Brazilian Dental Journal (2021) 32(6): 93-106
http://dx.doi.org/10.1590/0103-6440202104595

ISSN 0103-6440

Collagen cross-linking agents + dimethyl sulfoxide improving the adhesive properties of erosive lesion dentin

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To investigate the effect of the dimethyl sulfoxide combined with cross-linking agents on microtensile bond strength, silver nitrate penetration and in situ degree of conversion analysis of adhesives to the erosive dentin treatment with Cola-based soft drink. One hundred and sixty-six molars were assigned to 20 groups: (1) Treatment: Sound dentin; Erosive dentin; Erosive dentin treated with primer of dimethyl sulfoxide; Erosive dentin treated with DMSO primer containing proanthocyanidin and rivoflavin; (2) Adhesive systems: iBond Universal and Scotchbond Universal; and (3) adhesive strategy: etch-and-rinse or self-etch strategy. After restoration, specimens were sectioned into sticks to be tested. The data from microtensile bond strength (MPa), silver nitrate penetration (%) and in situ degree of conversion (%) were analyzed by (three- and two-factor ANOVA; Tukey's test α=5%). The application of dimethyl sulfoxide combined of not with cross-linkers improved all properties evaluated when compared to only erosive dentin treatment with Cola-based soft drink. However, only when dimethyl sulfoxide was combined to cross-linkers, the values of the microtensile bond strength, silver nitrate penetration and in situ degree of conversion in erosive dentin treatment with Cola-based soft drink was similar to sound dentin, for both adhesives and adhesive strategies. The application of dimethyl sulfoxide combined with the collagen cross-linking agent contributed to increasing the bond strength and degree of conversion in erosive lesion dentin, at the same time that significantly reduction of nanoleakage in this substrate.

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Key Words: Erosive tooth wear; Microtensile bond strength, Nanoleakage; Cross-linking agents, DMSO

Introduction

Preventive therapies based on fluoride application and improvements in hygiene practices have allowed a significant reduction in the incidence of caries (1) around the world. However, the increase acidic/erosive food ingestion, as is seen in many contemporary life , has led to an increment in the prevalence of lesion erosive (2).

Erosive tooth wear is a condition involving erosion with causes lying in attrition and abrasion, both of which impact quality of life, especially due to visibly shorter teeth and exposure of dentin, causing hypersensitivity (3). All these features complicate restorative management and contribute to the lower durability of restorations (3).

Erosive lesion dentin is a biological, chemical and structurally modified substrate (4) recognized by demineralization and successive alteration of the organic matrix (5). Furthermore, there are an increased micro- and nanoporosity and exposed denatured collagen fibers (4, 5). This process leads to occur a frequent pH drops and, consequently, promote higher proteolytic activity in the dentin matrix (5). All these changes compromise resinous monomers infiltration into to erosive lesion dentin (6).

To increase the mechanical properties of collagen fibrils and make them less susceptible to endogenous protease action, some researchers have focused their studies on the use of collagen crosslinking agents like as carbodiimide, glutaraldehyde, riboflavin and proanthocyanidins (7).

Recently published studies have shown promising adhesive results from the application of an additional water-based primer containing proanthocyanidin and riboflavin to eroded dentin (8, 9). However, according to Siqueira et al.,(8, 9), it was not possible to recover all adhesive and mechanical properties in eroded dentin compared to sound dentin. This may be due to the organic nature and higher molecule size of the collagen crosslinking agents. Both factors pose difficulties in the dissolution of polar solvents, limiting proper dissolution and infiltration within the demineralized collagen matrix; especially in eroded dentin wear (10).

An interesting alternative is the combination of collagen crosslinking agents with more potent solvents, such as dimethyl sulfoxide. It is a polyfunctional molecule, fully mixable in most solvents and in hydrophilic and hydrophobic monomers utilized in adhesive dentistry (11). The polar characteristic of dimethyl sulfoxide combined with the small, compact structure is responsible for its capacity to penetrate biological surfaces (11) and results in its ability to associate with water, proteins, carbohydrates, ionic substances and other constituents.

Additionally, dimethyl sulfoxide has the ability to contend with water molecules in an interpeptide hydrogen bond to dissociate extracellular collagen into a more dispersed network of fibrils (12), consequently improving the infiltration of demineralized dentin (13) and maintaining the bond to dentin (12). Due to the amphiphilic nature of dimethyl sulfoxide, it promotes better dissolution and infiltration of crosslinking agents into the demineralized matrix of eroded dentin (14). However, there is still a lack of knowledge; no research has evaluated an additional primer containing dimethyl sulfoxide combined with collagen crosslinking agents.

Therefore, this study evaluated the effect of dimethyl sulfoxide combined with collagen crosslinking agents on microtensile bond strength, silver nitrate penetration and *in situ* degree of conversion analysis of universal adhesive systems to the erosive dentin treatment with Cola-based soft drink. The experimental hypotheses evaluated were that regardless of the adhesive system, the application of dimethyl sulfoxide combined with the collagen crosslinking agents would (1) increase the bond strength, (2) decrease the silver nitrate penetration values of the adhesive interface and (3) improve *in situ* degree of conversion in erosive dentin treatment with Cola-based soft drink.

