Journal of Dentistry 136 (2023) 104643

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Glutaraldehyde-based desensitizers' influence on bonding performances and dentin enzymatic activity of universal adhesives

Edoardo Mancuso [a], Diego Durso [a], Claudia Mazzitelli [a], Tatjana Maravic [a], Uros Josic [a], Carlo D'alessandro [a], Luigi Generali [b], Vittorio Checchi [b], Lorenzo Breschi [a,*], Annalisa Mazzoni [a]

[a] Department of Biomedical and Neuromotor Sciences, DIBINEM, University of Bologna, Alma Mater Studiorum, Via San Vitale 59, Bologna 40125, Italy
[b] Department of Surgery, Medicine, Dentistry and Morphological Sciences, Unit of Dentistry and Oral-Maxillo-Facial Surgery, University of Modena and Reggio Emilia, Italy

ARTICLE INFO

Keywords:
Universal adhesive
Glutaraldehyde
Desensitizers
Mmp inhibition
Zymography
Dentin

ABSTRACT

Objectives: To evaluate the influence of two glutaraldehyde-based desensitizers (L: GLUMA Desensitizer, Heraeus Kulzer and G: GLUMA Desensitizer PowerGel) prior to the adhesive procedures on microtensile bond strength (μTBS) to dentin and endogenous enzymatic activity.

Methods: Noncarious human third molars ($N = 48$) were cut to expose middle coronal dentin. Six experimental groups were formed according to the dentin pre-treatment (L or G) and the universal adhesives (IBU - iBond universal, Kulzer or AU - Adhese Universal, Ivoclar Vivadent) used in the self-etch mode ($n = 8$): 1) L/IBU; 2) G/IBU; 3) IBU; 4) L/AU; 5) G/AU; 6) AU. Specimens were cut into sticks and stressed until failure after 24 h (T_0) or 1 yr of aging (T_{12}). Additional 4 teeth were used for in situ zymography evaluation and data were statistically analyzed ($\alpha = 0.05$).

Results: Dentin pre-treatment, adhesive and aging statistically influenced bond strength and enzymatic activity ($P<0.001$). AU demonstrated higher bond strength values than IBU ($P<0.001$). The L resulted in higher bond strength compared to the G and control groups ($P<0.001$). aging statistically influenced bonding performance, especially when no dentin pre-treatment was performed ($P<0.001$). In situ zymography revealed that at baseline the control groups exhibited lower interfacial fluorescence compared to the experimental groups, irrespective of the adhesive used ($P<0,001$). However, after 1 yr of artificial storage, no differences were found among the groups ($P>0.05$).

Conclusions: : Glutharldeadeyde-based products increased bond strength and determined a stabilization of the adhesive interface over time apparently not related to the MMPs inhibition.

Clinical Significance: The results of this in vitro study suggest that the application of glutaraldehyde-based desensitizers prior to the adhesive procedures when associated with universal adhesives could result in increased bond strength and stabilization of the adhesive interface over time.

1. Introduction

Removal of excessive sound tooth structure [1] and extensive cavity preparations cause the exposure of deep dentin substrate characterized by opened and widened dentinal tubules that, together with high heat production by dental instruments and dentin dehydration, are responsible for post-operative sensitivity (POS) [2]. POS has been frequently observed when etch-and-rinse (E&R) adhesive systems have been used for bonding procedures, due to the acid etching pre-treatment required before bonding application [3,2]. Attempts have been made over time to reduce the detrimental effects of dentin acid etching, such as reducing the etching times or the use of self-limiting etchants [4,5]. On the other hand, self-etch (SE) adhesive systems, as well as universal adhesives used in the SE mode have been associated with a lower incidence of POS when compared to their E&R counterparts [6–8]. Nonetheless, post-operatory dental sensibility has been reported to be present in 10% of placed restorations [9]. Therefore, given the interest in limiting dental sensitivity, a cause of discomfort for the patient and demotivating for the clinician, the use of desensitizing agents during and after restorative procedures has been suggested [10,11].

* Corresponding author.
E-mail address: lorenzo.breschi@unibo.it (L. Breschi).

https://doi.org/10.1016/j.jdent.2023.104643
Received 10 May 2023; Received in revised form 27 July 2023; Accepted 28 July 2023
Available online 29 July 2023

An additional drawback related to the E&R systems consists of uncomplete resin infiltration to the entire depth of the etched dentin, resulting in bare water-rich collagen fibrils at the bottom of the hybrid layer (HL) [12]. These portions of the collagen fibrils that are not infiltrated by the resin, represent sites prone to a series of microbiological (i.e. bacteria infiltration) [13], hydrodynamic [14] and enzymatic [15] phenomena all participating in the decrease of the longevity of the HL and increasing the risk of POS [2,16]. The acidic monomers comprised into the SE and universal adhesive systems are responsible for the partial dissolution of the smear layer, thus avoiding the complete exposure of the dentinal tubules, overcoming the problems related to incomplete resin infiltration, and reducing enzymatic activity within the HL [17]. However, regardless of the type of adhesive system (E&R or SE), their application has been associated with the activation of silenced enzymes usually present in dentin (i.e. matrix metalloproteinases – MMPs and cysteine cathepsins - CC), which can result in disruptive phenomena within the HL [13,17–19]. In order to inhibit the enzymatic activity and increase the longevity of the bond, several materials have been several materials have been investigated during the last 15 years (i.e. proanthocyanidin [18], genipin [19], tannic acid [20], chitosan [21] carbodiimide [22,23] glutaraldehyde [24], chlorhexidine [25]).

Among these agents, the benefits of dental usage of glutaraldehyde-based materials rely on their antimicrobial effect [26] and fixative properties [27,28] that together participate to fill in and seal the open dentinal orifices preventing the outward fluid flow through dentinal tubules and hence desensitize the tooth [29–34,6]. Glutaraldehyde has been used as a separate cross-linker on demineralized collagen matrix before adhesive application, reinforcing the collagen fibrils within the hybrid layer and promoting the longevity of the restoration [35,31,36]. According to previous studies, glutaraldehyde-based desensitizers (i.e. GLUMA desensitizer) used before E&R adhesives application did not impair bond strength [37,38]. However, these studies are dated and, in the meantime, updated products in terms of composition, viscosity and application methods have been introduced on the market. Moreover, little is known about the effect of glutaraldehyde used as dentin pretreatment on the bond strength and enzymatic activity of SE adhesives.

Taking into consideration the wide range of action of a substance such as glutaraldehyde, this laboratory study aimed to evaluate the effect of therapeutical dentin priming with glutaraldehyde-based solutions marketed as desensitizers and available in two consistencies (liquid and gel; Fig. 1) on the bonding performance and endogenous dentinal enzymatic activity of two simplified universal adhesives (UAs) used in the SE mode. Specifically, the null hypotheses tested were that: 1) dentin pretreatment with glutaraldehyde-based primers prior to adhesive procedures with two universal adhesives does not influence microtensile bond strength immediately or after aging; 2) dentin pretreatment with glutaraldehyde-based primers prior to adhesive procedures with two universal adhesives does not influence endogenous dentinal enzymatic activity immediately or after aging; and that 3) there are no differences between two tested universal adhesives in terms of bond strength and enzymatic activity.

2. Materials and methods

2.1. Microtensile bond strength test (μTBS)

Forty-eight freshly extracted non-carious human molars were obtained from anonymous individuals following their informed consent under a protocol approved by the Ethical Committee of the University of Bologna, Italy (protocol N°: 71/2019/OSS/AUSLBO). Tooth crowns were removed with a low-speed diamond saw under water cooling (Microremet, Remet, Casalecchio di Reno, Italy) to expose enamel-free middle/deep coronal dentin. Cut dentin surfaces were examined with a stereoscopical microscope to ensure that they were devoid of defects and enamel remnants. A standardized smear layer was created on each dentin surface using #320- grit wet silicon carbide paper and water lubrication.

In the experimental groups, prior to adhesive application, the dentin surfaces were pre-treated for 30 s with one of the following desensitizers available in 2 consistencies (Kulzer GmgH, Hanau, Germany): the liquid GLUMA Desensitizer (L) and the gel GLUMA Desensitizer Powergel (G). After application, the specimens were thoroughly water-rinsed as indicated by the manufacturer. In the control groups, no dentin pretreatment was performed. Then, two universal adhesives were employed for the bonding procedures in the SE mode: IBU - iBond universal (Kulzer), or AU - Adhese Universal (Ivoclar Vivadent, Schaan, Liechtenstein). In the end, the following groups were formed according to the desensitizer/adhesive combination ($n = 8$): 1) L/IBU; 2) G/IBU; 3) IBU (CTRL); 4) L/AU; 5) G/AU; 6 AU (CTRL). Complete details and instructions of the materials used in the study are presented in Table 1.

