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Concentration effect of DMSO-dry bonding on the stability of etch-and-rinse bonds

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ABSTRACT

Objectives: To examine whether lower dimethyl sulfoxide (DMSO) concentrations would affect long-term bond stability of simplified or multistep water-based adhesives to dry-etched dentin.
Methods: H_3PO_4-etched mid-coronal dentin surfaces from human molars were randomly blot- or air-dried for 30 s and pretreated or not with 5 or 50 % (v/v) ethanolic DMSO solutions. Untreated samples served as control. Samples were bonded with a two-step or a three-step etch-and-rinse adhesive. Restored crown segments (n = 5/group) were stored in distilled water for 24 h and sectioned for microtensile bond strength testing. Resin-dentin beams (0.8 mm^2) were tested under tension until fracture (0.5 mm/min) after 24 h and one year of storage in artificial saliva at 37 °C. Nanoleakage evaluation and hybrid layer characterization were performed by SEM. Bond strength data was examined by three-way ANOVA followed by the Tukey test ($\alpha = 0.05$). *Results:* Dry bonding produced significantly lower bond strengths than conventional wet bonding for both water-based adhesive systems ($p < 0.05$). DMSO-dry bonding restored bond strengths and reduced nanoleakage levels, regardless of adhesive type or DMSO concentration ($p < 0.05$). Bond strengths of DMSO-dry bonded samples were not significantly affected by long-term ageing regardless of adhesive type or DMSO concentration ($p < 0.05$).
Significance: Although bonding methacrylate-based resins to etched dentin is normally performed under wet conditions, hybridization of air-dried collagen can outperform conventional wet bonding by employing water-free DMSO solutions with concentrations as low as 5 %. Reduced moisture-related technique sensitivity, higher bonding performance and improved hybrid layer stability may contribute to extend the service life of resin-dentin bonding.

1. Introduction

In the past decades, adhesive dentistry has revolutionized restorative procedures and extended the service life of tooth-bonded treatments [1]. For such, adhesive procedures should maintain durable tooth-bonded interfaces for a number of years to attain clinical durability. While enamel bonding is predictable and enduring, considerable research effort has been devoted to extend the longevity of resin-dentin interfaces [1–5]. Despite improvements in resin formulations and better understanding of degradation mechanisms, achieving stable resin-dentin bonding under clinical conditions remains a challenge.

Resin-dentin bonding is a form of *in situ* dental tissue engineering, in which methacrylate-based monomers penetrate the dentin matrix, become entangled and upon polymerization (*e.g.* hybrid layer formation) provide micromechanical retention [6]. Hybrid layer stability is mainly dictated by the quality of both the formed dimethacrylate polymer networks and their interaction with dentin [1–5]. Most of the "hydrophilicity" of etched dentin is due to the presence of bound (*i.e.* 21 – 15 % w/w) and unbound (*i.e.* 75 – 79 % w/w) water molecules [7]. In H_3PO_4-etched dentin, hydroxyapatite minerals are initially dissolved and then replaced by water molecules (*i.e.* roughly 50 % v/v) [8]. Resin monomers must replace unbound water molecules for effective

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resin-dentin bonding. Considering that the molar concentration of resin monomers (0.3–5 mol/L) is only a fraction of the molar concertation of water (55.6 mol/L) [9], complete water replacement by resin monomers is not easily achieved. Failure to do so results in porous water-rich bonded interfaces highly susceptible to hydrolysis over time.

Currently, hybridization of etched dentin is normally performed under wet conditions. Water reduces collagen shrinkage by breaking interpeptide hydrogen bonding [10] maintaining interfibrillar spacing. This allows better diffusion of methacrylate-based monomers and higher collagen fibril encapsulation [11], albeit percolation through collagen fibrils is mostly by hydrophilic monomers [12]. There is a consensus that etch-and-rinse resins are highly sensitive to dentin moisture [1,6,13]. Both overdry or overwet conditions strongly affect dentin-bonding performance [14,15]. Water entrapment within the collagen matrix limits the diffusion of cross-linking hydrophobic monomers into hybrid layers [12], contributes to phase separation of monomers [2], reduces monomer conversion thus resulting in mechanically weaker polymers [16]. In fact, the critical amount of water necessary to produce optimal resin-dentin bonding is inferior than originally thought [17]. In attempt to reduce/eliminate the drawbacks of residual water on hybrid layer quality, dry bonding has regained attention [18–24]. The main problem resides on preventing or effectively re-expanding collapsed collagen fibrils after air-drying for adequate hybridization. Methacrylate-based bonding agents do not promote adequate re-expansion of dried collagen [10], so additional measures are necessary to allow proper monomer-collagen interactions. However, bonding commonly available resins to etched-dry dentin is possible following newly proposed protocols [18–24]. Dry bonding not only facilitates application procedures, but it can establish more reliable resin-dentin bonding given the appropriate conditions [18–21].

Among the recently proposed bonding techniques, dimethyl sulfoxide (DMSO)-dry bonding emerges as an alternative to bond relatively hydrophilic resins to extensively air-dried dentin [18–21]. Unlike previously proposed bonding protocols, which indeed produced improved outcomes [22–24], DMSO-dry bonding acts in several fronts to facilitate bonding to H_3PO_4-etched dentin. Improved hybrid layer formation [20], better collagen wettability [19], more uniform conversion [18] and lower endogenous collagenolytic activity [21] contribute to the extended durability of resin-dentin interfaces [18]. One main advantage is that DMSO-dry bonding can effectively displace residual water within collagen. This results in bonded interfaces with reduced water entrapment and lower porosity when water-free DMSO pretreatments are used [18,19]. To date, reports assessing DMSO-dry bonding have mainly focused on relatively high DMSO concentrations (*i.e.,* 50 % v/v) [18–21]. While substantially higher DMSO concentrations can be deleterious to resin-dentin interfaces [18], lower thresholds for dry bonding have not been investigated. Reducing DMSO content could potentially facilitate overall solvent removal and avoid any potential DMSO-related cytotoxicity issues, which may be empirically attributed to higher DMSO concentrations. Therefore, the primary aim of this study was to investigate whether DMSO-dry bonding using water-free low-DMSO contents would affect etch-and-rinse bonding of methacrylate-based resins. The objectives were to evaluate whether dentin pretreatments with reduced DMSO content would affect immediate or long-term bonding of different water-containing resins to extensively air-dried dentin. The tested null hypotheses were that DMSO-dry bonding with low-DMSO contents would have no effect on *(i)* resin-dentin bonding or *(ii)* hybrid layer quality and stability of etched dentin.

2. Materials and methods

Forty-eight sound third molars were selected for this study under a protocol approved by the Ethical Committee, University of Oulu, Finland (#23–2003). Teeth were extracted for clinical reasons that were not related to this study. Molars were stored in 0.5 % Chloramine-T immediately after extraction at 4 °C and used within 3 months of extractions.

