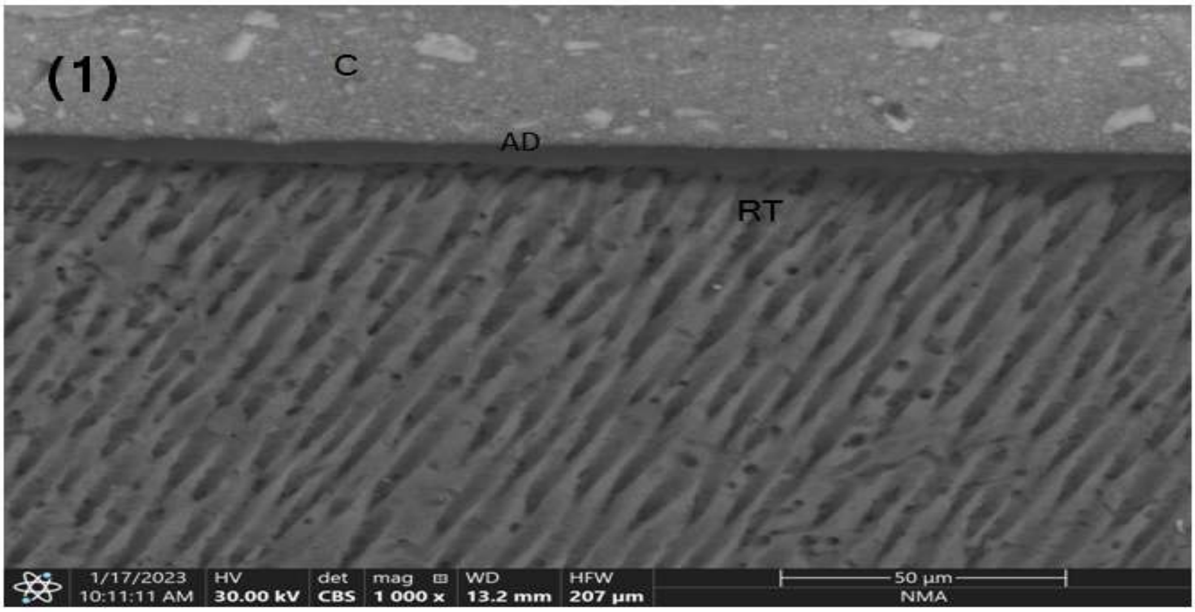
Each specimen was first cut vertically in a buccolingual direction, and two halves per specimen were obtained.Each half was polished using silicone carbide paper of different grit sizes (Sia Abrasives, Switzerland)(600 to 2500grit). Extra polishing was done using a polishing brush and pastes of different granulations. Specimens were copiously rinsed with distilled water, demineralized with a 10% orthophosphoric acid solution for 5 s, and then deproteinized with a 5% sodium hypochlorite solution for 5 min. Specimens were kept hydrated until the environmental scan electron microscopicpreparation (Prisma E model, Thermo Fisher Scientific, USA). Each specimen was imaged at 1000x to precisely analyze the resin/dentin interface.

III. RESULTS

Micromorphological pattern analysis at the resin/dentin interface

The descriptive environmental scan electron microscopic figures of the resin/dentin interface showed homogenous resin tags with some areas of microleakage or gap (in the control group) (Figures1 and 2).

<u>In the pretreated group</u>, therewere more noticeable, lengthy, funnel-shaped, and compact resin tags extending inside the dentinal tubules(Figures 3 and 4).