After polymerization of the respective adhesive, a build-up was created (two 2 mm-thick layers) with a nanohybrid resin composite (Venus Pearl, Kulzer). Light curing of the adhesive resin and each layer of composite resin was performed for 20 s with a light-emitting diode (LED) curing light (ELIPAR™ DeepCure-S, 3 M, St Paul, MN, USA; light output > 1000 mW/cm^2 and wavelength 430–480 nm).

The bonded specimens were serially sectioned to obtain sticks with ~0.9 mm × ~0.9 mm cross-sectional area, following the non-trimming technique of the microtensile bond strength test (μTBS). The exact dimension of the sticks was measured using a pair of digital calipers. Bond testing was performed after the sticks were aged in artificial saliva at 37 °C for 24 h (T_0) or 12 months (T_{12}). Each beam was stressed under tension to failure using a simplified bond testing machine (Shear Bond Tester; Bisco, Schaumburg, IL, USA) at a crosshead speed of 1 mm/min.

Fig. 1. Application on dentin of the two desensitizer, GLUMA Desensitizer (left) and GLUMA Desensitizer Powergel (right).

Table 1
Chemical composition of the materials used in the study and bonding procedures.

Material	Composition	pH	Mode of use
Adhese Universal Ivoclar Vivadent, Schaan, Liechtenstein	MDP, MCAP, HEMA, Bis-GMA, D3MA, Water, Ethanol Highly dispersed silicon dioxide Initiators and Stabilizers	2.5- 3	1. The adhesive is scrub on dentin for 20 s; 2. Air-spray with oil- and moisture-free compressed air until a glossy, immobile film layer results; 3. Light-cure using a LED light-curing unit for 20 s.
iBOND Universal Kulzer GmgH, Hanau, Germany	Phosphonic acid acrylate, HEMA, D3MA Highly dispersed silica Ethanol Catalysts, stabilizers, fluoride	1.6–1.8	1. The adhesive is scrub on dentin for 20 s; 2. Disperse the adhesive with an oil- and moisture-free compressed air until a glossy, immobile film layer result; 3. Light-cure using a LED light-curing unit for 10 s.
GLUMA Desensitizer Kulzer GmgH, Hanau, Germany	Purified water, (HEMA), glutardialdehyde, pyrogenic silicic	3.66	1. Apply the desensitizer for 30 - 60 s; 2. Thoroughly rinse off the desensitizer with water spray and dry with oil-free air; 3. Continue with the bonding procedures.
GLUMA Desensitizer Powergel Kulzer GmgH, Hanau, Germany	Purified water, (HEMA), glutardialdehyde, pyrogenic silicic	3.66	1. Apply the desensitizer for 30 - 60 s; 2. Thoroughly rinse off the desensitizer with water spray and dry with oil-free air; 3. Continue with the bonding procedures.
VENUS Pearl Kulzer GmgH, Hanau, Germany	Amorphous silica, triethylen glycol dimethacrylate, trimethoxysilylpropyl methacrylate, Phenyl-1,2-propandion, methyl methacrylate, acetic acid	Not defined	

The number of prematurely debonded specimens in each experimental group was recorded. Null bond strength values were not included in the statistical analysis because the number of prematurely debonded sticks did not exceed 3% of the total number of tested specimens and were similarly distributed within the groups [39]. A single observer evaluated each side of the fractured sticks with a stereomicroscope at 50× magnification to determine the mode of failure. Failure was classified as adhesive at the dentin interface (A), cohesive in dentin (CD), cohesive in composite (CC) or mixed failure (M; adhesive and cohesive fractures occurred simultaneously).

2.2. Scanning Electron Microscope (SEM) examination

After μTBS, two representative debonded sticks per group were selected (with a bond strength value close to the mean value of the group) and prepared for scanning electron microscopy (SEM) evaluation. Each specimen was fixated in a 2.5% glutaraldehyde 0.1 M cacodylate buffer (pH 7.4), dehydrated in ascending ethanol solutions (50%, 70%, 80%, 90%, 95% and 100%), and dried using hexamethyldisilazane. Then, they were mounted on aluminum specimen stubs, coated with 8÷10 nm gold particles and observed under a field-emission gun scanning electron microscope (FEG-SEM; Nova NanoSEM 450; FEI, Eindohoven, NL).

2.3. In situ zymography of resin-dentin interfaces

One-millimeter-thick slabs of middle/deep coronal dentin were obtained from four extracted human third molars using a low-speed saw (Micromet) under water-cooling. Two dentin slabs were obtained from each tooth. Each slab was further divided into 4 pieces so that testing of the six experimental groups was performed using the same dentin substrate. A standardized smear layer was created on each dentin surface using #600-grit silicon carbide paper under water cooling. Identical bonding procedures were performed as previously described for the μTBS forming the same 6 groups ($n = 4$). Resin-dentin interfaces were exposed by cutting the bonded specimens vertically into 1 mm-thick sticks using the slow-speed saw under water cooling. The sticks were fixed to glass slides with cyanoacrylate glue ground down and polished to obtain ~50 μm thick slabs using a series of wet silicon carbide papers. Self-quenched fluorescein-conjugated gelatin was used as the MMP substrate (E-12,055, Molecular Probes, Eugene, OR, USA) for in situ zymography at T_0 and T_{12} [40]. The fluorescent gelatin mixture was placed on top of each slab and covered with a glass coverslip. The slides were incubated in a humidified chamber at 37 °C overnight. During incubation, the assemblies were prevented from direct contact with water and were protected from exposure to light. After incubation, the microscopic slides were examined using a confocal laser scanning microscope (laser excitation wavelength 488 nm; emission wavelength 530 nm; Model A1-R; Nikon, Tokyo, Japan). To visualize the hydrolysis of the quenched fluorescein-conjugated gelatin substrate as an indicator of endogenous gelatinolytic activity, 3 z-stack images (~15 μm thick) were made per specimen. The steps between the optical sections were of 1 μm and the z-stacks were made from the top of the specimen and into the depth of ~15 μm. The images were made in the center and towards the two ends of the specimen, always making sure that the distance from the enamel (where present) was at least 1 mm. Enzymatic activity was quantified as the integrated density of the fluorescence signals by means of the ImageJ software (National Institutes of Health, Bethesda, MD, USA) using a rectangular selection (100×20 μm) placed over the hybrid layer and dentin (3 measurements per image). The quantification was performed after discarding several initial slices as to exclude parts of the specimen with the background noise in the signal attributed to the activated gelatin substrate on top of the specimen. After the removal of the initial slices also the differential interference contrast (DIC) image reached maximum focus and this point was considered repetible and ideally suited for the execution of the measurements in all the specimens.

2.4. Statistical analysis

Data sets obtained from bond strength testing and in situ zymography were first validated individually for their normality (Shapiro-Wilk test) and equality of variance (Brown-Forsythe test). For the μTBS test, as data were normally and equally distributed, the three-way analysis of variance (ANOVA) was performed to identify the effects of the 3 independent variables involved in the testing: adhesive systems, dentin desensitizer and aging time. Post-hoc pairwise comparisons were conducted using the Tukey test.

Regarding the in situ zymography results, since data were not normally distributed (Shapiro-Wilk test, $P<0.05$), they were analyzed using

the Kruskal-Wallis test and a Pairwise Multiple Comparison Procedures (Dunn's Method). All analyses were performed using a statistical software (Sigmaplot v.14.0.; StataCorp LLC, College Station, TX, USA) and the statistical significance was preset at $\alpha = 0.05$.

3. Results

3.1. Microtensile bond strength test (μTBS)

Mean microtensile bond strength results and standard deviations of the experimental groups tested at baseline and after 1 year of artificial storage are presented in Table 2. Statistical analysis revealed that dentin pre-treatment ($F = 20.131$; $P<0.001$), the type of adhesive ($F = 100.587$; $P<0.001$) and aging ($F = 25.905$; $P<0.001$) significantly influenced the results . The interaction between the adhesive and aging was also significant ($F = 3.931$; $P = 0.048$). AU showed higher bond strength compared with IBU ($P<0.001$). At baseline, specimens pre-treated with L resulted in higher bond strength compared to G ($P<0.001$) and control group ($P<0.001$). No statistically significant differences were found between G and the control groups ($P = 0.556$). Laboratory aging statistically decreased bonding values, irrespective of the experimental group ($P <0.001$). After 1 year of storage in artificial saliva no differences in bond strength were observed between L and G regardless of the adhesive ($P = 0.001$). However, pre-treating dentin with the two desensitizers resulted in higher bond strength when compared to the controls, independent of the adhesive product used for bonding procedures ($P<0.001$). Table 3 summarizes the percentage distribution of failure modes identified after μTBS test at T_0 and T_{12}. A predominance of CC failures, followed by the A ones was observed in all groups, irrespective of the adhesive and dentin pre-treatment. After storage, the percentage of the A failure mode decreased and the CC failures further increased for pre-treated groups (irrespective of whether it was L or G), while the control groups did not change tendency after aging (independent of the type of adhesive). A certain percentage of CD and M fractures were observed among groups, with an equal distribution independent of the adhesive, dentin pre-treatment and aging.