2.1. Experimental design and bonding procedures

The experimental design was composed of three study factors defined as: (*i*) "bonding protocol" at three levels (no treatment, 5 % or 50 % DMSO-dry bonding), (*ii*) "bonding resin" at two levels (two-step or three-step etch-and-rinse adhesive) and (*iii*) "storage time" at two levels (24 h or one year). Conventional wet bonding following manufacturer's instructions served as isolated control groups at 24 h and one year without DMSO pretreatments. Molars were mounted in acrylic resin 2 mm below the cement-enamel junction and then sectioned perpendicularly to their long axis under cooling to expose mid-coronal flat dentin surfaces with a diamond saw (Isomet 1000 Precision Saw, Lake Bluff, IL, USA) [25]. Absence of remaining enamel on the dentin surfaces was verified with a stereomicroscope (Leica M60, Leica Microsystems, Wetzlar, Germany) at 40 × magnification. Smear layer standardization was done using 320-grit silicon carbide grinding paper (Buehler-Met) for 60 s under water cooling. Crown segments (n = 5/group) were randomly allocated to 8 groups according to dry-bonding protocols and bonding resin. Dentin surfaces were etched for 15 s with 32 % phosphoric acid (Scotchbond Universal Etchant, 3 M ESPE, St. Paul, MN, USA), rinsed for 15 s and either blot-dried, leaving the surface partially wet (Control Wet), or air-dried for 30 s (dry bonding). DMSO 5 % and 50 % solutions (v/v) were prepared by mixing DMSO (Dimethyl sulfoxide, Sigma-Aldrich, St. Louis, MO, USA) with ethanol (Ethanol 99.8 %, Sigma-Aldrich). 50 μL [21] of DMSO solutions (5 or 50 %) were actively applied on the etched-dentin surfaces for 60 s according to bonding protocols. Moisture control was performed by either blot-drying, until paper filters presented no visible moisture creating a partially wet surface (Control Wet) or by air-drying for 30 s (Control Dry and DMSO-dry bonding) [18–21]. Two water-based bonding resins were used for hybridization either a simplified two-step etch-and-rinse adhesive (Adper Single Bond Plus, 3 M-ESPE; SB) or a three-step etch-and-rinse adhesive (Scotchbond Multi-Purpose, 3 M-ESPE; SBMP). Composition of adhesive systems and bonding procedures are shown in Table 1. All bonding resins were actively applied with light pressure of approximately 4 g

Table 1
Adhesive Systems, main components, and application mode of bonding agents.

Adhesive system	Components	Bonding protocol	Mode of application*
Scotchbond Multi-Purpose (3 M/ESPE; SBMP) Batch #	*Primer*: water (>40 %), HEMA, polyalkenoic acid methacrylate copolymer *Bond:* bis-GMA, HEMA, dimethacrylates photoinitiators	Control Wet Control Dry 5 % DMSO-dry 50 % DMSO-dry	*a, b, c, f, g, h, j* and *k* *a, b, d, f, g, h, j* and *k* *a, b, d, e; f, g, h, j* and *k*
Single Bond plus (3 M/ESPE; SB) Batch #	Ethyl alcohol; bis-GMA; silane-treated silica; HEMA; water (<10 %); copolymer of acrylic and itaconic acid; UDMA	Control Wet Control Dry 5 % DMSO-dry 50 % DMSO-dry	*a, b, c, i, g* and *k* *a, b, c, d, i, g* and *k* *a, b, d, e, i, g* and *k*
Scotchbond Universal Etchant (3 M-ESPE)	37 % phosphoric acid, fumared silica (pH 0.6)		*a* and *b*

Abbreviations: HEMA = 2-hydroxyethyl methacrylate; bis-GMA = bis-phenol diglycidylmethacrylate; UDMA = diurethane dimethacrylate
* *a*: dentin etching for 15 s; *b*: water rinsing for 15 s; *c*: blot-drying leaving dentin slight moist; *d*: air-drying for 30 s; *e*: DMSO-pretreatment application for 60 s according to bonding protocol; *f*: active *Primer* active application for 10 s; *g*: gentle blow-drying for 10 s; *h*: active *Bond* application for 10 s; *i*: active adhesive application for 10 s; *j*: air-drying for 5 s and *k*: light curing for 10 s

[26] in circular rubbing movements. For SB groups, SB was applied in a single coat for 10 s and gently air-blown for 10 s. For SBMP groups, the *Primer* was applied for 10 s and gently air-blown for 10 s. The *Bond* was subsequently applied for 10 s and gently air-blown for 5 s. Adhesive procedures were carried out in a controlled environment with a temperature of 23 ℃ and a relative humidity between 45 % and 55 %. Adhesives were light cured for 10 s using a LED light-curing unit (Elipar Deepcure, 3 M ESPE) at 1200 mW/cm^2. Composite blocks were built with a nanofilled composite resin (Filtek Supreme XTE, 3 M ESPE) in two increments of approximately 2 mm. Each increment was light cured for 20 s. All bonding procedures were carried out by a single operator. The restored crown segments were stored in distilled water for 24 h at 37 °C and longitudinally sectioned with a slow-speed diamond saw (Isomet, Buehler Ltd) into resin-dentin beams with cross sectional area of approximately 0.8 mm^2. A minimum of 18 resin-dentin beams were sectioned per tooth.

2.2. Resin-dentin beam storage

Resin-dentin beams were randomly selected for the microtensile test and nanoleakage analyses under two conditions: immediate, after 24 h of storage in distilled water, or long-term ageing, after one year storage in artificial saliva at 37 °C. Half of the beams were used for immediate testing and the other half for long-term ageing. The artificial saliva (pH 7.4) was composed of 5 mM HEPES, 2.5 mM $CaCl_2{\cdot}H_2O$, 0.05 mM $ZnCl_2$ and 0.3 mM NaN_3 at 37 °C [27]. It was changed biweekly to avoid pH changes [18,25].

2.3. Microtensile bond strength test (μTBS)

Microtensile bond strength testing followed the Academy of Dental Materials guidelines for non-trimmed μTBS testing [25]. A minimum of 7 beams per tooth (n = 5 teeth/group) were tested at each storage period. Beams were individually attached to a custom-made testing jig using a cyanoacrylate adhesive (Loctite 416, Henkel Corp., Dublin, Ireland) and tested under tension on a mechanical testing machine (Shimadzu, AGS-X, Maryland, USA) at a crosshead speed of 0.5 mm/min until failure. The cross-sectional area (CA) in mm^2 of each beam was measured with a digital caliper to nearest 0.01 mm. The formula uTBS = P/CA was used to calculate μTBS values in MPa. For the statistical analysis, pre-test failures were considered as 0 MPa. Since tooth was considered as the statistical unit, bond strengths of resin-dentin beams from each tooth were averaged to represent the mean bond strength value at each specific storage period [25]. All measurements were performed by a blinded operator. Fractured resin-dentin beams were analyzed with a stereomicroscope (Leica MD60, Leica Microsystems) at 40 × magnification to determine fracture patterns. Unidentifiable surfaces were examined by scanning electron microscopy (SEM) (Phenom ProX, Phenom-World, Eindhoven, Netherlands). Fracture modes were classified as (A) adhesive failure, (C) cohesive failure in composite or dentin, (M) mixed failure (failure at composite/dentin interface with cohesive failure of any substrates) and (P) pretest failure samples [18].