Materials and methods

Selection and Preparation of Teeth

One hundred and sixty-six extracted caries-free third molars were collected. After approved by the Commission for Medical Ethics (2.851.586), the molars were disinfected in 0.1% thymol solution and stored in deionized water for no more that 6 months. The occlusal third was removed using a diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Then, a standardized smear layer was created using 600-grit silicon carbide for 60s.

Experimental Groups

One hundred and sixty molars were randomly divided to 20 experimental conditions (n = 8 teeth for microtensile bond strength, silver nitrate penetration and *in situ* degree of conversion analysis inside the hybrid layer measurements, according to the combination of the independent variables: (1) *Treatment*: Sound dentin (control group); Erosive dentin treatment with Cola-based soft drink; Erosive dentin treated with DMSO primer of dimethyl sulfoxide (Sigma-Aldrich; St. Louis, MO, USA; pH 8.2); Erosive dentin treated with Primer containing 6.5% by weight proanthocyanidin (Mega Natural Gold, Madera, USA; Batch number 05592502-01), DMSO Primer containing 0.1% by weight riboflavin (Fisher Scientific GmbH, Schwerte, Germany) Batch number 070046); (2) Adhesive systems: iBond Universal (Heraeus Kulzer; Hanau, Germany); Scotchbond Universal (3M Oral Care; St Paul, MN, USA); and (3) *adhesive strategy*: etch-and-rinse or self-etch strategy. The materials used, batch numbers, composition, and application modes are detailed in box 1.

Box 1. Adhesive system (Batch number), groups, composition, and application modes

Adhesive system (batch number)	**Groups**	**Application mode**
		Etch-and-rinse
iBond Universal - IBU; Heraeus Kulzer (010024)	Sound dentin	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Apply adhesive as for self-etch mode.
	Erosive dentin treatment with Cola-based soft drink (ED)	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Apply adhesive as for self-etch mode.
	ED + DMSO	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Active application of 50% DMSO for 60 s; 5. Air stream for 5 s keep the surface slightly moist; 6. Apply adhesive as for self-etch mode without DMSO.

Adhesive system (batch number)	Groups	Application mode
		Etch-and-rinse
	ED + DMSO-PATC	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Active application of proanthocyanidin (Proanthocyanidin-grape seed extract 6.5% weight, 50% DMSO) for 60 s; 5. Air stream for 5 s keep the surface slightly moist; 6. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-RIB	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Active application of riboflavin (Riboflavin 0.1% weight, 50% DMSO) for 60 s; 5. Light-cure for 2 min; 6. Air stream for 5 s keep the surface slightly moist; 7. Apply adhesive as for self-etch mode without DMSO.
Scotchbond Universal -SBU; 3M Oral Care (638367)	Sound dentin	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Apply adhesive as for self-etch mode.
	Erosive dentin treatment with Cola-based soft drink (ED)	1. Apply etchant for 15 s; 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist; 4. Apply adhesive as for self-etch mode.
	ED + DMSO	1. Apply etchant for 15 s. 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist. 4. Active application of 50% DMSO for 60 s; 5. Air stream for 5 s keep the surface slightly moist; 6. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-PATC	1. Apply etchant for 15 s. 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist. 4. Active application of proanthocyanidin (Proanthocyanidin-grape seed extract 6.5% weight, 50% DMSO) for 60 s; 5. Air stream for 5 s keep the surface slightly moist; 6. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-RIB	1. Apply etchant for 15 s. 2. Rinse for 10 s; 3. Air-dry for 5 s to keep the surface slightly moist. 4. Active application of riboflavin (Riboflavin 0.1% weight, 50% DMSO) for 60 s; 5. Light-cure for 2 min; 6. Air stream for 5 s keep the surface slightly moist; 7. Apply adhesive as for self-etch mode without DMSO.

Box 1. continuation

Adhesive system (batch number)	Groups	Application mode
		Self-etch
iBond Universal - IBU; Heraeus Kulzer (010024)	Sound dentin	1. Keep dentin surface slightly moist; 2. Apply the adhesive to the entire preparation with a microbrush and rub it for 20 s; 3. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely; 4. Light cure for 10 s at 1200 mW/cm^2.
	Erosive dentin treatment with Cola-based soft drink (ED)	1. Keep dentin surface slightly moist; 2. Apply the adhesive to the entire preparation with a microbrush and rub it for 20 s; 3. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely; 4. Light cure for 10 s at 1200 mW/cm^2.