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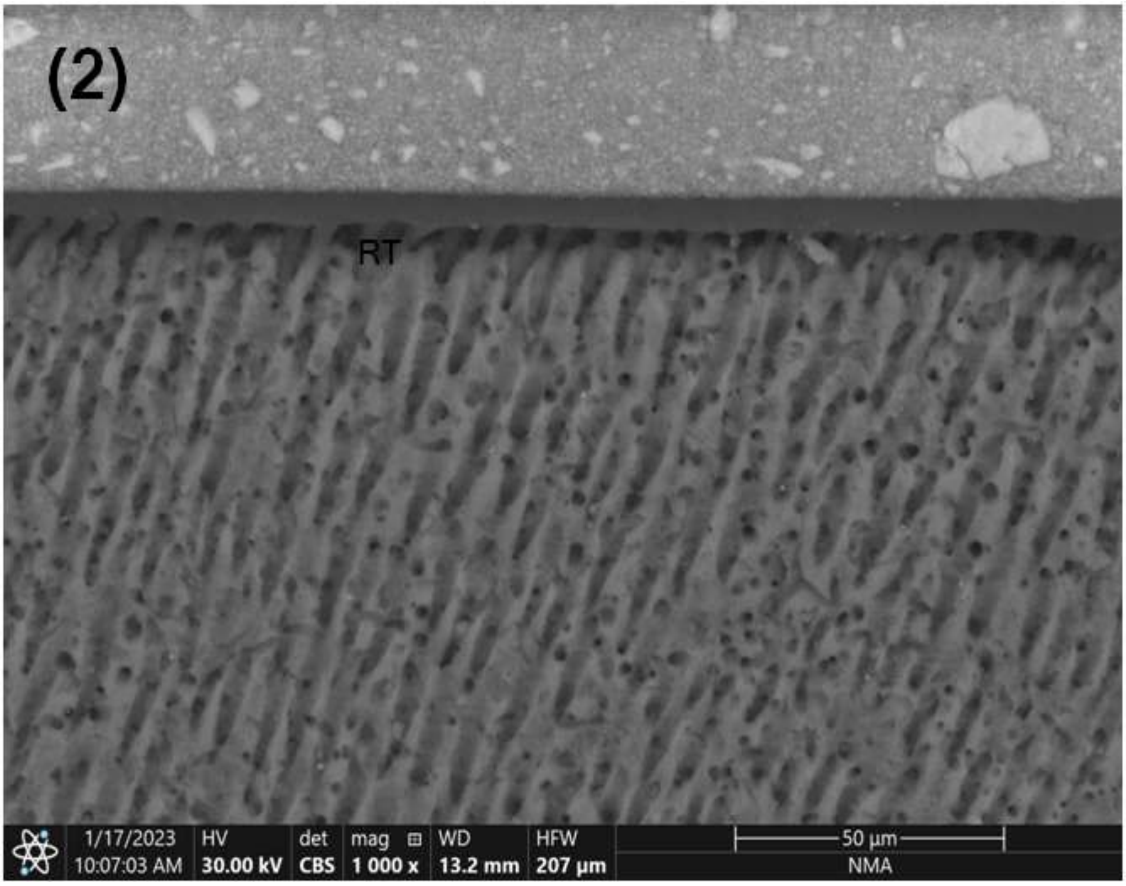
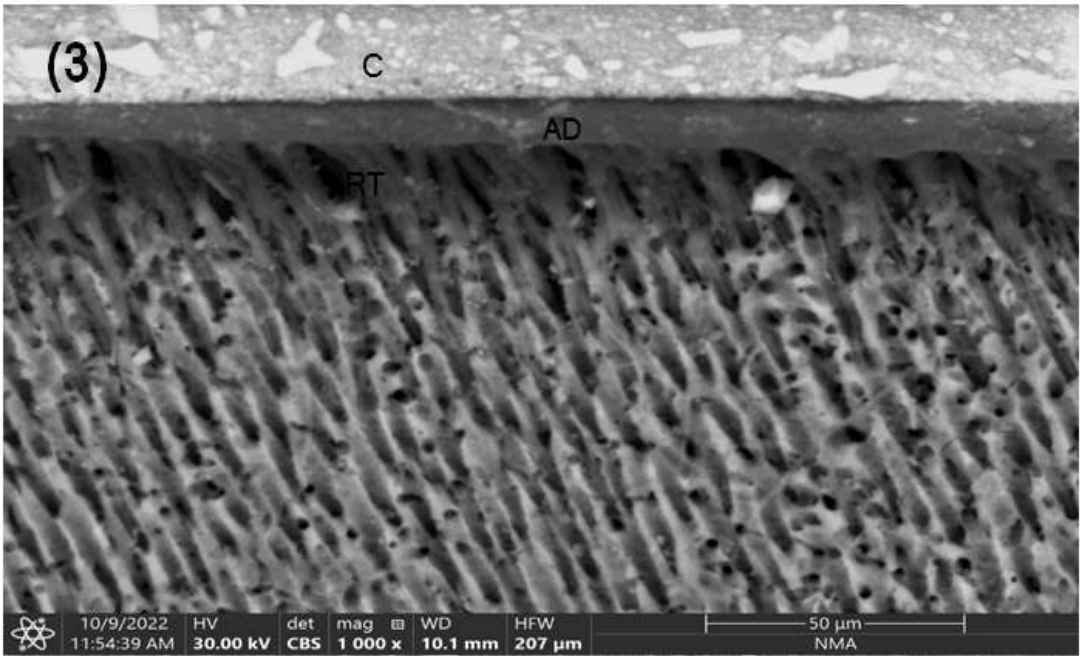


Figure 1 and 2 (The control group):C; composite resin, AD; adhesive layer, RT; resin tags