3.2. Scanning Electron Microscope (SEM) examinations

Representative SEM images at T_0 and T_{12} are shown in Fig. 2 and 3 respectively (magnifications 200x, 2000x and 5000x). SEM analysis showed the presence of smear layer when no dentin pre-treatment was performed (control groups in Figs 2C and G and Figs 3C and G), especially when compared to the experimental groups where the tubules orifices were mostly visible and wide (Figs 2A,B,D,E and Figs 3A,B,D,E). These situations were observed both at baseline (Fig. 2) and after laboratory storage (Fig. 3). Remnants of resin composite bulk were observed in all the experimental groups at baseline showing, however, rough and untight surfaces cautiously indicating mixed failures (Fig. 2). Higher concentration of dentinal tubules with larger amount of impregnated resin tags were observed at T_{12} for AU when compared to IBU, irrespective of the desensitizer used (Fig 3D and E). At the same timepoint, IBU revealed a softer and plastic appearance with sparsely distributed bubble formations when it was used after application of the liquid desensitizer or alone (CTRL group) (Fig. 2A and C, respectively). This conformation was only occasionally present when IBU was used after the application of G (Fig. 3B).

Table 2
Summary of microtensile bond strength results obtained from the six experimental groups immediately (T_0) and after 12 months of laboratory aging (T_{12}).

Densensitizer/Adhesive	T_0 (MPa) †	T_{12} (MPa) †
L/IBU	24.03 ± 7.43 ^{c,A}	24.37 ± 10.70 ^{a,b,A}
G/IBU	23.05 ± 14.26 ^{c,A}	18.57 ± 10.67 ^{b,A}
IBUCTRL	20.59 ± 6.86 ^{c,A}	16.59 ± 7.76 ^{b,A}
L/AU	43.16 ± 10.51 ^{a,A}	30.41 ± 9.55 ^{a,B}
G/AU	32.86 ± 1.63 ^{b,A}	27.54 ± 14.95 ^{a,A}
AUCTRL	31.50 ± 11.27 ^{b,A}	24.51 ± 11.72 ^{a,b,A}

Abbreviations: L – liquid desensitizing agent GLUMA Desensitizer; G – desensitizing agent GLUMA Desensitizer Powergel; IBU –iBond Universal; AU - Adhese Universal; CTRL – Control: no dentin pretreatment.
Different lower-case letters indicate significant differences within the same column ($p < 0.05$).
Different upper-case letters indicate significant differences within the same row ($p < 0.05$).
† Values are means ± standard deviations, in megaPascals (MPa).

Table 3
Failure mode distributions and their percentages identified among the experimental groups after bond strength testing at baseline (T_0) and 1 yr of laboratory storage in artificial saliva (T_{12}).

	Failure mode (%)							
	T_0				T_{12}			
	A	CC	CD	M	A	CC	CD	M
L/IBU	44%	49%	5%	2%	32%	61%	2%	5%
G/IBU	38%	51%	8%	3%	32%	57%	0%	11%
IBUCTRL	21%	64%	8%	8%	22%	63%	9%	6%
L/AU	34%	32%	19%	15%	18%	42%	22%	18%
G/AU	28%	51%	6%	15%	26%	60%	9%	5%
AUCTRL	25%	68%	3%	5%	19%	67%	10%	5%

Failure modes: A – adhesive at the dentin interface; CC - cohesive in composite; CD - cohesive in dentin; M – mixed.

3.3. In situ zymography

Representative confocal images of the tested groups are shown in Figs. 4 and 5 for time T_0 and T_{12}, respectively. The percentages of HLs exhibiting hydrolysis of the quenched fluorescein-conjugated gelatin at T_0 and T_{12} are shown in Fig. 6. The green fluorescence signals at T_0 identified from the AU control group were statistically lower compared to those exhibited by the L/AU group ($P<0,001$), whilst G/IBU showed statistically higher levels of fluorescence than IBU (CTRL) group ($P = 0,022$). In all the tested groups, the fluorescence density level was lower after aging. At T_{12} all the groups tested showed comparable endogenous enzymatic activity irrespective of the adhesive and the desensitizing agent used ($P>0.05$).

4. Discussion

According to our results, better stability of the hybrid layer was observed when the desensitizers were applied compared to the control group, irrespective of the type of adhesive. Therefore, since the type of dentin pre-treatment influenced bond strength results, the first null hypothesis must be rejected.

GLUMA (Kulzer) is a dentin desensitizer composed of glutaraldehyde and hydroxyethil methacrylate (HEMA) that has been used since 1991 with proven effectiveness and efficacy over time [41]. HEMA is a water-soluble monomer present in most current hydrophilic dental adhesives to facilitate resin infiltration in the moist dentin substrate and to facilitate the reaction with dentin collagen due to its ester and hydroxyl groups [42]. Indeed, HEMA was found to decrease surface tension of water molecules and subsequently increase monomer penetration into dentin facilitating the diffusion of the hydrophilic resin monomer into the tubules, and improving the efficacy of the resin-dentin bonds [43]. Previously, products containing glutaraldehyde and HEMA in their formulations have demonstrated the formation of a collagen-glutaraldehyde layer at the dentin/desensitizer interfaces ready to chemically interact with HEMA molecules [32]. Eventually, copolymerization occurs between the adhesive and HEMA complexes increasing the resin-dentin bonding performances [32,29], and this may explain the enhanced experimental groups' bond strength in the present study.