2.4. Interfacial nanoleakage evaluation (SEM)

Two resin-dentin beams per tooth (n = 5 teeth/group) were randomly selected to evaluate silver nitrate uptake at bonded interfaces after 24 h or one-year storage in artificial saliva at 37 °C. Nanoleakage evaluation was performed according to a protocol previously described by Tay et al. [28]. Resin-dentin beams were initially wet polished with 600-grit and 2000-grit SiC paper and coated with two layers of nail varnish applied up to 1 mm of the bonded interfaces. Coated samples were rehydrated in distilled water for 1 h and immersed in 50 % (w/v) ammoniacal silver nitrate (pH 9.5) for 24 h. Samples were thoroughly rinsed in distilled water for 120 s and immersed in photo-developing solution (Kodak Professional D-76 developer, Kodak Rochester, NY, USA) for 8 h under fluorescent light. Silver impregnated resin-dentin beams were embedded in epoxy resin, wet polished with 600-, 1000-, and 2000-grit SiC paper (Carbimet, Buehler Ltd.,) and 1, 0.25 (MetaDi, Buehler Ltd) and 0.05 μm (MasterPrep, Buehler Ltd) polishing pastes, ultrasonically cleaned in distilled water after each polishing step for 2 min, air-dried for 2 h, mounted on aluminum stubs, dried in silica overnight and carbon sputtered. Nanoleakage was qualitatively analyzed using a scanning electron microscope (Phenom ProX, Phenom-World) on backscattering mode at 10 kV. Silver uptake patterns and extensions were evaluated by a blinded operator at magnifications ranging from 1000 – 10,000×.

2.5. Hybrid layer characterization (SEM)

Two central resin-dentin beams from each tooth were randomly selected for hybrid layer evaluation under SEM. Beams were embedded in epoxy resin and wet-polished with 600-, 1200-, 2000- and 4000-grit SiC paper (Buehler Ltd., Lake Bluff, IL, USA). Specimens were ultrasonically cleaned in distilled water after each polishing step for 5 min. Bonded interfaces were then treated with 50 % H_3PO_4 for 5 s and 3 % NaOCl for 10 min followed by dehydration in ascending ethanol series (50, 70, 80, 90 and 3 × 100 %), fixed in hexamethyldisilazane, sputtered with gold/palladium and analyzed on backscattering mode at 10 kV (Phenom ProX, Phenom-World). A series of sequential micrographs of the entire bonded interfaces (5000 × magnification) were obtained from each resin-dentin beam. Three randomly selected areas on each micrograph, located between adjacent resin tags, were analyzed and measured by a blinded examiner. Hybrid layer thickness was measured using an open-source image software (ImageJ, National Institute of Health, Bethesda, MD, USA). Measurements obtained from beams were averaged and corresponded for the hybrid layer thickness of each specific tooth (n = 5).

2.6. Statistical analysis

Bond strength data was normally distributed (Shapiro-Wilk; $p = 0.091$) and homoscedastic (Levene Test; $p = 0.064$). Three-way ANOVA followed by the Tukey test. Hybrid layer thickness data (Levene Test; $p = 0.137$) was analyzed by two-way ANOVA followed by the Tukey test. Statistical significance was set at $\alpha = 0.05$. Calculations were performed by IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Microtensile bond strength

The cross-sectional area of resin-dentin beams (0.84 mm^2 ± 0.1) ranged from 0.70 to 0.99 mm^2 without significant differences regarding specimen size between groups ($p = 0.092$). Bond strengths are reported in Fig. 1. Three-way ANOVA revealed that “bonding protocol” ($p < 0.001$; $\eta^2 = 0.908$), “bonding resin” ($p < 0.001$; $\eta^2 = 0.258$), “storage time” ($p = 0.005$; $\eta^2 = 0.115$) and the interactions between “bonding protocol” * “storage time” ($p = 0.046$; $\eta^2 = 0.116$) had significant effects on resin-dentin bond strengths. No significant differences were observed between the two-step (SB) and three-step (SBMP) etch-and-rinse adhesives under wet bonding at 24 h. Dry bonding (Control Dry) produced the lowest bond strengths. Significant reductions in the order of roughly 85 % ($p < 0.05$) were observed for both bonding resins at 24 h compared to the conventional wet-bonding technique (Control Wet). DMSO-dry bonding protocols produced significantly higher bond strengths than dry bonding (Control Dry) for both adhesives ($p < 0.05$). For the two-step etch-and-rinse adhesive (SB), both 5 % and 50 % DMSO-dry bonding produced comparable immediate bond strengths to wet bonding (Control Wet). While ageing for one year significantly reduced bond strengths of the two-step etch-and-rinse adhesive by roughly 46 %

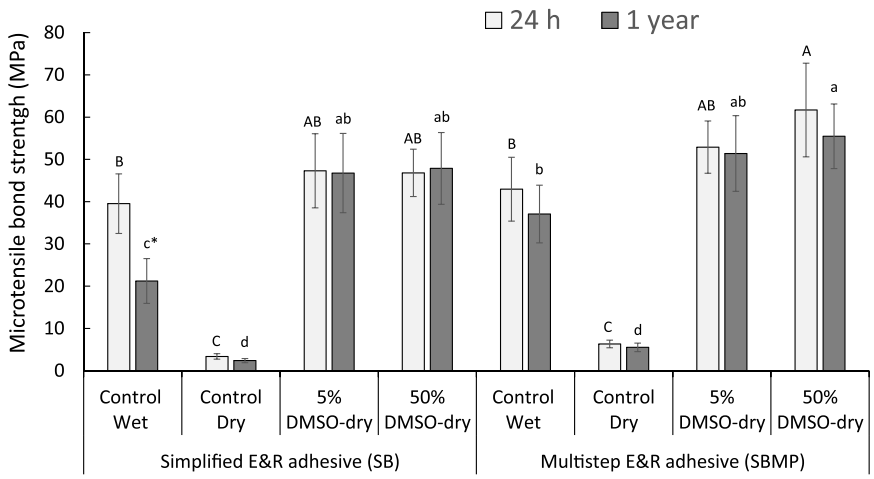

Fig. 1. Microtensile bond strength (MPa) means and standard deviations of resin-dentin interfaces bonded to dry dentin using 5 % or 50 % DMSO/EtOH solutions as pretreatments. A two-step (SB) or a three-step (SBMP) etch-and-rinse adhesive were used for bonding. Resin-dentin beams were tested at 24 h or after 1 year of ageing in artificial saliva at 37 °C. Tooth was considered the statistical unit (n = 5/group). Different upper-case letters indicate significant differences between groups within the 24 h testing period. Different lower-case letters indicate significant differences between groups after ageing for 1 year. * indicates significant differences between ageing periods within similar treatments. Statistical comparisons were performed by the Tukey test (α = 0.05).

($p < 0.05$) under wet bonding (Control Wet), no significant reductions occurred for DMSO-dry bonded samples regardless of DMSO concentration ($p < 0.05$). For the three-step etch-and-rinse adhesive, 5 % DMSO-dry bonding produced comparable immediate bond strengths to wet bonding ($p > 0.05$) and 50 % DMSO-dry bonding produced significantly higher values ($p < 0.05$). Ageing for one year had no significant effects on the bond strengths of the three-step etch-and-rinse adhesive under wet bonding ($p > 0.05$); however, 50 % DMSO-dry bonding produced significantly higher bond strengths than wet bonding in order of 50 % ($p < 0.05$). Fracture pattern distributions (%) for all groups are shown in Fig. 2 and Table 2 shows the number of fractured resin-dentin beams. The predominant failure mode at 24 h was mixed failure for both adhesive systems for all bonding protocols except for dry bonding (Control Dry), which presented a high number of pre-test failures. After