Adhesive system (batch number)	Groups	Application mode
		Self-etch
	ED + DMSO	1. Keep dentin surface slightly moist; 2. Active application of 50% DMSO for 60 s; 3. Air stream for 5 s keep the surface slightly moist; 4. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-PATC	1. Keep dentin surface slightly moist; 2. Active application of proanthocyanidin (Proanthocyanidin-grape seed extract 6.5% weight, 50% DMSO) for 60 s; 3. Air stream for 5 s keep the surface slightly moist; 4. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-RIB	1. Keep dentin surface slightly moist; 2. Active application of riboflavin (Riboflavin 0.1% weight, 50% DMSO) for 60 s; 3. Light-cure for 2 min; 4. Air stream for 5 s keep the surface slightly moist; 5. Apply adhesive as for self-etch mode without DMSO.
Scotchbond Universal - SBU; 3M Oral Care (638367)	Sound dentin	1. Keep dentin surface slightly moist; 2. Apply the adhesive to the entire preparation and leave undisturbed for 20 s; 3. Direct a gentle stream of air over the liquid for approximately 5 s until it no longer moves and the solvent evaporates completely; 4. Light cure for 10 s at 1200 mW/cm^2.
	Erosive dentin treatment with Cola-based soft drink (ED)	1. Keep dentin surface slightly moist; 2. Apply the adhesive to the entire preparation and leave undisturbed for 20 s; 3. Direct a gentle stream of air over the liquid for approximately 5 s until it no longer moves and the solvent evaporates completely; 4. Light cure for 10 s at 1200 mW/cm^2.
	ED + DMSO	1. Keep dentin surface slightly moist; 2. Active application of 50% DMSO for 60 s; 3. Air stream for 5 s keep the surface slightly moist; 4. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-PATC	1. Keep dentin surface slightly moist; 2. Active application of proanthocyanidin (Proanthocyanidin-grape seed extract 6.5% weight, 50% DMSO) for 60 s; 3. Air stream for 5 s keep the surface slightly moist; 4. Apply adhesive as for self-etch mode without DMSO.
	ED + DMSO-RIB	1. Keep dentin surface slightly moist; 2. Active application of riboflavin (Riboflavin 0.1% weight, 50% DMSO) for 60 s; 3. Light-cure for 2 min; 4. Air stream for 5 s keep the surface slightly moist; 5. Apply adhesive as for self-etch mode without DMSO.

(*) The materials were applied according to the recommendations of their respective manufacturers.
Abbreviation: ED: erosive dentin treatment with Cola-based soft drink; DMSO: dimethyl sulfoxide; PATC: proanthocyanidins RIB: riboflavin-UVA

Sample size calculation

The sample size calculation was performed on a free website, www.sealedenvelope.com. The sample size was determined considering the microtensile bond strength values of the Scotchbond Universal. The mean and standard deviation of Scotchbond Universal reported in the literature are 49.8 ± 5.3 MPa. (15-17). To detect a difference of 8 MPa among the tested groups at a significance level of 5%, a power of 80%, and using a two-sided test, the minimum sample size was 8 teeth per group.

Erosion model

Before erosive model, the lateral and root areas were covered with nail varnish, allowing erosive demineralization to occur only on the occlusal surface. The specimens were immersed in a cola drink (Coca-Cola, pH 2.6) 4 times a day for 90 s each (10 ml/specimen) for 5 days (9). The cola drink was renewed after each erosive demineralization. Then, the specimens were washed in deionized water (10 s) and immersed in a remineralizing solution (pH 6.7, 10 ml/specimen) for 1 h (9), between erosive demineralization. The remineralization solution was replaced daily. The pH levels of all solutions were checked periodically.

Restorative procedures

After erosive model, the teeth were distributed according to the combination of variables. For groups where the primer of the dimethyl sulfoxide alone or combined to the cross-linking agents were applied, the experimental primers containing dimethyl sulfoxide was done according to Stape et al., (12). For this, 50 µL of dimethyl sulfoxide (Sigma-Aldrich; St. Louis, MO, USA; pH 8.2) was mixed to a base of water 50% (v/v) added or not the 6.5 wt% proanthocyanidin or 0.1 wt% riboflavin. The primers were renewed daily.

For etch-and-rinse strategy, the dimethyl sulfoxide primer was applied after acid-etching. The teeth were conditioned with 37% phosphoric acid etching (Condac, FGM Dental Products; Joinville, SC, Brazil) for 15 s, rinsed and kept slightly moist. For both strategies, the experimental primers were then applied using a microbrush by 60 s (Brush, KG Sorensen, Cotia, SP, Brazil). An air stream was used for 5 to 10 s to remove the excess. For the dimethyl sulfoxide associated with riboflavin primer application, the dentin surfaces were photoactivated using a curing-light unit set at 1200 mW/cm^2 (Radii, SDI; Bayswater, Victoria, Australia) (9) and air-dried. Independent of the group, the dentin surface was kept slightly moist before the application of the adhesive (Box 1).

The adhesive systems were applied following the manufacturer's recommendations; further, composite resin buildup (Opallis, FGM, Joinville, Brazil) were placed in layer of 2 mm each, and individually photoactivated for 40 s (Radii, SDI; Bayswater, Victoria, Australia). After 24 h, composite-dentin bonded sticks (cross-sectional area 0.8 mm^2) were prepared using a slow-speed diamond saw (Isomet, Buehler) and measured by a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan) to calculate the bond strength in MPa. The number of sticks showing premature failure (PF) during specimen preparation was recorded for each tooth.

Two composite-dentin bonded sticks per tooth from each experimental group were used to evaluate the *in situ* degree of conversion within the adhesive/hybrid layers. Three composite-dentin bonded sticks per tooth were used to evaluate silver nitrate penetration, and the remaining composite-dentin bonded sticks were tested for microtensile bond strength.

Microtensile bond strength

The composite-dentin bonded sticks were fixed to a Geraldeli's jig using cyanoacrylate glue and stressed under tension (Instron, Instron Inc., Canton, USA) at 1.0 mm/min until fracture occurred. The microtensile bond strength values (MPa) derived by dividing the imposed force by bonding area.

The fracture mode of the composite-dentin bonded sticks were examined with a light microscope at 100X magnification (Olympus SZ40, Tokyo, Japan) and categorized as cohesive (failure exclusively within the dentin or the resin composite) or adhesive (failure at the resin-dentin interface or with partial cohesive failure of the neighboring substrates). Specimens with premature fracture were included in the tooth mean for statistical analysis.