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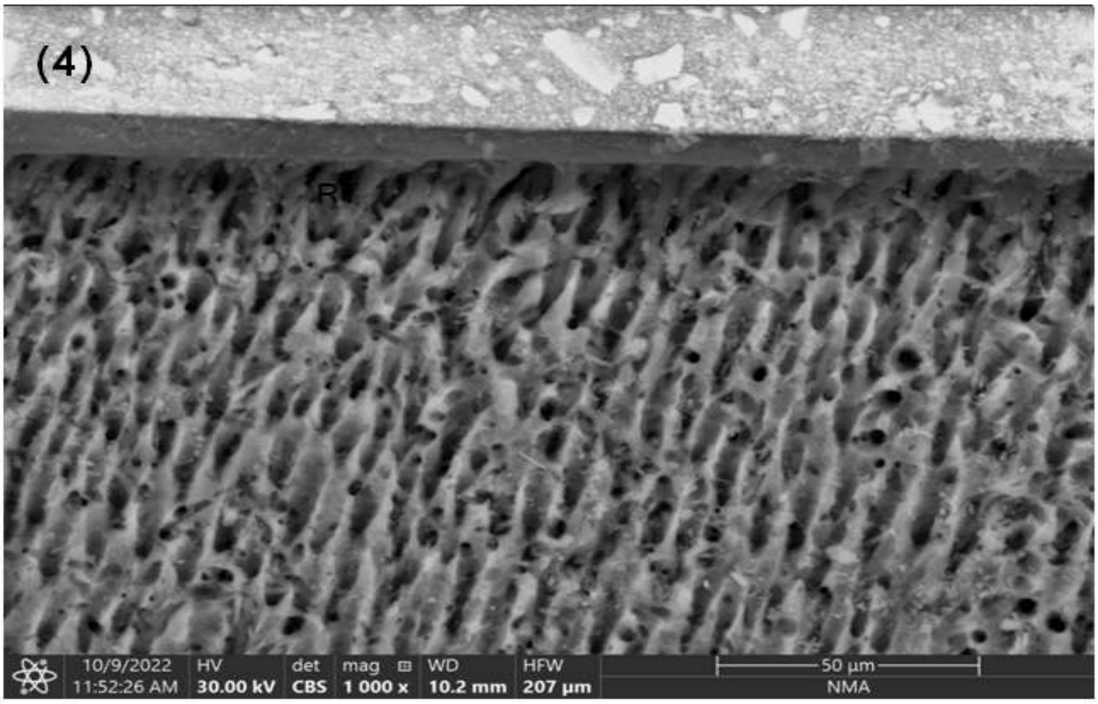

Figure 3 and 4 (DMSO-pretreated group):C; composite resin, AD; adhesive layer, RT; resin tags

IV. DISCUSSION

The durability of the adhesive-dentin bond is directly related to the quality of the hybrid layer that joins the bulk adhesive to the adjacent dentin. Water in dentin bonding assumes the role of a double-edged sword, posing as a sanctuary or complication. Water-associated artifacts may be manifested as water-infused hybrid or adhesive layers, water channels, bubbles, or discrete water films between the adhesive and resin composite. This is in addition to increasing interfacial permeability just after bonding.

In this study, DMSO was used for its proper wetting capability, especially for porous irregular surfaces(14). The presence of DMSO in the collagen matrix may reduce the number of water molecules between polymers by lowering the water cohesive forces and surface tension. In addition to being a penetration enhancer and a carrier for the monomers into collagen interfibrillar spaces, dimethyl sulfoxide is also a diluent for the resin viscosity, which in turn reduces phase separation during hybridization.

Instead of using epoxy resin dies, artificial teeth, or bovine teeth, human natural teeth were used to replicate the physiological oral scenarios. For a full day at 37 °C, specimens were submerged in distilled water to facilitate water sorption and finish the polymerization of composite resin and post-operative adhesive (15).

RT

As the acid etch eliminates the smear layer and increases the surface energy, resin tags and a hybrid layer were seen, according to the results of the micromorphological investigation (16). The majority of the scanning images showed thin adhesive and hybrid layers without a clear separation line between them, which is due to the phase separation and high vapor pressure brought on by the acetone content, which was in line with the conclusions of Takamizawa et al. (15). After applying dimethyl sulfoxide to moist dentin, an increase in the length, quantity, and depth of resin tag penetration into dentin was observed. These results may be associated with the actions of dimethyl sulfoxide, which enhance penetration and wetting. These results were similar to those ofGuo et al. (16), who assessed the integrity of the hybrid layer and conducted experiments with water, ethanol, and DMSO-wet bonding. In contrast to the shorter and more ragged resintags observed with water-wet bonding, longer resintags were discovered with DMSO-wet bonding.

Through this study, it could be concluded that the application of dimethyl sulfoxide as a dentin surface pretreatment can improve the resin-dentin interface integrity. However, the findings of this study were limited to studying dimethyl sulfoxide's immediate effects. Therefore, its effect over the longterm still needs to be evaluated. In addition, its effect on other adhesive formulas isalso required in futureresearch.

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