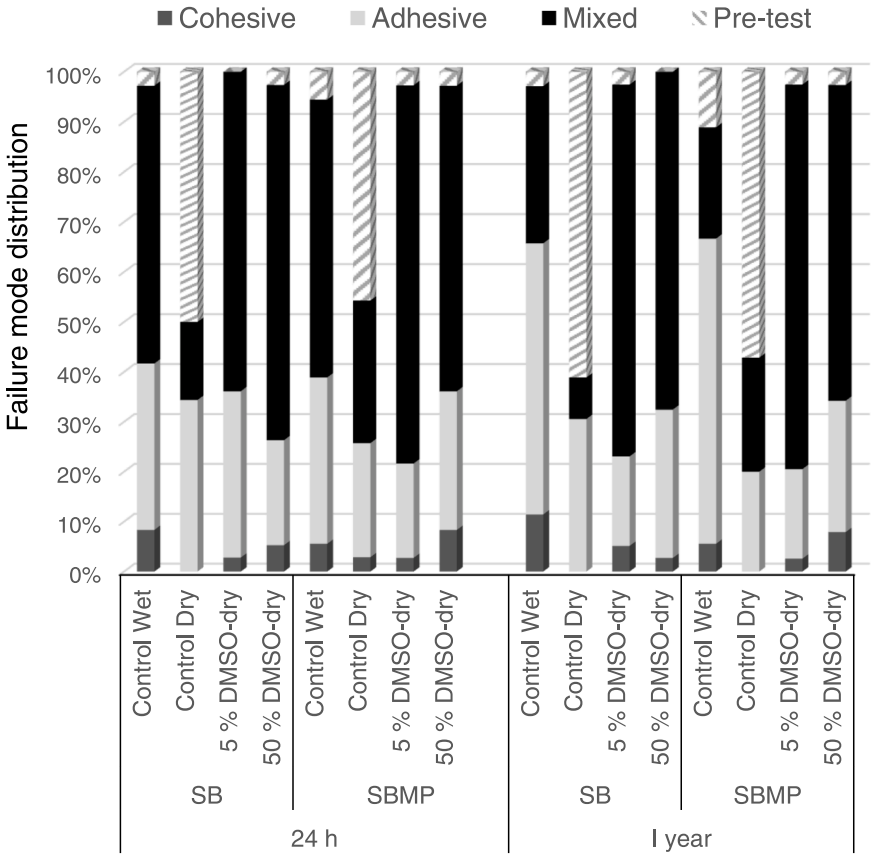

Fig. 2. Fracture patterns in percentages (%) of tested specimens after the bond strength test at 24 h or 1 year of aging in artificial saliva at 37 °C. Fracture patterns were classified as: cohesive failure = failure exclusive within dentin or resin composite; adhesive failure = failure at resin/dentin interface and mixed failure = failure at resin/dentin interface with cohesive failure of the neighboring substrates.

Table 2
Microtensile bond strength means (MPa), standard deviations (± SD) and number of specimens according to fracture mode.

	Simplified E&R adhesive (SB)				Multistep E&R adhesive (SBMP)			
	Control Wet	Control Dry	5 %DMSO-dry	50 %DMSO-dry	Control Wet	Control Dry	5 %DMSO -dry	50 %DMSO-dry
24 h	39.52 ± 7.05 (3/12/20/ 1/ 36)	3.37 ± 0.65 (0/11/5/ 16/ 32)	47.3 ± 8.77 (1/12/23/ 0/ 36)	46.81 ± 5.6 (2/8/27/ 1/38)	42.94 ± 7.55 (2/12/20/ 2/ 36)	6.32 ± 0.9 (1/8/10/ 16/ 35)	52.90 ± 6.9 (1/7/28/ 1/ 37)	61.68 ± 10.3 (3/10/22/ 1/ 36)
1 year	21.22 ± 5.3 (4/19/11/ 1/ 35)	2.38 ± 0.49 (0/11/3/ 22/ 36)	46.76 ± 9.38 (2/7/29/ 1/39)	47.86 ± 8.49 (1/11/25/ 0/ 37)	37.07 ± 6.83 (2/22/8/ 4/36)	5.51 ± 1.01 (0/7/8/ 20/ 35)	51.39 ± 8.96 (1/7/30/ 1/ 39)	55.46 ± 7.64 (3/10/24/ 1/ 38)

Tooth was considered the statistical unit (n = 5). Numbers between quotation marks represent the number of specimens (resin-dentin beams) following the fracture mode classification (1/2/3/4/5): (1) cohesive failure; (2) adhesive failure; (3) mixed failure; (4) pre-test failure and (5) total number of tested specimens.

Table 3
Hybrid layer thickness (μm) and standard deviations.

Adhesive	*Control Wet*	*Control Dry*	*5 % DMSO-dry*	*50 % DMSO-dry*
Simplified E&R (SB)	3.23^{Aa} (±0.38)	1.02^{Ba} (±0.11)	3.06^{Aa} (±0.34)	3.27^{Aa} (±0.32)
Multistep E&R (SBMP)	3.58^{Aa} (±0.45)	1.10^{Ba} (±0.13)	3.14^{Aa} (±0.3)	3.39^{Aa} (±0.32)

Different upper-case letters indicate significant differences between dentin pretreatments (row). Different lower-case letters indicate significant differences between adhesives (column). Statistical comparisons were performed by the Tukey test (α = 0.05).

ageing, wet-bonded samples presented adhesive failures as the predominant pattern and DMSO-dry bonded samples did not present substantial changes in fracture patterns.

3.2. Interfacial nanoleakage evaluation (SEM)

Representative backscattered SEM micrographs showing silver infiltration across hybrid layers are shown in Fig. 3. All bonding protocols presented silver deposits in the peritubular region of dentin tubules surrounding resin tags. Dry bonding (Control Dry) presented the highest levels of silver uptake showing hybrid layers heavily impregnated by heavy silver deposits. Such high nanoleakage levels were characterized by heavy reticular silver deposits depicting large extensions of water-filled porous zones. The conventional wet-bonding technique (Control Wet) produced considerably lower silver uptake than dry bonding (Control Dry). The two-step etch-and-rinse adhesive (SB) presented higher nanoleakage levels than the three-step etch-and-rinse adhesive (SBMP) under wet bonding. Nanoleakage patterns were mostly reticular silver deposits located at the bottom of hybrid layers, albeit spotted patterns were frequently observed for both adhesives. DMSO-dry bonded samples presented lower nanoleakage extensions than wet bonding, characterized by spotted silver deposits lightly distributed along hybrid layers for both adhesives. No substantial differences were detected between 5 % and 50 % DMSO-dry bonded samples considering the same bonding resin at the same storage period.

After ageing, higher nanoleakage levels were identified for all bonding protocols: Control Dry > Control Wet > 5 % DMSO-dry = 50 % DMSO-dry regardless of adhesive type. Under wet bonding (Control Wet), the three-step etch-and-rinse adhesive (SBMP) presented considerably less silver uptake than the two-step adhesive (SB). Silver uptake consisted of mostly reticular deposits with varying extensions along the hybrid layers. DMSO-dry bonding reduced nanoleakage at bonded interfaces for both etch-and-rinse adhesives regardless of DMSO concentration. Areas with complete-dense hybrid layer impregnation by silver were hardly identified on DMSO-dry treated samples, characterized mostly by spotted silver patterns.