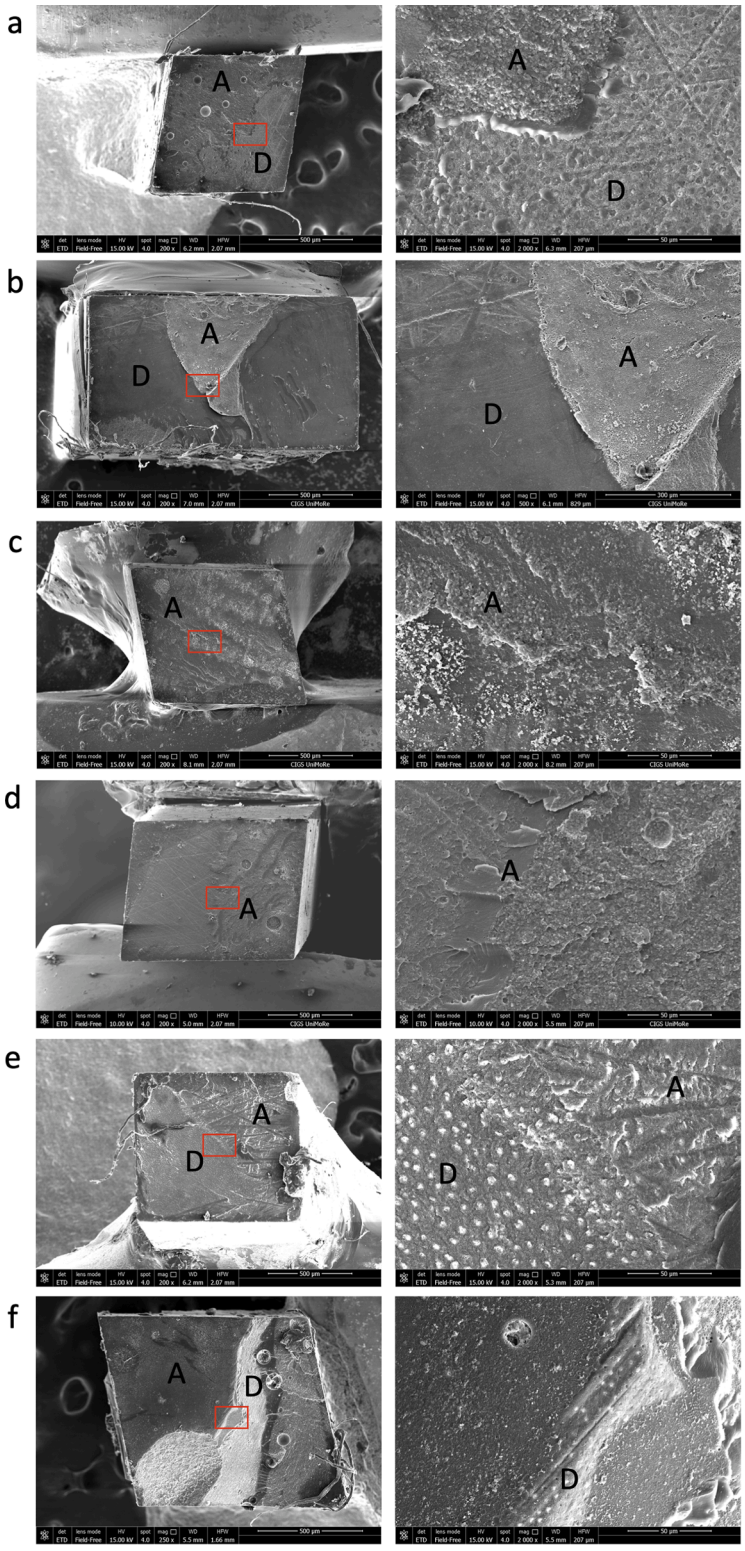

Fig. 2. Representative field emission scanning electron microscope micrographs of the adhesive interfaces of fractured microtensile bond strength sticks (the dentin side) at baseline (T_0): left - view of the whole adhesive surface; right - enlarged view of the area marked with the red selection. Magnification: 200 x, left; 2000 x, right. (a) L/IBU: mixed failure with the presence of remnants of resin composite. (b) G/IBU: mixed debondings with the present of resin composite still attached on the dentin surface. (c) IBUCTRL: mixed failure, the presence of soft-appearance material was noted over the dentin surface. (d)L/AU: this group showed the highest percentage of mixed failures among groups at baseline. (e) G/AU: mixed failure, with the presence of sparsely distributed resin tags occluding dentinal tubules. (f) AUCTRL: mixed failures. *A* = adhesive resin; *D* = dentin.

Regarding the desensitizer used in this study, GLUMA, in both its consistencies, has a pH of ≈3. When it comes to the evaluation of the effect of acidity on smear layer modification, we can cautiously hypothesize that this desensitizer acted similarly to a mild SE adhesive which only superficially/partially demineralized the dentin surface, leaving the hydroxyapatite remnants still available for chemical bonding [44]. In partial support of this statement, the SEM images showed a greater dissolution of the smear layer in the groups treated with the desensitizers, irrespective of the consistency of the product (Fig. 3). It would seem that the application of an acidic universal adhesive resin after GLUMA pretreatment has provided higher smear layer dissolution, dentin interaction and possibly chemical interaction with the underlying dentin substrates. Due to the complex mechanism of bonding to dentin, the latter information can only be assumed, as this was not taken into consideration in our methodology, thus requiring further evaluations and future studies to be performed to compare this

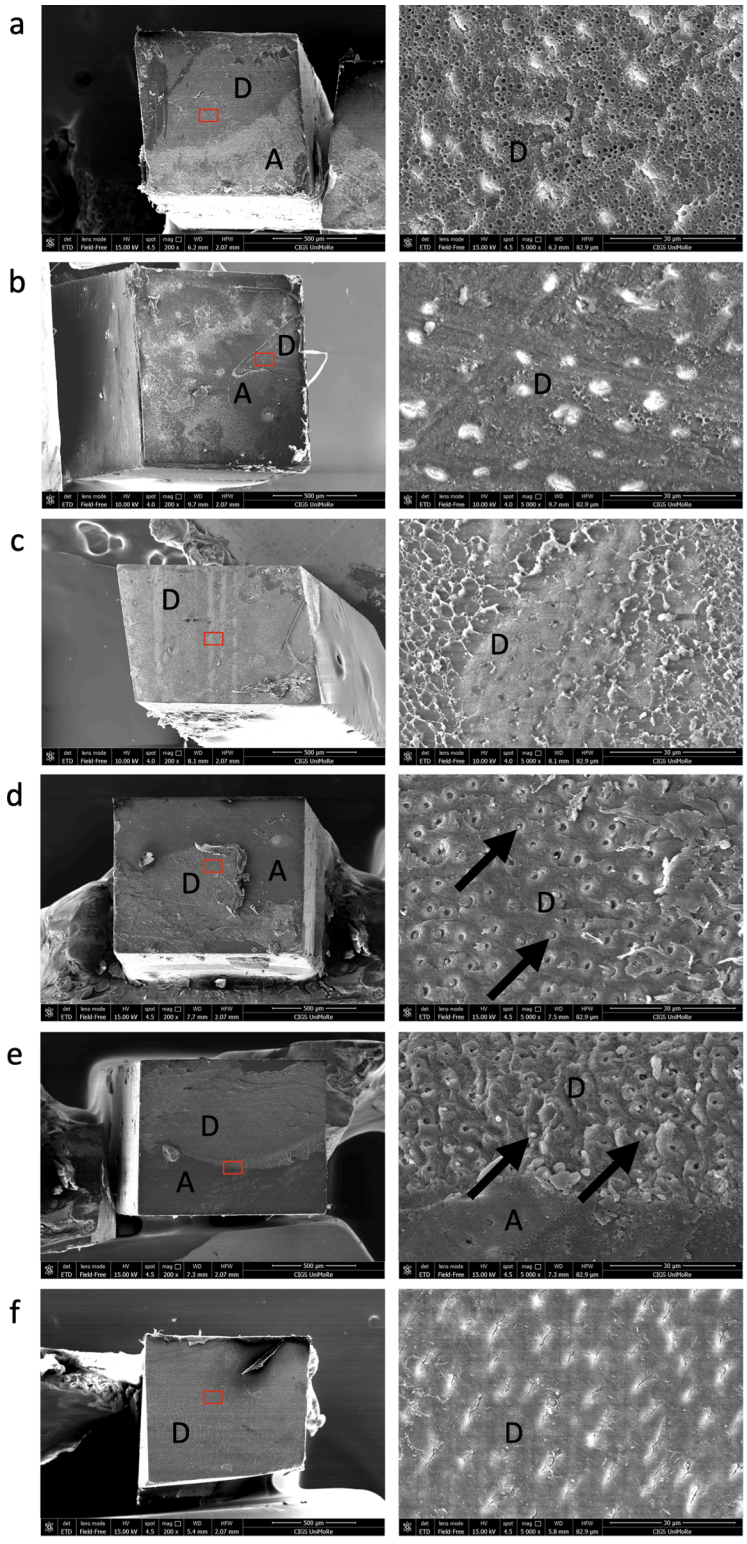

Fig. 3. Representative field emission scanning electron microscope micrographs of the adhesive interfaces of aged (T_{12}) fractured microtensile bond strength sticks (the dentin side): left, view of the whole adhesive surface; right, enlarged view of the area marked with the red selection. Magnification: 200 x, left; 5000 x, right. (a) L/IBU: mixed fracture, with the presence of a porous with internmittent bubbles cross overing the resin. (b) G/IBU: mixed failure with some resin tags and sparsely distributed soft resin remnants. (c) IBUCTRL: mixed fracture with some opened dentinal tubules with no evident resin penetration. The surface was also characterized by the presence of plastic filaments, rendering the surface rough and irregular. (d) L/AU: mixed failure showing exposed dentinal tubules with thin extruding resin tags formations. (e) G/AU: mixed failure with the presence of opened dentinal tubules intermittently occluded by thin and sparse resin impregnation. (f) AUCTRL: mixed failure with the presence of smear layer on the dentin surface occluding the underneath tubule orifices. *A* = adhesive resin; *D* = dentin; Black arrow = resin tags in the dentinal tubule.

potential etching efficacy with other dentin pre-treatment methods, such as phosphoric acid or EDTA [45]. Apart from the possible chemical interaction with the dental substrate, in the presence of open dentinal tubules filled with HEMA, as observed after GLUMA application, resin penetration into the dentin could be improved, leading to higher bond strength compared to the control groups independent of the testing times (Table 2), and this was in accordance with previous studies [51, 104]. It could be suggested that GLUMA could work as an intermediate between an acid etching and demineralized agent, thus providing more than partial smear layer dissolution and aperture of dentinal tubules ready to interact with the subsequent adhesive resin. Thus, the smear layer removal and the resin penetration might have been improved with the subsequent application of the universal adhesives after GLUMA, and this might be another reason explaining the higher results in the bonding strength of the experimental groups [51,104].