Silver nitrate penetration analysis

Composite-dentin bonded sticks (n = 3 per each tooth) were immersed in an aqueous solution of ammoniacal silver nitrate solution for 24 h, followed by 8h in photo-developing solution under a fluorescent lamp. Specimens were wet-polished using SiC papers and polished used diamond paste (Buehler Ltd., Lake Bluff, IL, USA).

The composite-dentin interfaces were observed using a field-emission scanning electron microscope (VEGA 3 TESCAN, Shimadzu, Tokyo, Japan) at 15 kV with backscattering mode. The amount of SNP within the adhesive layer, hybrid layer of each stick was measured in three regions (5 µm × 5 µm) of the bonded stick. Acquisition mode of images and calculate percentage of SNP was according to Hass et al., (18). ImageJ software were used to calculate the percentage of SNP within hybrid layers in each specimen.

In situ degree of conversion by Micro-Raman analysis

Composite-dentin bonded sticks (n = 2 per each tooth) were prepared as previously described by Hass et al., (18). *In situ* degree of conversion analysis was measured inside the hybrid layer of the adhesive interfaces using a micro-Raman spectrometer (XploRA ONETM Raman microscope, HORIBA Scientific, New Jersey, NY, USA). Previously, the micro-Raman spectrometer was calibrated for zero. Them, the Raman spectrometer was configured to use a 638-nm diode laser, 100x objective, 600-lines/mm grafting centered between 500 and 1800 cm-1, using 100 mW power, spatial resolution of approximately 3 µm, spectral resolution of 5 cm-1, and accumulation time of 25 s with 3 co-additions. The spectra were acquired at 3 different sites for each specimen, in the middle of the hybrid layer, and the values averaged for statistical purpose. Post-processing of the spectra was performed using the Opus Spectroscopy Software version 6.5. The average of the values was used for statistical analysis and the spectra of uncured adhesives were considered as references.

The ratio of the double-bond content of monomer to polymer in the adhesive were quantified by calculating ratio derived from the aliphatic C=C (vinyl) absorption (1638 cm-1) to the aromatic C=C absorption (1608 cm-1) signals for both polymerized and unpolymerized samples. The DC was calculated, according to the following formula:

$$DC\ (\%) = (1-[R\mathrm{cured}/R\mathrm{uncured}]) \times 100,$$

Where "*R*" is the ratio of aliphatic and aromatic peak intensities at 1638 cm^{-1} and 1608 cm^{-1} in cured and uncured adhesives, in accordance with Hass et al. (18). In addition, the more intense peaks observed for all materials and the corresponding chemical bonding were recorded.

Occlusal and lateral morphological modification by erosion model

Six teeth were used in this part of the study. The teeth were sectioned parallel to the occlusal surface using a low-speed diamond saw (IsoMet 1000; Buehler, Lake Bluff, IL, USA) under cooling water to expose the mid-coronal dentin. After that, each tooth was transversely sectioned in a buccal-to-lingual direction to obtain two halves per teeth (n=12 specimens). For lateral analysis of the morphological modification, in the half the teeth a precut groove was made on the pulpar side to allow segmentation.

Specimens from each tooth were divided according to sound dentin and erosive dentin treatment with Cola-based soft drink. To allowing erosive demineralization to occur only on the occlusal surface, lateral and pulpar areas were covered with two layers of nail varnish. After that, the specimens were submitted to erosive demineralization according to erosion model section.

After erosive demineralization, the surfaces were rinsed with tap water for 30 seconds and air-dried for 5 seconds, keeping the dentin moist. The specimens were treated according to Siqueira et al. (19) and Kenshima et al. (20) and the entire surface was examined under a scanning electron microscope (MIRA3 LM, Tescan Orsay Holding, Warrendale, PA, USA). Three photomicrographs of representative occlusal and lateral areas were taken at 5000X and 20.000X magnification. It was possible to see a totally removal of the smear layer with a higher opening of the dentin tubules in dentin submitted to erosion model in comparison to sound dentin (Figure 1).

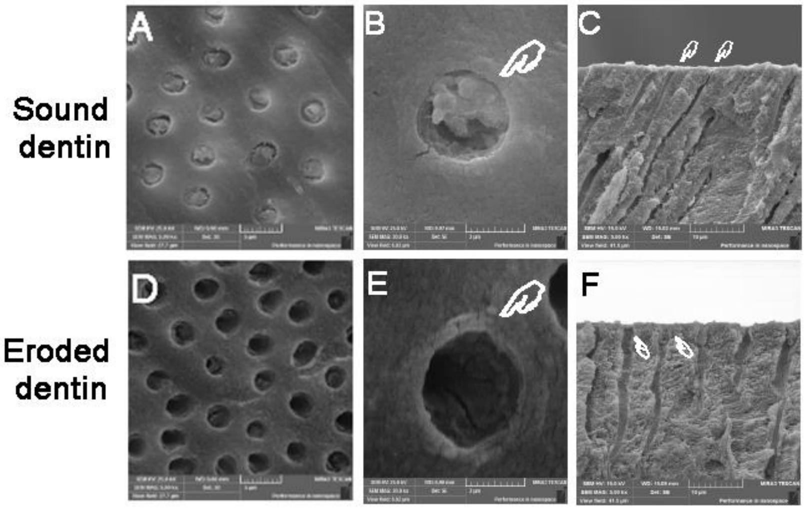

Figure 1. Representative SEM images of the occlusal and cross-sectional view of sound (A to C) and eroded (D to F) dentin specimens. In sound dentin, it was possible to see an obliteration of the dentin tubules (see B and white hands in C). On the other hand, after the erosive model, it was occurring a totally removal of the smear layer with a higher opening of the dentin tubules (see E and white hands in F). This confirm that the present model produced the desired erosive effect on dentin.