3.3. Hybrid layer characterization (SEM)

Representative SEM micrographs showing hybrid layers of resin-dentin interfaces are shown in Fig. 4. Well-defined hybrid layers ranging from 1.1 to 3.58 μm were identified for all groups. Two-way ANOVA revealed that "bonding resin" had no significant effect on hybrid layer thickness. Differently, "bonding protocol" ($p < 0.001$; $\eta 2 = 0.922$) significantly affect hybrid layer formation. Dry bonding (Control Dry) produced the lowest thickness values. Wet bonding produced a significantly 3-fold increase in thicker hybrid layer thickness compared to dry bonding (Control Dry) regardless of bonding resin ($p < 0.05$). No significant differences were observed between DMSO-dry bonded samples (5 or 50 % DMSO) and wet-bonded samples (Control Wet) regardless of bonding resin ($p > 0.05$). Dry-bonded samples (Control Dry) were characterized by hybrid layers with shorter (8 – 21 μm) and thinner resin tags sparsely distributed along irregularly bonded interfaces for both simplified and multistep etch-and-rinse adhesives. The two-step etch-and-rinse adhesive displayed silica-filler agglomerates mostly across the adhesive layer, which were not affected by DMSO-dry bonding protocols. No substantial differences in hybrid layer morphology were observed between conventional wet bonding (Control Wet) and DMSO-dry bonding (5 or 50 % DMSO). Resin-impregnated intertubular dentin continuously extended into deep-penetrating resin tags (16 – 38 μm) uniformly distributed along the entire extension of bonded interfaces.

4. Discussion

Since lower DMSO fractions had a positive effect on etch-and-rinse bonding to extensively air-dried dentin (*i.e.,* 30 s), the first null hypothesis was rejected. Bonding methacrylate monomers to dry collagen is not an easy task [10]. Although wet bonding benefits from low DMSO concentrations [29–32], DMSO-dry bonding has otherwise relied on relatively higher DMSO concentrations (*i.e.,* 50 % w/w) to bond resins under dry conditions [18–21]. The rationale for originally using high DMSO fractions was to benefit from DMSO's concentration-dependent ability to dissociate highly cross-linked collagen into a sparser network of fibrils [33] and increase the wettability of dry collagen [19, 34]. It has been shown that high DMSO concentrations can eliminate the negative impact of air-drying on collagen wetting by commonly employed methacrylate-based hydrophilic resin blends [19]. Findings presented here confirm the ability of 50 % DMSO pretreatments to effectively bond multistep [18–21], as well as simplified etch-and-rinse adhesives, to dry dentin, improving long-term *in vitro* performance [18–21]. Nonetheless, this study provides evidence that high DMSO concentrations are not necessarily mandatory to achieve long-lasting bonding to dry dentin.

Water-free DMSO solutions as low as 5 % (v/v) not only restored immediate bond strengths to dry collagen, but also prevented bond strength degradation over time. The selection of 5 % DMSO was based on DMSO's low toxicity at concentrations <10 % (v/v) [35,36].

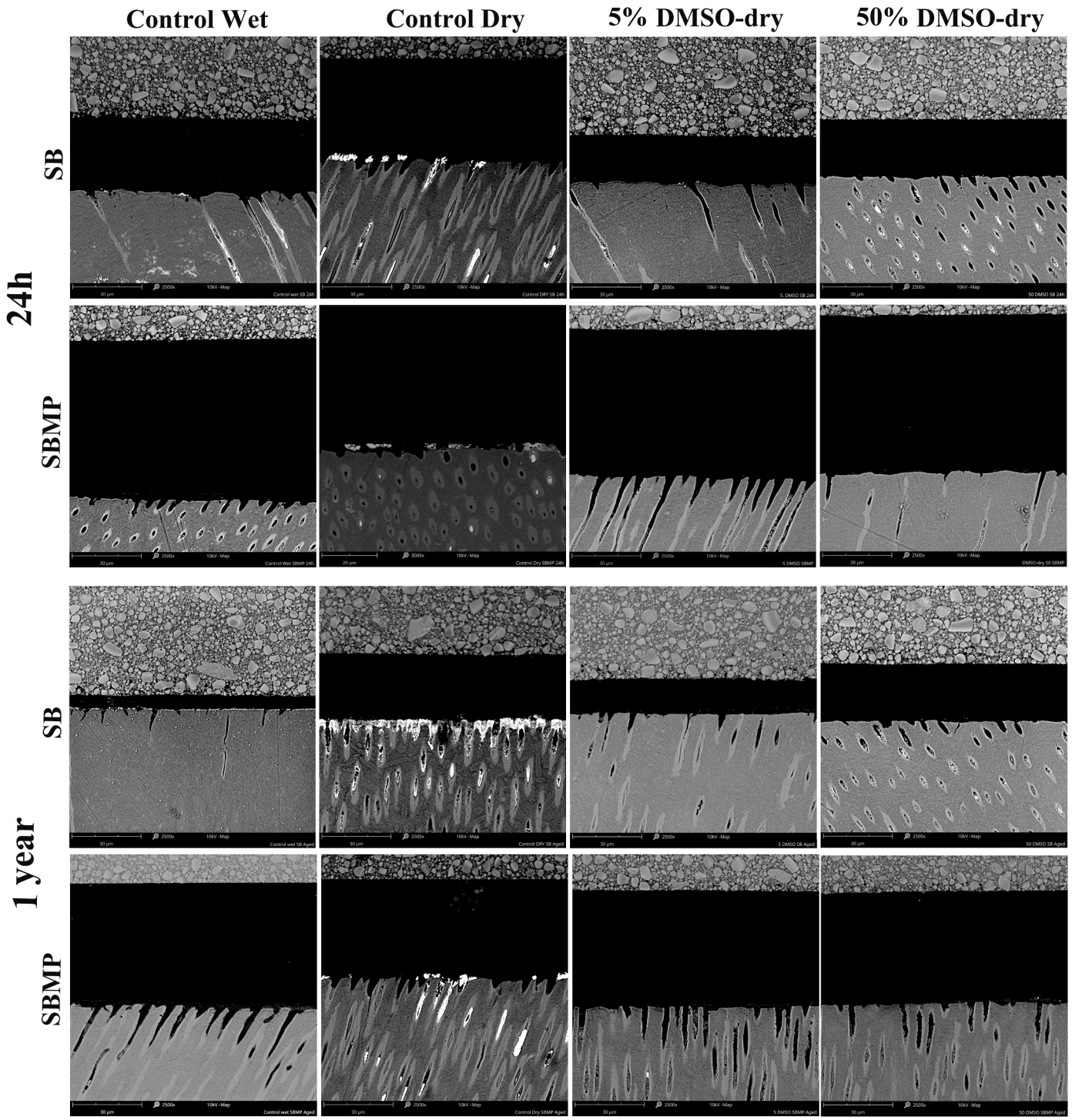

Fig. 3. Representative SEM micrographs showing nanoleakage extensions of resin-dentin interfaces bonded to dry dentin using 5 % or 50 % DMSO/EtOH solutions as pretreatments. A two-step (SB) or a three-step (SBMP) etch-and-rinse adhesive were used for bonding. Resin-dentin beams were tested at 24 h or after 1 year of ageing in artificial saliva at 37 °C.