When the liquid version of GLUMA was used before adhesive

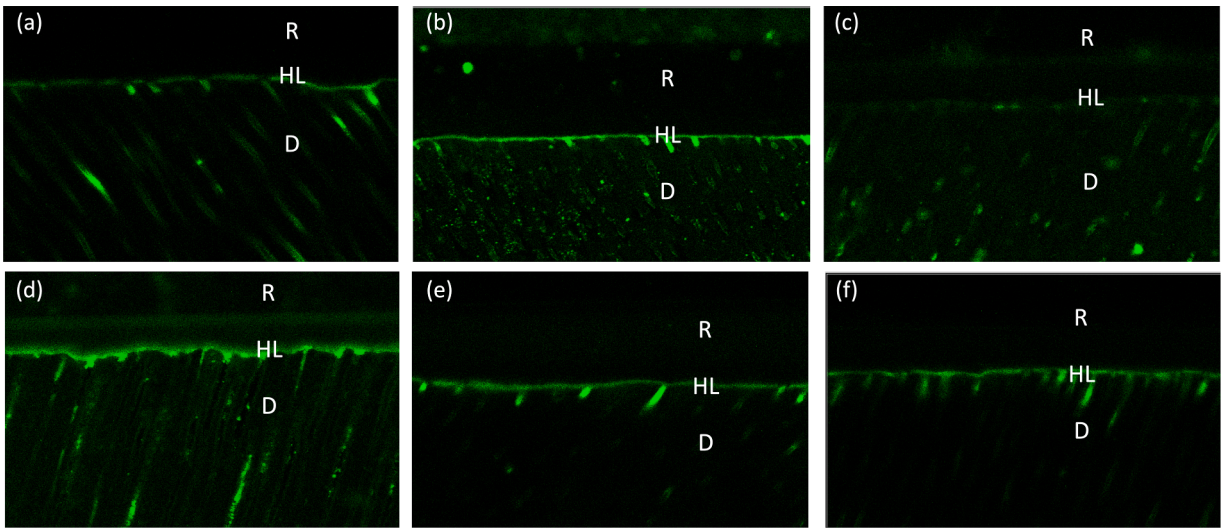

Fig. 4. Resin-bonded mid-coronal dentin interfaces prepared with L/IBU (a) G/IBU (b), IBUCTRL (c), L/AU (d), G/AU (e) and AUCTRL (f) at T_0, incubated with quenched fluorescein-labeled gelatin. All the images were acquired in green channel, showing fluorescence (identifying intense endogenous enzymatic activity) in dentinal tubules and within the HL. *D* = Dentin; HL = Hybrid Layer; *R* = Resin Composite.

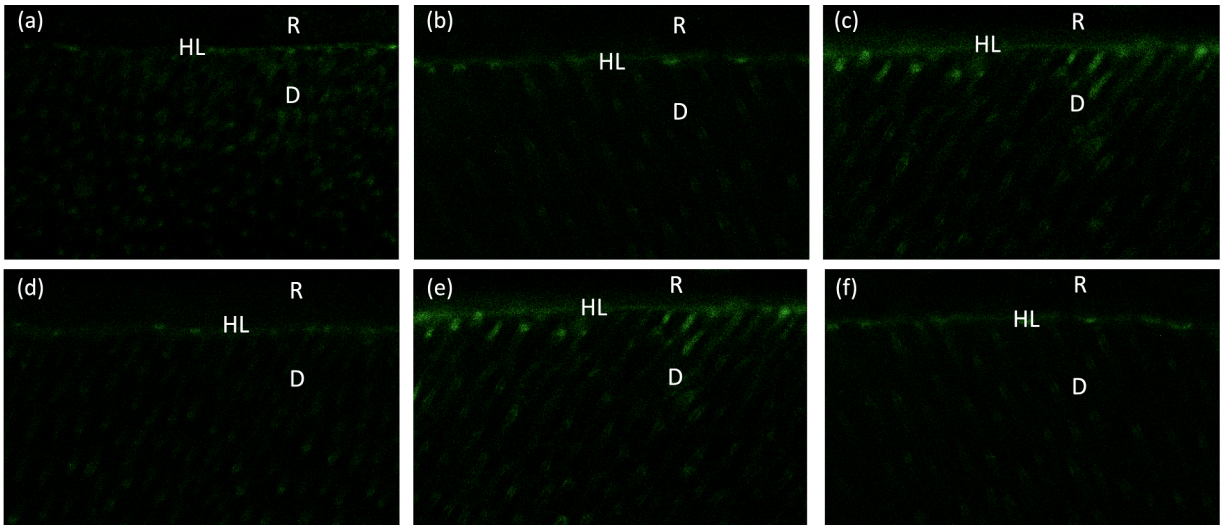

Fig. 5. Resin-bonded mid-coronal dentin interfaces prepared with L/IBU (a) G/IBU (b), IBUCTRL (c), L/AU (d), G/AU (e) and AUCTRL (f) at T_{12}, incubated with quenched fluorescein-labeled gelatin. All the images are acquired in green channel, showing fluorescence (identifying intense endogenous enzymatic activity) in dentinal tubules and within the HL. *D* = Dentin; HL = Hybrid Layer; *R* = Resin Composite.

application, statistically higher bonding values were recorded when compared to gel consistency (Table 2). These results are in accordance with the study of Lee and Sabatini where the two formulations of GLUMA were tested with an etch-and-rinse adhesive [31]. It is worth mentioning that the two forms of the desensitizer, liquid and gel, possess identical formulations in terms of glutaraldehyde and HEMA content, as well as the same application modality (in terms of application time and the removal method). Therefore, it could be speculated that the flowability of the liquid version has enhanced its ability to diffuse among the dentin collagen fibrils and the smear layer compared to the more viscous gel. The gel formulation could be also more difficult to rinse off from the dentin surface, leaving remnants of the material on the surface, possibly hindering the subsequent bonding mechanisms of resin penetration and polymerization.

In this study, the application of the desensitizing agents influenced dentin MMPs activity only at T_0, whilst after 1 year of aging no statistical significance was found among the groups. Therefore, the second null hypothesis is partially rejected.

Glutaraldehyde, in addition to its well-known disinfectant and desensitizing properties, is also widely known as a potent protein cross-linker reacting with the amino groups of proteins within minutes [46]. As previously observed, GLUMA was able to inactivate matrix-bound dentin proteinases in demineralized dentin matrices almost entirely in a short time application (within 30 s of exposure) [36]. The inactivation of MMPs induced by a collagen cross-linker is a non-specific mechanism involving covalent bonds that are claimed to be very stable over time [47]. Studies have shown a significant decrease in MMPs activation after the treatment of demineralized dentin with glutaraldehyde solutions [35,48].

In the present study, however, the application of GLUMA for 30 s prior to the SE adhesive procedures resulted in a statistically significant increase in the MMPs expression in the HL. Differently from the cited studies where the dentin was etched and then only primed with GLUMA, in this research, GLUMA was applied for 30 s on mineralized dentin prior to the universal adhesive application in the SE mode. Thus, the application of GLUMA was not performed on demineralized dentin but on a

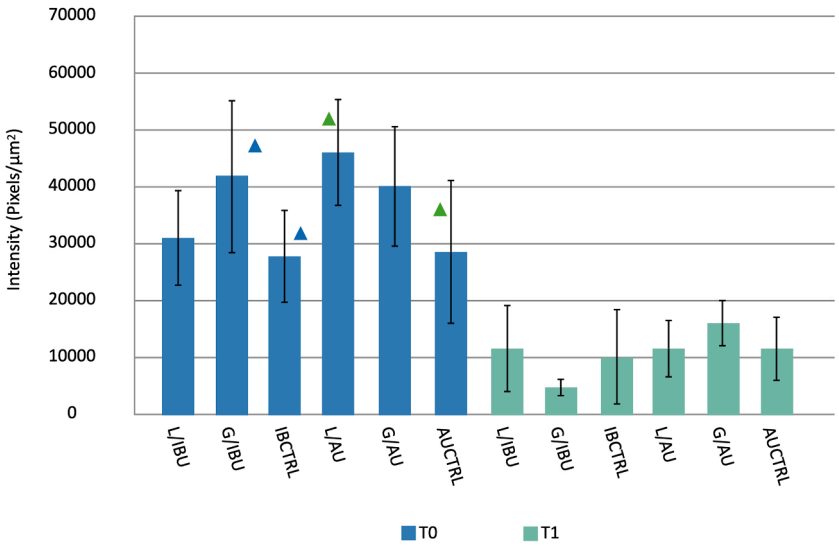

Fig. 6. Quantification (Pixels/um2) of the fluorescence obtained in the different groups at T0 and T12 by means of in situ zymography showing reduced enzymatic activity in all T12 groups irrespective from the adhesive and desensitizing agent used.

mineralized substrates. This should be considered, as Qin et al. [29] stated that the glutaraldehyde present in GLUMA could not form cross-links with mineralized dentin. Indeed, the enzymatic inhibition of glutaraldehyde requires contact with collagen fibrils. Its application before the demineralization procedures could result in the presence of a smear layer preventing contact with the fibrils and prone to nullify the cross-linking ability of GLUMA [33]. Another recent study [49] demonstrated that a cross-linker used before a SE adhesive was not able to inactivate the MMPs, or improve the longevity of the hybrid layer as efficiently as in the case of the etch-and-rinse adhesives.

GLUMA, as reported by the manufacturers, in both its formulation possess a pH of ≈ 3. This mild acidity can activate latent forms of MMPs (pro-MMPs) via the cysteine-switch mechanism [50–52]. The latency of the enzyme is maintained by an unpaired cysteine sulfhydryl group in the propeptide domain, which interacts with the active site zinc ion. Low pH perturbs this cysteine-zinc interaction liberating the active site zinc to bind a water molecule that can then attack the peptide bonds of the protein substrate [52]. Acidic resin monomers may also provide excellent conditions for the activation of the protease's cysteine cathepsins [40,47,53]. This might explain the further activation of MMPs and the consequent activation of the enzymes at T_0.