Statistical analysis

The Shapiro-Will test was employed to assess whether the data from these tests followed a normal distribution. The Barlett's test was performed to determine the validity of the assumption of equal variances. After confirmed the normal distribution and homoscedasticity, the mean of microtensile bond strength (MPa), silver nitrate penetration (%) and *in situ* degree of conversion (%) of all bonded sticks from the same tooth were averaged for statistical purposes. Therefore, the experimental unit in this study was the tooth. The value attributed to PFs specimens was according to described in the previous studies (18, 21). In this study, the value was 4.2 MPa. The microtensile bond strength (MPa), silver nitrate penetration (%) and *in situ* degree of conversion (%) means for every test group resulted from the average of the eight teeth used per group. The microtensile bond strength (MPa) and silver nitrate penetration (%) data were analyzed by three-factor ANOVA (treatment vs. adhesive systems vs. adhesive strategies). The *in situ* degree of conversion (%) data for each adhesive were analyzed by two-factor ANOVA (treatment vs. adhesive strategies). For all tests, a post-hoc Tukey's test with of significance (alpha=0.05) was applied.

Results

Microtensile bond strength

Approximately 20-25 composite-dentin bonded sticks were obtained per tooth, including the premature failures. Regarding microtensile bond strength values, the cross-product interaction was not significant, nor was the main factor adhesive strategy (Table 1; p = 0.42 and p = 0.72, respectively). However, the main factors treatment (p = 0.002) and adhesive (p = 0.0001) were statistically significant (Table 1). A significantly lower microtensile bond strength value was observed in erosive dentin treatment with Cola-based soft drink compared to dimethyl sulfoxide groups (Table 1; p = 0.002). However, a significant increase in microtensile bond strength values was observed when dimethyl sulfoxide was combined with proanthocyanidin and riboflavin-UVA in comparison to only dimethyl sulfoxide (Table 1; p = 0.002). Actually, when dimethyl sulfoxide was combined with proanthocyanidin and riboflavin-UVA similar microtensile bond strength results between erosive dentin treatment with Cola-based soft drink and sound dentin were observed (Table 1; p > 0.05). Usually, SBU showed higher microtensile bond strength values compared to IBU (Table 1; p = 0.001).

Table 1. Means and standard deviations of resin-dentin bond strength values, as well as statistical analysis (MPa) for all experimental groups (*).

Adhesive systems	Treatment									
	Sound dentin		ED		ED + DMSO		ED + DMSO-PATC		ED + DMSO-RIB	
	ER	SE	ER	SE	ER	SE	ER	SE	ER	SE
IBU	37.3±2.9B	36.3±1.2B	22.6±4.1D	24.9±3.7D	32.1±3.1C	33.2±3.6C	36.1±4.2B	35.3±2.8B	38.5±2.9B	37.1±4.1B
SBU	48.0±4.8A	48.3±4.6A	32.6±3.9C	28.3±2.9CD	38.2±3.5B	38.4±3.2B	45.4±3.5A	42.3±3.2A	47.2±3.4A	45.5 4.3A

(*) Different letters are means differences statistically significant between groups (Three-way ANOVA; Tukey test, p < 0.05).

Abbreviations: ED: erosive dentin treatment with Cola-based soft drink ; DMSO: dimethyl sulfoxide; PATC: proanthocyanidins RIB: riboflavin-UVA; ER: Etch-and-rinse, SE: self-etch.

The most common fracture mode observed was adhesive/mixed for all experimental groups (Table 2). For Sound dentin, 99% of failures were considered A/M and only 1% were PF (Table 2). For eroded dentin, higher number of premature failures were observed (26%) (Table 2). When eroded dentin was treated with dimethyl sulfoxide or combined with proanthocyanidin and riboflavin-UVA only 2% of premature failures were observed (Table 2).

Table 2. Number of specimens (%) according to fracture mode.

	Treatment (**)																													
	Sound dentin						ED						ED + DMSO						ED + DMSO-PATC						ED + DMSO-RIB					
	ER			SE			ER			SE			ER			SE			ER			SE			ER			SE		
	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP	A/M	C	FP
IBU	139 (99)	0 (0)	1 (1)	125 (99)	0 (0)	1 (1)	120 (95)	0 (0)	4 (5)	120 (93)	0 (0)	9 (7)	137 (98)	0 (0)	3 (2)	129 (99)	0 (0)	1 (1)	136 (97)	4 (3)	0 (0)	143 (99)	1 (1)	0 (0)	133 (97)	3 (2)	1 (1)	128 (97)	0 (0)	4 (3)
SBU	130 (100)	0 (1)	0 (0)	127 (100)	0 (0)	0 (0)	122 (94)	0 (0)	8 (6)	139 (96)	0 (0)	6 (4)	144 (96)	0 (0)	6 (4)	154 (96)	0 (0)	6 (4)	145 (100)	0 (0)	0 (0)	152 (100)	0 (0)	0 (0)	152 (98)	1 (1)	1 (1)	142 (99)	1 (1)	0 (0)