Although substantially higher DMSO concentrations have been employed in the medical field, DMSO can induce cell apoptosis due to pore formation on plasma membranes [35,36]. Hence, attempts to minimize potential cytotoxic effects for dental applications remain valid. Using 5 % DMSO (v/v) as a threshold further increased safety margins still resulting in improved bonding to dry dentin. DMSO's ability to bond methacrylate-based resins to dry dentin and its protective effect on long-term bonding were not concentration dependent between 5 % and 50 % (v/v). This clearly shows that DMSO-dry bonding protocols may not require such high DMSO concentrations as initially thought. Using ethanol-based pretreatments containing low DMSO fractions invariably opens new possibilities to dry bonding. It is interesting how the incorporation of relatively low DMSO fractions modified ethanol-collagen interactions allowing effective bonding under dry conditions. The 5 % DMSO pretreatment was composed mostly by ethanol (*i.e.,* 95 % v/v), which was air-dried for 30 s before adhesive application. Normally, bonding resins to chemically dehydrated ethanol-saturated dentin (*i.e.,* ethanol-wet bonding [10]) must be

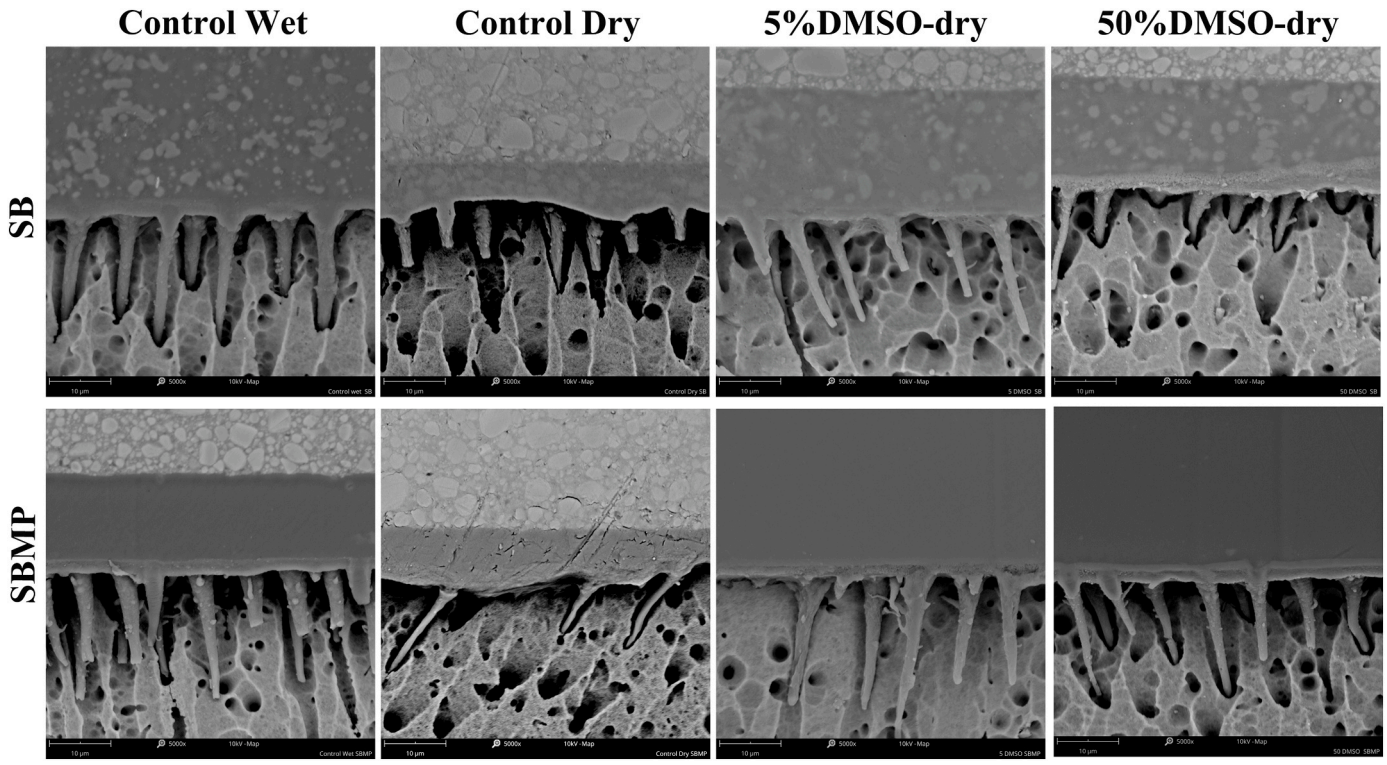

Fig. 4. Representative SEM micrographs showing hybrid layers of resin-dentin interfaces bonded to dry dentin using 5 % or 50 % DMSO/EtOH solutions as pre-treatments. A two-step (SB) or a three-step (SBMP) etch-and-rinse adhesive were used for bonding.

performed following a quite laborious-sensitive technique [37]. Collagen must be initially moist to prevent interpeptide hydrogen bonding for subsequent dehydration with a series of ascending ethanol solutions [10]. It is crucial for collagen to be fully embedded in ethanol before adhesive application [10]. Failure to prevent initial collapse of collagen compromises bonding efficacy [10]. This was not impeditive for high-, as previously shown [18–21], nor now for low-percentage DMSO-dry bonding. No significant differences on bond strengths occurred between low or high DMSO concentrations. Conceptually, DMSO-dry bonding differs from ethanol-wet bonding in the sense that collagen does not need to be fully immersed in ethanol before hybridization [18]. Air-drying high- or low-content DMSO before hybridization did not hinder bonding efficiency. It is clear that even at lower concentrations, DMSO broadened the moisture spectrum for resin bonding [19] bringing more flexibility and long-term stability to dry bonding protocols.

The central aim of this study was to investigate clinically feasible dry-bonding approaches to ultimately reduce the overall water entrapment within hybrid layers. However, two water-based adhesives (*i.e.,* SB <10 and SBMP >40 % water content) were chosen. Bonding with water-free adhesives would indeed lower water entrapment within resin-dentin interfaces. However, full re-expansion of air-dried collagen is not easily accomplished by commonly used adhesive solvents applied under clinically feasible times [10]. The higher the Hoy's solubility parameters for hydrogen bonding forces (δh) of a polar solvent above 14.8, the greater is the degree of collagen re-expansion [10]. Water is indeed the strongest known hydrogen bonding solvent (δh 37.3 $(J/cm^3)^{1/2}$). As such, it effectively breaks interpeptide bonds of air-dried collagen (δh 14.8 $(J/cm^3)^{1/2}$), promptly re-expanding and increasing interfibrillar spaces [10]. Previously used aqueous solutions containing 50 % DMSO (δh 26.8 $(J/cm^3)^{1/2}$) result in modest re-expansion (roughly 70 %) of dry collagen [34]. The tested water-free DMSO pretreatments present even lower δh, which further reduces their ability to re-expand dry collagen. Incomplete collagen re-expansion before hybridization impairs resin infiltration and compromise resin-dentin bonding [6,10]. The obtained low bond strengths to untreated-dry dentin (Control Dry) corroborates the inefficiency of water-based resins to re-expand [10] and properly bond to air-dried collagen [26,38]. Considering the extreme dry-bonding conditions, it seemed reasonable to select water-based adhesives to complement collagen re-expansion produced by water-free DMSO pretreatments as in previous studies [18–21]. In such manner, DMSO breaks self-associative tendencies of water [39] and acts as a surfactant, facilitating the diffusion of adhesive components into collagen [40]. The combined collagen re-expansion produced by DMSO pretreatments (*i.e.,* high or low content) and water-based adhesives seemed sufficient for adequate bonding. SEM analyses of hybrid layers provided indirect evidence that resin infiltration of collagen matrices was not hampered by lower DMSO concentrations of pretreatments nor by reducing water content of adhesives. While conventional dry bonding showed clear limitations in adhesive diffusion, due to severe collagen collapse, no significant differences were observed between hybrid layer thicknesses or bond strengths between wet (Control Wet) and DMSO-dry bonded protocols. This indicates that the water content of bonding resins and DMSO concentrations can be reduced, without negative impacts on resin-dentin interactions. Future studies should focus on determining the lowest water content of bonding resins to effectively bond hydrophilic resins using the DMSO-dry bonding approach. It is tempting to speculate the possibility of bonding water-free resins to extensively air-dried etched dentin employing protocols that are more technique forgiving.