Aging negatively affected bonding performances and determined a reduction of enzymatic activity in the tested groups. As no significant differences in terms of endogenous enzymatic activity were found irrespective of the adhesive and the desensitizing agent used, this reduction seems to be independent of the influence of the MMPs, and prevalently due to the hydrolytic degradation of the adhesive resin. Both adhesives employed in the study, IBU and AU, contain functional monomers. It has been postulated that the Ca salts created by these functional monomers on the dentin surface may inhibit MMP activity reducing the enzymatic activity over time [54,55], explaining the decrease of the activity of the samples in all groups after 1 y storage in artificial saliva.

As the two adhesives employed in the study have shown bond strength differences but exhibited no differences in terms of enzymatic activity, the third null hypothesis must also be partially rejected. AU performed statistically better than IBU immediately and after aging in terms of bonding performance, irrespective of the dentin pretreatment with the desensitizer. The two adhesives differ in terms of their acidity and composition. IBU has a pH of ≈ 1.4–1.8 and it is classified as a strong universal adhesive while AU is reported to have a pH of around 2.6 and it is classified as a mild universal adhesive. Dentin bond strength of intermediately strong universal adhesives has been indicated as less stable over time [56]. These lower values could be explained by the presence of unpolymerized monomers remaining after light activation, which continue to demineralize the dentin due to their high level of acidity, thus promoting dentin-adhesive interfaces with low hydrolytic stability and low-stability chemical interactions with the collagen [17]. Furthermore, these two systems have different solvents. While AU contains ethanol, IBU contains acetone and water. Acetone-based adhesives are more prone to hydrolytic degradation [57]. In fact, IBU previously demonstrated higher hydrophilicity, resulting in swallowing phenomena and plasticization after polymerization [58,59], as also observed in the present SEM images.

The desensitizers employed in the study, as recommended by the manufactures, should be applied before the adhesive procedure, both in the E&R and SE approaches. Future investigations could explore the effects of the desensitizer application on demineralized or partially demineralized dentin after the etching step, when used in combination with E&R or 2-step SE adhesives. Such approach would aim to enhance collagen fibril interaction and harness the crosslinking potential of the glutaraldehyde compound.

Previous studies have reported potential cytotoxicity of glutheraldehyde when combined with HEMA, as in the GLUMA Desensitizer, particularly when applied on demineralized dentin [60–62]. However, other findings suggest that when applied on 1-mm-thick etched dentin specimens, GLUMA Desensitizer exhibited non-cytotoxic properties [63]. Furthermore, it is essential to consider that the recommended application of these materials is on mineralized dentin, where the smear layer has not been removed. In such cases, dentin permeability towards the pulp chamber is surely reduced. This distinction in the clinical context may make a demarcation between biocompatibility and cytotoxic effects of the GLUMA Desensitizer, reinforcing the importance of case-specific evaluations in dental applications.

It is worth considering that the etching effect induced by SE adhesive and GLUMA could potentially increase the permeability of dentin tubules. This higher permeability might facilitate fluid movement from the pulp to the hybrid layer through the tubules, thereby potentially leading to increased hydrolytic degradation at the bonding interface [52]. In order to gain a more comprehensive understanding of these dynamics, future studies should be conducted by subjecting the specimens to simulated pulpal pressure, also investigating the alterations in dentin permeability subsequent to the application of these materials to further clarifying their effects on the dentin-pulp complex [64,65].

The tested material GLUMA is indicated and has proven its efficacy,

prior to adhesive restorations, in those cases where, due to extended cavity preparation, or proximity with the pulp, postoperative sensibility may develop. Contrarily to other crosslinkers, that require an additional clinical step solely to achieve improved bonding and stability of the hybrid layer, GLUMA could combine in a single step beneficial desensitizing effect and act as a bonding enhancer and stabilizer. This however does not seem to be due to the cross-linking and MMPs inactivation potential of glutaraldehyde, but more likely due to the priming effect these HEMA-containing mildly acidic solutions possess, thus improving the adhesive resin penetration in dentin.

5. Conclusions

Dentin pre-treatment with the GLUMA desensitezers prior to the application of two universal adhesives in the self-etch mode, could increase the bond strength immediately and after laboratory aging. However the stabilization of the adhesive interface seems not to be related to the MMPs activity.

Edoardo Mancuso, Diego Durso, Claudia Mazzitelli, Tatjana Maravic, Uros Josic, Carlo D'alessandro, Luigi Generali, Vittorio Checchi, Lorenzo Breschi, Annalisa Mazzoni.

Credit author statement

Edoardo Mancuso: Conceptualization; Investigation; Writing - Original Draft. **Diego D'urso:** Investigation, Methodology, Writing - Review & Editing. **Claudia Mazzitelli:** Methodology, Writing - Review & Editing. **Tatjana Maravic:** Formal analysis, Writing - Review & Editing. **Uros Josic** Investigation, Writing - Review & Editing. **Carlo D'alessandro:** Software, Writing - Review & Editing. **Luigi Generali:** Methodology, Writing - Review & Editing. **Vittorio Checchi:** Software, Writing - Review & Editing. **Annalisa Mazzoni:** Supervision, Validation, Writing - Review & Editing. **Lorenzo Breschi:** Conceptualization, Resources, Methodology, Project administration, Writing - Original Draft. All authors gave their final approval and agreed to be accountable for all aspects of the work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the University of Modena and Reggio Emilia for using the Leica SP8 microscope and the FEG-SEM NovaNanoSEM at the imaging facility Centro Interdipartimentale Grandi Strumenti (CIGS).

References

[1] E. Mancuso, C. Mazzitelli, T. Maravic, J. Pitta, A. Mengozzi, A. Comba, A. Baldi, N. Scotti, A. Mazzoni, V. Fehmer, I. Sailer, L. Breschi, The influence of finishing lines and margin location on enamel and dentin removal for indirect partial restorations: a micro-CT quantitative evaluation, J. Dent. 127 (2022), https://doi.org/10.1016/J.JDENT.2022.104334.

[2] A. Reis, A. Dourado Loguercio, M. Schroeder, I. Luque-Martinez, D. Masterson, L. Cople Maia, Does the adhesive strategy influence the post-operative sensitivity in adult patients with posterior resin composite restorations?: a systematic review and meta-analysis, Dent. Mater. 31 (2015) 1052–1067, https://doi.org/10.1016/J.DENTAL.2015.06.001.

[3] N. Opdam, A. Feilzer, J. Roeters, I. Smale, Class I occlusal composite resin restorations: in vivo post-operative sensitivity, wall adaptation, and microleakage, Am. J. Dent. 11 (1998) 229–234. Am J Dent. 11 (1988) 229–34.

[4] E. Mancuso, A. Comba, C. Mazzitelli, T. Maravic, U. Josic, F. Del Bianco, F.R. Tay, L. Breschi, A. Mazzoni, Bonding to dentin using an experimental zirconium oxynitrate etchant, J. Dent. 108 (2021), 103641, https://doi.org/10.1016/J.JDENT.2021.103641.

[5] M. Cadenaro, U. Josic, T. Maravić, C. Mazzitelli, G. Marchesi, E. Mancuso, L. Breschi, A. Mazzoni, Progress in dental adhesive materials, J. Dent. Res. 102 (2023), 002203452211456, https://doi.org/10.1177/00220345221145673.

[6] C.M. Marto, A. Baptista Paula, T. Nunes, M. Pimenta, A.M. Abrantes, A.S. Pires, M. Laranjo, A. Coelho, H. Donato, M.F. Botelho, M. Marques Ferreira, E. Carrilho, Evaluation of the efficacy of dentin hypersensitivity treatments-A systematic review and follow-up analysis, J. Oral Rehabil. 46 (2019) 952–990, https://doi.org/10.1111/JOOR.12842.

[7] U. Josic, M. Sebold, R. Lins, J. Savovic, C. Mazzitelli, T. Maravic, A. Mazzoni, L. Breschi, Does immediate dentin sealing influence postoperative sensitivity in teeth restored with indirect restorations? A systematic review and meta-analysis, J. Esthet. Restor. Dent. 34 (2022) 55–64.

[8] U. Josic, C. Mazzitelli, T. Maravic, I. Radovic, J. Jacimovic, E. Mancuso, F. Florenzano, L. Breschi, A. Mazzoni, The influence of selective enamel etch and self-etch mode of universal adhesives' application on clinical behavior of composite restorations placed on non-carious cervical lesions: a systematic review and meta-analysis, Dent. Mater. 38 (2022) 472–488, https://doi.org/10.1016/J.DENTAL.2022.01.002.