Abbreviations: ED: erosive dentin treatment with COca-based soft drink; DMSO: dimethyl sulfoxide; PATC: proanthocyanidins; RIB: riboflavin-UVA ER: Etch-and-rinse; SE: Self-etch; A/M: adhesive/mixed fracture mode; C: cohesive fracture mode; PF: premature failures

Silver nitrate penetration analysis

Neither the cross-product interaction nor the main factor adhesive strategy was significant (Table 3; p = 0.47 and p = 0.55, respectively). On the other side, treatment (p = 0.02) and adhesive (p = 0.01), as main factors, were considered statistically significant (Table 3). A significantly higher silver nitrate penetration value was observed in eroded dentin compared to dimethyl sulfoxide groups (Table 3; p = 0.02; Figure 2). Contrastingly, the application of dimethyl sulfoxide decreased silver nitrate penetration values in eroded dentin, combined or not with proanthocyanidins and riboflavin-UVA (Table 3; p = 0.02; Figure 2). However, the addition of proanthocyanidins and riboflavin-UVA showed silver nitrate penetration values compared to observed in sound dentin (Table 3; p > 0.05). For all comparisons, Scotchbond Universal showed lower silver nitrate penetration values compared to IBond Universal (Table 3; p = 0.02).

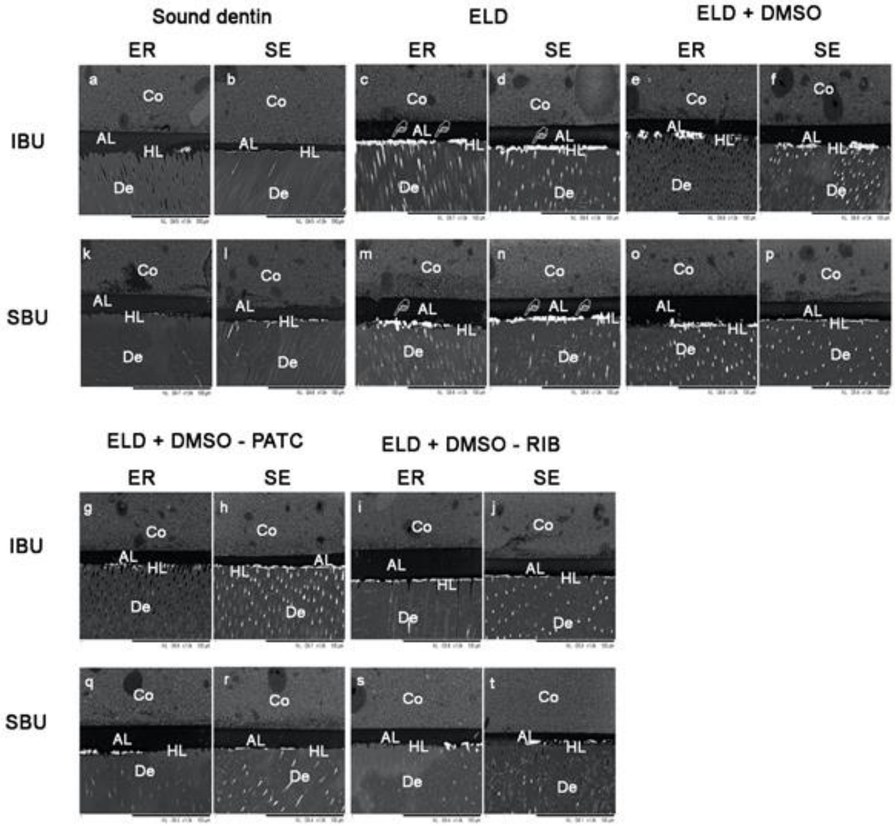

Figure 2. Representative SEM images of the resin-dentin interfaces of the experimental groups. Silver nitrate deposit was observed. However, this deposition was more pronounced in ED (white hands) when compared to sound dentin groups, as well as DMSO or DMSO combination to collagen cross-linking agent. (Co = composite resin; AL = adhesive layer, HL = hybrid layer, De = dentin).

Table 3. Means and standard deviations of silver nitrate penetration values (%), as well as statistical analysis for all experimental groups (*).

Adhesive systems	Treatment									
	Sound dentin		ED		ED + DMSO		ED + DMSO-PATC		ED + DMSO-RIB	
	ER	SE	ER	SE	ER	SE	ER	SE	ER	SE
IBU	12.6±4.7B	14.8±3.5B	22.7±3.9C	20.9±3.2C	17.4±3.2B	16.8±2.7B	16.0±3.2B	15.5±2.1B	16.8±2.0B	15.9±2.0B
SBU	7.8±2.4A	7.3±3.2A	15.3±3.1B	17.5±3.4B	12.1±4.1A	11.2±3.2A	10.2±2.0A	8.9±2.5A	9.3±3.4A	8.1±1.4A

(*) Different letters are means differences statistically significant between groups (Three-way ANOVA; Tukey test, $p < 0.05$).
Abbreviation: ED: erosive dentin treatment with Cola-based soft drink; DMSO: dimethyl sulfoxide; PATC: proanthocyanidins RIB: riboflavin-UVA; ER: Etch-and-rinse, SE: self-etch.