Successful resin-dentin bonding is strongly related to the stability of bonded interfaces over time. Adhesive composition plays a major role on handling and dentin bonding performance [1,5]. While the wet bonded (Control Wet) simplified etch-and-rinse adhesive (SB) presented significant bond strength reductions after ageing, the tested multistep etch-and-rinse adhesive (SBMP) did not. The superiority of multistep over simplified adhesives has been well stablished [1,6,41,42]. Multi-step adhesives make use of a separate more hydrophobic resin that improves the mechanical stability of hybrid layers [6,43] and reduces hydrolytic degradation of polymers over time [42]. Bonding resins with

higher ratios of hydrophobic dimethacrylate monomers tend to result in stronger cross-linked polymer networks [44] with lower water solubility [45,46], making multistep adhesives less prone to degradation [1,6,41,42]. Noteworthy, DMSO-dry bonding equiparated aged bond strengths between simplified and multistep etch-and-rinse adhesives regardless of DMSO concentrations. The protective mechanisms conferred by DMSO certainly contributed to higher stability of bond strengths and improved hybrid layer quality depicted by the nanoleakage analyses. After ageing, hybrid layers of DMSO-dry bonded samples presented less water-filled zones, shown as silver deposits, than untreated wet- or dry-bonded samples. This was especially true for the simplified adhesive. DMSO has the ability to reduce enzymatic activity [21], improve monomer conversion [18], increase monomer diffusion within collagen [40] and reduce nano-porosities within hybrid layers originated by long-term ageing [19]. DMSO-dry bonding circumvents major degradation mechanisms of resin-dentin interfaces extending their durability. Additionally, the resultant interaction between DMSO and collagen disrupts water layers surrounding collagen fibrils, allowing better monomer-collagen interactions [47]. We speculate that such water-disruption mechanism takes place at lower DMSO concentrations in a relevant manner considering similarities in bonding and nanoleakage outcomes for both high and low DMSO concentrations. Clearly, the use of lower DMSO concentrations can be characterized as an effective approach to restore and improve resin-dentin interfaces under dry conditions. Nonetheless, it is important to consider that multistep adhesives may additionally benefit, at least to some extent, from higher DMSO concentrations. While bond strengths of 5 % DMSO-dry bonding were not significantly different from untreated-wet dentin (Control Wet), 50 % DMSO-dry bonding indeed produced higher values. Future studies should determine the extent in which monomeric composition of bonding resins affect DMSO-dry bonding.

5. Conclusion

Bonding water-based simplified or multistep adhesives to extensively air-dried dentin is possible given the right conditions. Water-free DMSO pre-treatments with concentrations as low as 5 % not only restores bond strengths of dry-etched dentin, but also prevents long-term resin-dentin degradation. Higher DMSO concentrations may be more advantageous for the overall dentin bonding performance of multistep adhesives. Nonetheless, dry bonding with lower DMSO concentrations unfolds as a valid alternative to reduce moisture-related technique sensitivity of etch-and-rinse bonding resins regardless of adhesive type. Even at lower DMSO concentrations, DMSO-dry bonding may extend the service life of resin-dentin interfaces outperforming conventional wet-bonding protocols.

Disclosure statement

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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References

[1] van Meerbeek B, Yoshihara K, van Landuyt K, Yoshida Y, Peumans M. From buonocore's pioneering acid-etch technique to self-adhering restoratlves. A status perspective of rapidly advancing dentai adheslve technology. J Adhes Dent 2020; 22:7–34. https://doi.org/10.3290/j.jad.a43994.

[2] Tjäderhane L. Dentin bonding: can we make it last? Oper Dent 2015;40:4–18. https://doi.org/10.2341/14-095-BL.

[3] Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer - a review. Dent Mater 2013;29:999–1011. https://doi.org/10.1016/j.dental.2013.07.016.

[4] Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116–35. https://doi.org/10.1016/j.dental.2012.08.004.

[5] Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, et al. Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018;34:78–96. https://doi.org/10.1016/j.dental.2017.11.005.

[6] Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1–16. https://doi.org/10.1016/j.dental.2010.10.016.

[7] Agee KA, Prakki A, Abu-Haimed T, Naguib GH, Nawareg MA, Tezvergil-Mutluay A, et al. Water distribution in dentin matrices: bound vs. unbound water. Dent Mater 2015;31:205–16. https://doi.org/10.1016/j.dental.2014.12.007.

[8] Kinney JH, Marshall SJ, Marshall GW. The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature. Crit Rev Oral Biol Med 2003;14:13–29. https://doi.org/10.1177/154411130301400103.

[9] Nishitani Y, Yoshiyama M, Donnelly AMM, Agee KAA, Sword J, Tay FRR, et al. Effects of resin hydrophilicity on dentin bond strength. J Dent Res 2006;85:1016–21. https://doi.org/10.1177/154405910608501108.

[10] Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007;20:7–20.

[11] Spencer P, Wang Y, Katz JL. Identification of collagen encapsulation at the dentin/adhesive interface. J Adhes Dent 2004;6:91–5.

[12] Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. J Dent Res 2003;82:141–5. https://doi.org/10.1177/154405910308200213.

[13] Tay FR, Gwinnett AJ, Pang KM, Wei SHY, Gwinnett JA, Pang P, et al. Resin permeation into acid-conditioned, moist, and dry dentin: a paradigm using water-free adhesive primers. J Dent Res 1996;75:1034–44. https://doi.org/10.1177/00220345960750040601.

[14] Tay FR, Gwinnett JA, Wei SHY. The overwet phenomenon in two-component acetone-based primers containing aryl amine and carboxylic acid monomers. Dent Mater 1997;13:118–27. https://doi.org/10.1016/s0109-5641(97)80021-8.

[15] Tay FR, Gwinnett JA, Wei SHY. Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free, acetone-based, single-bottle primer/adhesives. Dent Mater 1996;12:236–44. https://doi.org/10.1016/S0109-5641(96)80029-7.

[16] Loguercio AD, Loeblein F, Cherobin T, Ogliari F, Piva E, Reis A. Effect of solvent removal on adhesive properties of simplified etch-and-rinse systems and on bond strengths to dry and wet dentin. J Adhes Dent 2009;11:213–9. https://doi.org/10.3290/j.jad.a15627.

[17] Reis A, Loguercio AD, Azevedo CLN, de Carvalho RM, da Julio Singer M, Grande RHM. Moisture spectrum of demineralized dentin for adhesive systems with different solvent bases. J Adhes Dent 2003;5:183–92.

[18] Stape THS, Mutluay MM, Tjäderhane L, Uurasjärvi E, Koistinen A, Tezvergil-Mutluay A. The pursuit of resin-dentin bond durability: simultaneous enhancement of collagen structure and polymer network formation in hybrid layers. Dent Mater 2021;37:1083–95. https://doi.org/10.1016/j.dental.2021.03.010.

[19] Stape THS, Uctasli M, Cibelik HS, Tjäderhane L, Tezvergil-Mutluay A. Dry bonding to dentin: broadening the moisture spectrum and increasing wettability of etch-and-rinse adhesives. Dent Mater 2021;37:1676–87. https://doi.org/10.1016/j.dental.2021.08.021.