[9] G. Berkowitz, H. Spielman, A. Matthews, D. Vena, R. Craig, F. Curro, V. Thompson, Postoperative hypersensitivity and its relationship to preparation variables in class i resin-based composite restorations: findings from the Practitioners Engaged in Applied Research and Learning (PEARL) network. Part 1, Compend. Contin. Educ. Dent. 34 (2013) e44, https://doi.org/10.12816/0010811.

[10] A.O. Lopes, C. de Paula Eduardo, A.C.C. Aranha, Evaluation of different treatment protocols for dentin hypersensitivity: an 18-month randomized clinical trial, Lasers Med. Sci. 32 (2017) 1023–1030, https://doi.org/10.1007/S10103-017-2203-0.

[11] S. Sauro, T.F. Watson, F. Mannocci, K. Miyake, B.P. Huffman, F.R. Tay, D. H. Pashley, Two-photon laser confocal microscopy of micropermeability of resin-dentin bonds made with water or ethanol wet bonding, J. Biomed. Mater. Res. B Appl. Biomater. 90 B (2009), https://doi.org/10.1002/jbm.b.31290.

[12] C.A.G. Arrais, D.C.N. Chan, M. Giannini, Effects of desensitizing agents on dentinal tubule occlusion, J. Appl. Oral Sci. 12 (2004) 144–148, https://doi.org/10.1590/S1678-77572004000200012.

[13] Z. Ergücü, K.A. Hiller, G. Schmalz, Influence of dentin on the effectiveness of antibacterial agents, J. Endod. 31 (2005) 124–129, https://doi.org/10.1097/01.DON.0000137640.77772.40.

[14] C. Prati, D.H. Pashley, Dentin wetness, permeability and thickness and bond strength of adhesive systems, Am. J. Dent. 5 (1992) 33–38.

[15] L. Breschi, T. Maravic, S.R. Cunha, A. Comba, M. Cadenaro, L. Tjäderhane, D. H. Pashley, F.R. Tay, A. Mazzoni, Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications, Dental Mater. 34 (2018) 78–96, https://doi.org/10.1016/j.dental.2017.11.005.

[16] D. Cobb, J. Reinhardt, M. Vargas, Effect of HEMA-containing dentin desensitizers on shear bond strength of a resin cement, Am. J. Dent. 10 (1997) 62–65.

[17] B. Van Meerbeek, K. Yoshihara, Y. Yoshida, A. Mine, J. De Munck, K.L. Van Landuyt, State of the art of self-etch adhesives, Dental Mater. 27 (2011) 17–28, https://doi.org/10.1016/j.dental.2010.10.023.

[18] L. He, C. Mu, J. Shi, Q. Zhang, B. Shi, W. Lin, Modification of collagen with a natural cross-linker, procyanidin, Int. J. Biol. Macromol. 48 (2011), https://doi.org/10.1016/j.ijbiomac.2010.12.012.

[19] A.K.B. Bedran-Russo, P.N.R. Pereira, W.R. Duarte, J.L. Drummond, M. Yamaychi, Application of crosslinkers to dentin collagen enhances the ultimate tensile strength, J. Biomed. Mater. Res. B Appl. Biomater. 80 (2007) 268–272, https://doi.org/10.1002/JBM.B.30593.

[20] A.K.B. Bedran-Russo, K.J. Yoo, K.C. Ema, D.H. Pashley, Mechanical properties of tannic-acid-treated dentin matrix, J. Dent. Res. 88 (2009) 807–811, https://doi.org/10.1177/0022034509342556.

[21] T. Maravić, E. Baena, C. Mazzitelli, U. Josić, E. Mancuso, V. Checchi, L. Generali, L. Ceballos, L. Breschi, A. Mazzoni, Endogenous enzymatic activity in dentin treated with a chitosan primer, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/IJMS22168852.

[22] A. Comba, T. Maravić, V. Villalta, S. Tozzola, C. Mazzitelli, V. Checchi, E. Mancuso, N. Scotti, F.R. Tay, L. Breschi, A. Mazzoni, Effect of an ethanol cross-linker on universal adhesive, Dental Mater. 36 (2020) 1645–1654, https://doi.org/10.1016/j.dental.2020.10.004.

[23] A. Comba, N. Scotti, A. Mazzoni, T. Maravic, S. Ribeiro Cunha, R. Michelotto Tempesta, M. Carossa, D.H. Pashley, F.R. Tay, L. Breschi, Carbodiimide inactivation of matrix metalloproteinases in radicular dentine, J. Dent. 82 (2019) 56–62, https://doi.org/10.1016/j.jdent.2019.01.007.

[24] D. Cheung, N. Perelman, E. Ko, M. Nimni, Mechanism of crosslinking of proteins by glutaraldehyde III: reaction with collagen in tissues, Connect. Tissue Res. 13 (1985) 109–115.

[25] L. Breschi, T. Maravic, A. Comba, S.R. Cunha, A.D. Loguercio, A. Reis, V. Hass, M. Cadenaro, E. Mancuso, E. Mayer-Santos, L. Niu, D.H. Pashley, F.R. Tay, A. Mazzoni, Chlorhexidine preserves the hybrid layer in vitro after 10-years aging, Dental Mater. 36 (2020) 672–680, https://doi.org/10.1016/j.dental.2020.03.009.

[26] T.D. Larson, Clinical uses of glutaraldehyde/2-hydroxyethylmethacrylate (GLUMA), Northwest Dent. 92 (2013) 27–30.

[27] G.E.H.M. Dijkman, W.L. Jongebloed, J. de Vries, B. Ögaard, J. Arends, Closing of dentinal tubules by glutardialdehyde treatment, a scanning electron microscopy study, Scand. J. Dent. Res. 102 (1994) 144–150, https://doi.org/10.1111/J.1600-0722.1994.TB01170.X.

[28] G. Dondi dall'Orologio, A. Lone, W. Finger, Clinical evaluation of the role of glutardialdehyde in a one-bottle adhesive, Am. J. Dent. 15 (2002) 330–334.

[29] C. Qin, J. Xu, Y. Zhang, Spectroscopic investigation of the function of aqueous 2-hydroxyethylmethacrylate/glutaraldehyde solution as a dentin desensitizer, Eur. J.