In situ degree of conversion by Micro-Raman analysis

Regarding *in situ* degree of conversion values, the cross-product interaction and main factor adhesive strategy were not significant for each adhesive (Table 4; p > 0.38 and p > 0.53, respectively). However, the main factor treatment was considered statistically significant (Table 4; p = 0.01 for both adhesives). A significantly lower *in situ* degree of conversion value was observed in eroded dentin compared to dimethyl sulfoxide groups (Table 4; $p = 0.01$), while the application of dimethyl sulfoxide increased *in situ* degree of conversion values in eroded dentin, independently combination with proanthocyanidin and riboflavin-UVA (Table 4; $p = 0.01$). Also, the addition of dimethyl sulfoxide in eroded dentin, independently combination with proanthocyanidin and riboflavin-UVA, showed similar *in situ* degree of conversion results when compared to sound dentin for both adhesives (Table 4; $p > 0.05$).

Table 4. Means and standard deviations of *in situ* degree of conversion by micro-raman values (%), as well as statistical analysis for all experimental groups (*)

Adhesive systems	Treatment									
	Sound dentin		ED		ED + DMSO		ED + DMSO-PATC		ED + DMSO-RIB	
	ER	SE	ER	SE	ER	SE	ER	SE	ER	SE
IBU	55.4±2.1 A	52.7±3.2A,B	42.1±3.2C	44.2±2.7C	50.1±2.2B	51.1±3.2B	51.2±0.6B	50.9±0.5B	51.0±0.2B	50.8±0.9B
SBU	62.6±2.4a	59.8±2.7a	50.2±3.4b	52.1±2.7b	60.5±2.1a	61.9±3.1a	60.6±0.3a	60.4±0.7a	60.7±0.2a	60.9±0.2a

(*) For each adhesive, different capital or lower case letters are means differences statistically significant between groups (columns; three-way ANOVA; Tukey test, $p < 0.05$).

Abbreviation: ED: erosive dentin treatment with Cola-based soft drink; DMSO: dimethyl sulfoxide; PATC: proanthocyanidins RIB: riboflavin-UVA; ER: Etch-and-rinse, SE: self-etch

Discussion

The results presented in this study showed that the application of dimethyl sulfoxide as an additional primer prior to an adhesive procedure on eroded dentin significantly increased the microtensile bond strength and *in situ* degree of conversion as well as decreasing the silver nitrate penetration values compared to the erosive dentin treatment with Cola-based soft drink group, leading to acceptance of all hypotheses of the present study.

As mentioned in the introduction, erosive tooth wear promotes demineralization and dissolution of the mineral component, and continued progression induces formation of a zone of dense, the fibrous collagen network (5). An increased loss of collagen periodicity occurs in the collagen matrix (21), and these spaces between the collagen fibrils are occupied by water (5).

All these features hinder adequate infiltration by the adhesive monomers into the underlying eroded dentin (6, 8, 9). Therefore, the hybrid layer formed in eroded dentin could result in areas of hydrophilic predominance and demineralized zones with collagen fibrils incompletely encapsulated by resin monomers (22), leading to the formation of a structurally imperfect and highly porous hybrid layer (22). These characteristics significantly affect the bonding performance of adhesive systems in eroded dentin (6, 8, 9). These results can also confirm by major amount of premature failure for erosive dentin treatment with Cola-based soft drink group when compared with sound dentin.

Dimethyl sulfoxide is an ideal solvent for medical purposes with a special ability to penetrate the biological surface (11). Due to this characteristic, dimethyl sulfoxide has been recommended to increase adhesive penetration into the exposed collagen matrix (12) and, consequently, significantly increase the microtensile bond strength values compared to a erosive dentin treatment with Cola-based soft drink group when compared to sound dentin, as well as previously observed by several authors (12, 23).

Furthermore, dimethyl sulfoxide is a special solvent that dissolves both non-polar and polar compounds. It is a polyfunctional molecule with a highly polar S=O group and two hydrophobic groups. The partial negative charge of the oxygen atom of the dimethyl sulfoxide molecule favors the formation of hydrogen bridges with water molecules (23), thereby reducing the self-associative tendency of water (24). Also, taking into account the silver nitrate penetration within the hybrid layer representing water-rich interfacial regions, the interaction between dimethyl sulfoxide and water could be responsible for the lower silver nitrate penetration values when compared to the erosive dentin treatment with Cola-based soft drink group. In addition, the application of dimethyl sulfoxide groups in erosive dentin treatment with Cola-based soft drink showed similar silver nitrate penetration as sound dentin, in accordance with a previously published study (23).

Once dimethyl sulfoxide reduces the number of water molecules entrapped between polymeric chains, an increase in the degree of conversion would be expected (25), as observed in the present study. However, despite improvement in bonding performance promoted by dimethyl sulfoxide in an eroded-dentin interface, there is no cross-linking effect reinforcing the mechanical properties of the dentinal matrix (10). Thus, this is the first study evaluating the effect of dimethyl sulfoxide combined with collagen crosslinking agents on the bonding performance of universal adhesives in eroded dentin.

According to the results of this study, the combination of dimethyl sulfoxide with riboflavin or proanthocyanidin agents promoted a significant increase of microtensile bond strength values compared erosive dentin treatment with Cola-based soft drink group or even when only dimethyl sulfoxide was applied. An increase in bond strength values occurred for all dimethyl sulfoxide groups. However, only in the dimethyl sulfoxide-combined cross-linking agents, the results in the erosive dentin treatment with Cola-based soft drink were similar to sound dentin.