[20] Stape THS, Tjäderhane L, Abuna G, Sinhoreti MAC, Martins LRM, Tezvergil-Mutluay A. Optimization of the etch-and-rinse technique: new perspectives to improve resin–dentin bonding and hybrid layer integrity by reducing residual water using dimethyl sulfoxide pretreatments. Dent Mater 2018;34:967–77. https://doi.org/10.1016/j.dental.2018.03.010.

[21] Stape THS, Seseogullari-Dirihan R, Tjäderhane L, Abuna G, Martins LRM, Tezvergil-Mutluay A. A novel dry-bonding approach to reduce collagen degradation and optimize resin-dentin interfaces. Sci Rep 2018;8:16890. https://doi.org/10.1038/s41598-018-34726-8.

[22] Mai S, Wei CC, Gu L, sha, Tian F, Arola cong, Chen DD, hua J, et al. Extrafibrillar collagen demineralization-based chelate-and-rinse technique bridges the gap between wet and dry dentin bonding. Acta Biomater 2017;57:435–48. https://doi.org/10.1016/j.actbio.2017.05.017.

[23] Sebold M, André CB, Carvalho RM, Giannini M. Dry-bonding to dentin using alternative conditioners based on iron-containing solutions or nitric acid. J Mech Behav Biomed Mater 2019;94:238–48. https://doi.org/10.1016/j.jmbbm.2019.03.015.

[24] Zhou J, Chiba A, Scheffel DLS, Hebling J, Agee K, Tagami J, et al. Cross-linked dry bonding: a new etch-and-rinse technique. Dent Mater 2016;32:1124–32. https://doi.org/10.1016/j.dental.2016.06.014.

[25] Armstrong S, Breschi L, Özcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μTBS) approach. Dent Mater 2017;33:133–43. https://doi.org/10.1016/j.dental.2016.11.015.

[26] Reis A, Pellizzaro A, Dal-Bianco K, Gomes OM, Patzlaff R, Loguercio AD. Impact of adhesive application to wet and dry dentin on long-term resin-dentin bond strengths. Oper Dent 2007;32:380–7. https://doi.org/10.2341/06-107.

[27] Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjäderhane L, et al. The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dent Mater 2010;26:1059–67. https://doi.org/10.1016/j.dental.2010.07.006.

[28] Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472–6. https://doi.org/10.1177/154405910208100708.

[29] Stape THS, Tezvergil-Mutluay A, Mutluay MM, Martins LRM, do Prado RL, Pizi ECG, et al. Influence of dimethyl sulfoxide used as a solvent on the physical properties and long-term dentin bonding of hydrophilic resins. J Mech Behav Biomed Mater 2016;64:220–8. https://doi.org/10.1016/j.jmbbm.2016.07.003.

[30] Tjäderhane L, Mehtälä P, Scaffa P, Vidal C, Pääkkönen V, Breschi L, et al. The effect of dimethyl sulfoxide (DMSO) on dentin bonding and nanoleakage of etch-and-rinse adhesives. Dent Mater 2013;29:1055–62. https://doi.org/10.1016/j.dental.2013.07.014.

[31] Szesz AL, Pereira G de MA, Siqueira FSF, de, Cardenas AFM, Bandeca MC, Armas-Vega A, et al. Effect of addition of dimethyl sulfoxide to simplified adhesives on dentin bond durability after three years of water storage. J Adhes Dent 2021;23:159–65. https://doi.org/10.3290/j.jad.b1079585.

[32] Speranza Zabeu G, Candia Scaffa PM, Ciccone Giacomini M, de Mattos Pimenta Vidal C, Tjäderhane L, Wang L. Gelatinolytic activity after dentin pretreatment with dimethyl sulfoxide (DMSO) combined to dental bonding systems: perspectives for biological responses. J Mech Behav Biomed Mater 2022;130:105188. https://doi.org/10.1016/j.jmbbm.2022.105188.

[33] Zimmerley M, McClure RA, Choi B, Potma EO. Following dimethyl sulfoxide skin optical clearing dynamics with quantitative nonlinear multimodal microscopy. Appl Opt 2009;48:D79. https://doi.org/10.1364/AO.48.000D79.

[34] Mehtälä P, Pashley DH, Tjäderhane L. Effect of dimethyl sulfoxide on dentin collagen. Dent Mater 2017;33:915–22. https://doi.org/10.1016/j.dental.2017.04.018.

[35] de Ménorval MA, Mir LM, Fernández ML, Reigada R. Effects of dimethyl sulfoxide in cholesterol-containing lipid membranes: a comparative study of experiments in silico and with cells. PLoS One 2012;7:1–12. https://doi.org/10.1371/journal.pone.0041733.

[36] Notman R, Noro M, O'Malley B, Anwar J. Molecular basis for dimethylsulfoxide (DMSO) action on lipid membranes. J Am Chem Soc 2006;128:13982–3. https://doi.org/10.1021/ja063363t.

[37] Sadek FT, Mazzoni A, Breschi L, Tay FR, Braga RR. Six-month evaluation of adhesives interface created by a hydrophobic adhesive to acid-etched ethanol-wet bonded dentine with simplified dehydration protocols. J Dent 2010;38:276–83. https://doi.org/10.1016/j.jdent.2009.11.007.

[38] Manso AP, Marquezini L, Silva SMA, Pashley DH, Tay FR, Carvalho RM. Stability of wet versus dry bonding with different solvent-based adhesives. Dent Mater 2008;24:476–82. https://doi.org/10.1016/j.dental.2007.04.009.

[39] Vishnyakov A, Lyubartsev AP, Laaksonen A. Molecular dynamics simulations of dimethyl sulfoxide and dimethyl sulfoxide-water mixture. J Phys Chem A 2001;105(10):1702. https://doi.org/10.1021/jp0007336.

[40] Stape THS, Tjäderhane L, Marques MR, Aguiar FHB, Martins LRM. Effect of dimethyl sulfoxide wet-bonding technique on hybrid layer quality and dentin bond strength. Dent Mater 2015;31:676–83. https://doi.org/10.1016/j.dental.2015.03.008.

[41] Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010;26:e100–21. https://doi.org/10.1016/j.dental.2009.11.148.

[42] De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 2005;84:118–32. https://doi.org/10.1177/154405910508400204.

[43] Carrilho MRDO, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM. Mechanical stability of resin-dentin bond components. Dent Mater 2005;21:232–41. https://doi.org/10.1016/j.dental.2004.06.001.

[44] Chiaraputt S, Mai S, Huffman BP, Kapur R, Agee KA, Yiu CKY, et al. Changes in resin-infiltrated dentin stiffness after water storage. J Dent Res 2008;87:655–60. https://doi.org/10.1177/154405910808700704.

[45] Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005;26:6449–59. https://doi.org/10.1016/j.biomaterials.2005.04.052.

[46] Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, et al. Water sorption/solubility of dental adhesive resins. Dent Mater 2006;22:973–80. https://doi.org/10.1016/j.dental.2005.11.020.

[47] Stape THS, Tjäderhane L, Tezvergil-Mutluay A, Yanikian CRF, Szesz AL, Loguercio AD, et al. Dentin bond optimization using the dimethyl sulfoxide-wet bonding strategy: a 2-year in vitro study. Dent Mater 2016;32:1472–81. https://doi.org/10.1016/j.dental.2016.09.015.