Oral Sci. 114 (2006) 354–359, https://doi.org/10.1111/J.1600-0722.2006.00382.X.
[30] N. Gupta, U.N. Reddy, P. Leela Vasundhar, K. Sita Ramarao, K.V.V. Pratap Varma, V. Vinod, Effectiveness of desensitizing agents in relieving the pre- and postcementation sensitivity for full coverage restorations: a clinical evaluation, J. Contemp. Dent. Pract. 14 (2013) 858–865, https://doi.org/10.5005/JP-JOURNALS-10024-1416.
[31] J. Lee, C. Sabatini, Glutaraldehyde collagen cross-linking stabilizes resin-dentin interfaces and reduces bond degradation, Eur. J. Oral Sci. 125 (2017) 63–71, https://doi.org/10.1111/EOS.12317.
[32] E.C. Munksgaard, E. Asmussen, Bond strength between dentin and restorative resins mediated by mixtures of HEMA and glutaraldehyde, J. Dent. Res. 63 (1984), https://doi.org/10.1177/00220345840630081701.
[33] P. Schüpbach, F. Lutz, W.J. Finger, Closing of dentinal tubules by Gluma desensitizer, Eur. J. Oral Sci. 105 (1997) 414–421, https://doi.org/10.1111/J.1600-0722.1997.TB02138.X.
[34] I.L.M. de Oliveira, T.A. Hanzen, A.M. de Paula, J. Perdigão, M.A.J.R. Montes, A. D. Loguercio, G.Q. de, M. Monteiro, Postoperative sensitivity in posterior resin composite restorations with prior application of a glutaraldehyde-based desensitizing solution: a randomized clinical trial, J. Dent. 117 (2022), https://doi.org/10.1016/J.JDENT.2021.103918.
[35] V. Hass, I.V. Luque-Martinez, M.F. Gutierrez, C.G. Moreira, V.B. Gotti, V.P. Feitosa, G. Koller, M.F. Otuki, A.D. Loguercio, A. Reis, Collagen cross-linkers on dentin bonding: stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition, Dent. Mater. 32 (2016) 732–741, https://doi.org/10.1016/J.DENTAL.2016.03.008.
[36] C. Sabatini, D.L.S. Scheffel, R.H. Scheffel, K.A. Agee, K. Rouch, M. Takahashi, L. Breschi, A. Mazzoni, L. Tjäderhane, F.R. Tay, D.H. Pashley, Inhibition of endogenous human dentin MMPs by Gluma, Dental Mater. 30 (2014), https://doi.org/10.1016/j.dental.2014.04.006.
[37] I. Sailer, A.E.H. Oendra, B. Stawarczyk, C.H.F. Hämmerle, The effects of desensitizing resin, resin sealing, and provisional cement on the bond strength of dentin luted with self-adhesive and conventional resincements, J. Prosthet. Dent. 107 (2012) 252–260, https://doi.org/10.1016/S0022-3913(12)60070-5.
[38] A.C.C. Aranha, A.D.S. Siqueira Junior, L.M.A. Cavalcante, L.A.F. Pimenta, G. M. Marchi, Microtensile bond strengths of composite to dentin treated with desensitizer products, J. Adhes. Dent. 8 (2006) 85–90.
[39] P. Maciel Pires, A. Dávila-Sánchez, V. Faus-Matoses, J.M. Nuñez Martí, L. Lo Muzio, S. Sauro, Bonding performance and ultramorphology of the resin-dentine interface of contemporary universal adhesives, Clin. Oral Investig. 26 (2022) 4391–4405, https://doi.org/10.1007/s00784-022-04402-3.
[40] A. Mazzoni, P. Scaffa, M. Carrilho, L. Tjäderhane, R. Di Lenarda, A. Polimeni, A. Tezvergil-Mutluay, F.R. Tay, D.H. Pashley, L. Breschi, Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9, J. Dent. Res. 92 (2013) 82–86, https://doi.org/10.1177/0022034512467034.
[41] D.A. Felton, G. Bergenholtz, B.E. Kanoy, Evaluation of the desensitizing effect of gluma dentin bond on teeth prepared for complete-coverage restorations, Int. J. Prosthodont. 4 (1991) 292–298.
[42] J. Xu, I. Stangel, I.S. Butler, D.F.R. Gilson, An FT-Raman spectroscopic investigation of dentin and collagen surfaces modified by 2-hydroxyethylmethacrylate, J. Dent. Res. 76 (1997), https://doi.org/10.1177/00220345970760011101.
[43] N. Nakabayashi, A. Watanabe, N.J. Gendusa, Dentin adhesion of "modified" 4-META/MMA-TBB resin: function of HEMA, Dental Mater. 8 (1992), https://doi.org/10.1016/0109-5641(92)90096-U.
[44] M. Giannini, P. Makishi, A.P.A. Ayres, P.M. Vermelho, B.M. Fronza, T. Nikaido, J. Tagami, Self-Etch adhesive systems: a literature review, Braz. Dent. J. 26 (2015), https://doi.org/10.1590/0103-6440201302442.
[45] C. Mazzitelli, F. Monticelli, M. Toledano, M. Ferrari, R. Osorio, Dentin treatment effects on the bonding performance of self-adhesive resin cements, Eur. J. Oral Sci. 118 (2010) 80–86, https://doi.org/10.1111/J.1600-0722.2009.00703.X.
[46] J.A. Kiernan, Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do, Micros. Today 8 (2000), https://doi.org/10.1017/s1551929500057060.
[47] I.L. Tersariol, S. Geraldeli, C.L. Minciotti, F.D. Nascimento, V. Pääkkönen, M. T. Martins, M.R. Carrilho, D.H. Pashley, F.R. Tay, T. Salo, L. Tjäderhane, Cysteine cathepsins in human dentin-pulp complex, J. Endod. 36 (2010), https://doi.org/10.1016/j.joen.2009.12.034.
[48] C. Chen, C. Mao, J. Sun, Y. Chen, W. Wang, H. Pan, R. Tang, X. Gu, Glutaraldehyde-induced remineralization improves the mechanical properties and biostability of dentin collagen, Mater. Sci. Eng. C 67 (2016), https://doi.org/10.1016/j.msec.2016.05.076.
[49] A. Mazzoni, V. Angeloni, A. Comba, T. Maravic, M. Cadenaro, A. Tezvergil-Mutluay, D.H. Pashley, F.R. Tay, L. Breschi, Cross-linking effect on dentin bond strength and MMPs activity, Dental Mater. 34 (2018) 288–295, https://doi.org/10.1016/j.dental.2017.11.009.
[50] E.L. Davis, G. Wieczkowski, X.Y. Yu, R.B. Joynt, J. Gallo, Adhesion of dentin bonding agents after smear layer treatments, Am. J. Dent. 5 (1992).
[51] L. Tjäderhane, H. Larjava, T. Sorsa, V.J. Uitto, M. Larmas, T. Salo, The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions, J. Dent. Res. 77 (1998), https://doi.org/10.1177/00220345980770081001.
[52] C. Chaussain-Miller, F. Fioretti, M. Goldberg, S. Menashi, The role of matrix metalloproteinases (MMPs) in human caries, J. Dent. Res. 85 (2006), https://doi.org/10.1177/154405910608500104.
[53] C. Tallant, A. Marrero, F.X. Gomis-Rüth, Matrix metalloproteinases: fold and function of their catalytic domains, Biochim. Biophys. Acta Mol. Cell Res. (2010) 1803, https://doi.org/10.1016/j.bbamcr.2009.04.003.
[54] J. Shen, H. Xie, Q. Wang, X. Wu, J. Yang, C. Chen, Evaluation of the interaction of chlorhexidine and MDP and its effects on the durability of dentin bonding, Dental Mater. 36 (2020), https://doi.org/10.1016/j.dental.2020.10.006.
[55] X. Yuan, Q. Wang, Q. Zhao, Z. Bai, C. Chen, H. Xie, Inhibition of matrix metalloproteinase-9 by 10-MDP and its calcium salt contributes to improved dentin-bonding durability, Int. J. Adhes. Adhes. 120 (2023), 103302, https://doi.org/10.1016/J.IJADHADH.2022.103302.
[56] C.E. Cuevas-Suárez, W.L.O. da Rosa, R.G. Lund, A.F. da Silva, E. Piva, Bonding performance of universal adhesives: an updated systematic review and meta-analysis, J. Adhes. Dent. 21 (2019), https://doi.org/10.3290/j.jad.a41975.
[57] L. Tjäderhane, F.D. Nascimento, L. Breschi, A. Mazzoni, I.L.S. Tersariol, S. Geraldeli, A. Tezvergil-Mutluay, M. Carrilho, R.M. Carvalho, F.R. Tay, D. H. Pashley, Strategies to prevent hydrolytic degradation of the hybrid layer—A review, Dental Mater. 29 (2013) 999–1011, https://doi.org/10.1016/j.dental.2013.07.016.
[58] C. Mazzitelli, T. Maravic, E. Mancuso, U. Josic, L. Generali, A. Comba, A. Mazzoni, L. Breschi, Influence of the activation mode on long-term bond strength and endogenous enzymatic activity of dual-cure resin cements, Clin. Oral Investig. 26 (2022), https://doi.org/10.1007/s00784-021-04141-x.
[59] C. Mazzitelli, T. Maravic, M. Sebold, V. Checchi, U. Josic, L. Breschi, A. Mazzoni, Effect of shelf-life of a universal adhesive to dentin, Int. J. Adhes. Adhes. 102 (2020), https://doi.org/10.1016/j.ijadhadh.2020.102673.
[60] D.L.S. Scheffel, L. Bianchi, D.G. Soares, F.G. Basso, C. Sabatini, C.A.D.S. Costa, D. H. Pashley, J. Hebling, Transdentinal cytotoxicity of carbodiimide (EDC) and glutaraldehyde on odontoblast-like cells, Oper. Dent. (2015) 40, https://doi.org/10.2341/13-338-L.
[61] G.B. Eyüboğlu, C. Yeşilyurt, M. Ertürk, Evaluation of cytotoxicity of dentin desensitizing products, Oper. Dent. (2015) 40, https://doi.org/10.2341/13-334-L.
[62] R. Jiang, Y. Xu, F. Wang, H. Lin, Effectiveness and cytotoxicity of two desensitizing agents: a dentin permeability measurement and dentin barrier testing in vitro study, BMC Oral Health 22 (2022), https://doi.org/10.1186/s12903-022-02424-7.
[63] A. Wiegand, K. Buchholz, C. Werner, T. Attin, In vitro cytotoxicity of different desensitizers under simulated pulpal flow conditions, J. Adhes. Dent. 10 (2008).
[64] M. Cadenaro, F. Antoniolli, S. Sauro, F.R. Tay, R. Di Lenarda, C. Prati, M. Biasotto, L. Contardo, L. Breschi, Degree of conversion and permeability of dental adhesives, Eur. J. Oral Sci. 113 (2005) 525–530, https://doi.org/10.1111/j.1600-0722.2005.00251.x.
[65] L. Breschi, M. Cadenaro, F. Antoniolli, S. Sauro, M. Biasotto, C. Prati, F.R. Tay, R. Di Lenarda, Polymerization kinetics of dental adhesives cured with LED: correlation between extent of conversion and permeability, Dental Mater. 23 (2007), https://doi.org/10.1016/j.dental.2006.06.040.