This improvement in bond strength could be attributed to the cross-linking effect of both collagen crosslinking agents used in the present study. Riboflavin is a known collagen crosslinking agent when combined with UVA or LED lights (24). Due to the photo-oxidative cross-linking effect, riboflavin showed stability and resistance to collagen degradation via the increased mechanical properties of dentin collagen (26). On the other hand, proanthocyanidins improves the biomechanical properties of collagen fibrils through several chemical mechanisms (27), which produce collagen cross-linking. This increase of the mechanical properties of dentin collagen was also reflected in the increase in adhesive/mixture failures in these experimental groups when compared to erosive dentin treatment with Cola-based soft drink.

However, the combination of dimethyl sulfoxide with riboflavin or proanthocyanidin agents did not significantly affect the silver nitrate penetration or *in situ* degree of conversion values. These results were expected, as the collagen crosslinking agents tested herein cannot alter the hydrophilic property of adhesive, improve adhesive evaporation or produce more permeable adhesive layers (12). Also, due to the molecular size of organic substances such as riboflavin and proanthocyanidin, dissolution in polar solvents is difficult, limiting proper infiltration within the demineralized collagen matrix (10).

Actually, some collagen crosslinking agents have potential to reduce the polymerization of adhesive systems (28). For instance, proanthocyanidins donate H^+ ions to free radicals and inhibit the initiation and propagation of the polymerization process (28). However, at concentrations as low as those used, proanthocyanidins did not interfere with the degree of conversion, as previously observed in vitro studies (9, 12).

On the other hand, this study provided evidence for better performance of Scotchbond Universal than IBond Universal for all comparisons. This is in agreement with a Jacker-Guhr et al., (29). According to this study, due to presence of acetone and absence of 2-hydroxyethyl methacrylate (HEMA), the IBond Universal application needs a strict protocol regarding correct moisture management (29), mainly because HEMA-free universal adhesives are more susceptible to phase separation at the adhesive interface (29).

In terms of self-etch strategy, it is worth mentioning that IBond Universal is an intermediary strong acidic adhesive in comparison to Scotchbond Universal, a mildly acidic adhesive. According to Van Meerbeek et al., (30), the most favorable adhesive performance in dentin was obtained with mild adhesives; intermediary strong adhesives showed more zones of partially demineralized but uninfiltrated dentine beneath hybrid layers, as observed in the silver nitrate penetration results of the present study. These areas are considered locations of potential degradation mechanisms with these intermediary strong adhesives (30).

The results of the present study may promote the development of novel strategies to improve eroded-dentin bonding performance. However, microtensile bond strength and chemical evaluations of adhesive interfaces can only provide limited information related to the interactions of these crosslinking agents in eroded dentin. Thus, more investigations are necessary to present the real advantages of the combination of dimethyl sulfoxide with cross-linking agents, especially after long-term water storage.

In the conclusion, the application of dimethyl sulfoxide combined with collagen crosslinking agents contributed to increasing the microtensile bond strength and *in situ* degree of conversion with a significant reduction of silver nitrate penetration in erosive dentin treatment with Cola-based soft drink.

Acknowledgment

Authors are grateful to State Foundation of Support to Research, Scientific and Technological Development of Maranhão (FAPEMA) and Central de Laboratórios Multiusuários/Universidade Estadual de Ponta Grossa (CLABMU/UEPG) for the support provided. Also, this study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 303332/2017-4 and 308286/2019-7, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Resumo

Este estudo investigou o efeito do dimetil sulfóxido combinado a agentes de reticulação de colágeno na resistência de união à microtração, infiltração de nitrato de prata e análise do grau de conversão por Micro-Raman de sistemas adesivos universais para a dentina erosionada por refrigerante a base de Cola. Cento e sessenta molares foram divididos em 20 grupos: (1) Tratamento: Dentina sadia; Dentina erosionada; Dentina erosionada tratada com primer de dimetil sulfóxido; Dentina erosionada tratada com primer contendo 6,5% de proantocianidina e; Dentina erosionada tratada com primer contendo 0,1% de rivoflavina; (2) Sistemas adesivos: iBond Universal e Scotchbond Universal; e (3) estratégia adesiva: estratégia condicionamento e lavagem ou autocondicionate. Após a restauração, os espécimes foram seccionados em palitos e testados. Os dados dos três testes foram analisados estatisticamente (ANOVA de 2 e 3 fatores e teste de Tukey; $\alpha = 5\%$). A aplicação de dimetil sulfóxido combinado ou não agentes de reticulação de colágeno melhorou todas as propriedades avaliadas quando comparado a dentina erosionada. Entretanto, apenas quando o dimetil sulfóxido foi combinado com agentes de reticulação de colágeno, os valores de adesão a dentina, infiltração de nitrato de prata e grau de conversão em dentina erosionada foi semelhante a dentina sadia, para os dois adesivos e estratégias adesivas. A aplicação de dimetil sulfóxido combinado com agentes de reticulação de colágeno contribuiu para aumentar a resistência de união e o grau de conversão dentro da camada híbrida na dentina erodida, ao mesmo tempo que reduziu significativamente a nanoinfiltração neste substrato.

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Received: 06/07/2021
Accepted: 13/09/2021