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An in-vitro investigation of the bond strength of experimental ion-releasing dental adhesives to caries-affected dentine after 1 year of water storage.

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Short title: Conventional and experimental adhesives bonded to caries-affected dentine

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Keywords: interface degradation, selective caries removal, caries-affected dentine, ion-releasing adhesives, bioglass, phosphoproteins.

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ABSTRACT

Objective. The aim of this study was to evaluate the bonding performance after 1 year storage of an experimental dental adhesive containing analogues of phosphoproteins and fluoride-doped bioglass (EXP), applied in self-etching (SE) or etch & rinse (ER) mode, to caries-affected dentine after selective caries removal.

Materials and Methods. Fifty human molars with dentine carious lesions were excavated selectively using Carisolv™ gel and then connected to simulated pulpal pressure system. Teeth were divided randomly into five groups based on the tested materials: EXP-SE, EXP-ER, a resin-modified glass-ionomer cement (RMGIC), a three-step adhesive system (OPT) and a universal adhesive applied in SE mode (UA-SE). The specimens were submitted to different analytical tests (μ TBS, SEM fractographic analysis and dye-enhanced confocal microscopy) at baseline (T0) and after 1 year (T1).

Results. At T0 there was no difference in bond strength between the tested materials ($p>0.05$). At T1, EXP-SE and EXP-ER were the only materials to show no significant reduction in bond strength ($p<0.05$). The SEM showed a clear presence of minerals deposited on the dentine surface after bonding in the EXP-SE and EXP-ER groups. The specimens in the RMGIC group failed primarily cohesively with no exposure of the dentine surface. The OPT and UA-SE specimens showed clear signs of degradation at the interface. Confocal microscopy imaged mineral precipitation at the interface of the EXP groups.

Conclusion. After selective carious dentine removal, conventional adhesive systems may not maintain a long-lasting bond strength *in vitro*.

Clinical significance. The innovative ion-releasing adhesives tested in this study may represent an appropriate strategy to remineralise the adhesive interface and achieve a stable bond over time to caries-affected, demineralised dentine.

1. INTRODUCTION

Although significant progress in prevention and treatment of dental caries has been made in the last three decades, the replacement of resin composite restorations due to interface degradation and secondary caries remains one of the largest clinical priorities in dentistry [1,2]. Therefore, improved restorative techniques and materials with reduced failure rates, which increase the longevity of the tooth-restoration complex, are needed. It has been reported that the formation of a high-quality hybrid layer between the dental adhesive and dentine has been linked to the durability of resin composite restorations in function [3] and the type adhesive system employed and the protection of the collagen over time representing key determinants for success [4]. Moreover, failure of the resin-dentine interface is associated clinically to secondary caries [caries associated with restoration and sealants (CARS)] and restoration fracture [2]. Excessive exposure of dentine collagen during the etching and bonding process is responsible for the activation of natural proteases such as metalloproteinases' cysteine cathepsin, which cause the degradation of the hybrid layer over time [5].

Minimally invasive operative intervention of cavitated deep carious lesions involves the selective removal of the heavily bacterially contaminated superficial caries-infected dentine caries with retention of the deeper caries-affected dentine close to the vital pulp. This cavity can then receive a restoration using biocompatible ion-releasing, bio-interactive materials. Unfortunately, most of the contemporary restorative techniques using resin-based materials (*e.g.* adhesives and resin composites) are not capable of remineralising and/or protecting the mineral-depleted dentine that is often present at the tooth-restoration interface [6–8]. Conversely, with the advent of modern “bio-interactive” materials, it may be possible to replace dental hard tissues and reduce the

susceptibility of tooth mineral to dissolution and/or to recover part of their mechanical properties *via* remineralisation [9].

Currently, there is still no adhesive restorative material capable of remineralising the hybrid layer (HL), fully restoring the modulus of elasticity of the underlying dental collagen [10]. Moreover, it has been reported that the bond strength of resin composite to caries-affected dentine (CAD) is generally lower (20–50%) than the bond strength achieved when bonding directly to sound dentine (SD) [11–14]. This is a consequence of the fact that caries-affected dentine has a reduced mineral content, so increasing porosities, structural alteration of the collagen, non-collagenous proteins and water content [15,16]. The hybrid layer created within CAD is often thicker and poorly resin infiltrated, regardless of the adhesive system employed during the restorative procedure. All these aspects directly affect the strength and durability of the resin–dentine interface [12]. Conversely, it has been recently demonstrated that simulated-caries-affected dentine can be completely remineralised in presence of ion-releasing materials and biomimetic analogues of matrix phosphoprotein such as a low molecular weight polyacrylic acid (PAA) and sodium trimetaphosphate (TPM). Indeed, PAA is able to stabilise $\text{Ca}^{2+}/\text{PO}_4^{3-}$ into liquid-like nano-precursors of metastable amorphous calcium phosphates, which can penetrate within collagen fibrils. However, their remineralisation occurs at an intra-fibrillar and extra-fibrillar level thanks to the chemical phosphorylation attained in presence of TPM; this also fossilise dentine proteases so reducing the enzymatic degradation of demineralised fibrils [19,20].

However, there is little information on the effects that biomimetic analogues of matrix phosphoprotein such as PAA and TPM may have on the immediate and long-term bonding performance of bioglass-doped adhesives applied on natural caries-affected

dentine. Thus, the aim of this study *in vitro* study was to evaluate the adhesive performance after one year of storage of an experimental bonding system containing biomimetic analogues (PAA and TMP) of phosphoproteins and fluoride-doped bioglass (EXP). The experimental adhesive was applied in self-etch (SE) or total-etch (ER) modes on caries-affected dentine after selective caries removal and compared to two commercial adhesives and a resin-modified glass-ionomer cement (RMGIC). Moreover, fractographic analysis was also performed using FE-SEM, as well as ultra-morphology analysis of the adhesive interface through dye-enhanced confocal microscopy.

The hypothesis tested in this study was that the experimental adhesive applied in ER and SE mode after selective caries removal would preserve the bond strength after 1 year storage compared to the commercial products used as control groups in this study.

2. MATERIALS AND METHODS

2.1. Project Overview And Experimental Design

Fifty human molars with natural cavitated deep carious lesions, but with no involvement of the pulp (Pre-selection was performed through a clinical dental Xray system) were collected according to the guidelines of the local Ethics Committee and under an institutional approved protocol number (H1537947515352) and after obtaining a signed consent agreement from donors. The teeth were stored in 1 wt% thymol/water solution at 5°C for no longer than 3 months. The teeth were then divided randomly into five experimental groups (n= 10/group) based on the different materials tested and each group was divided in subgroups (n= 5/group) based on the time of storage under pulpal pressure while immersed in artificial saliva (T0: 24h or T1: 1 year). In group "OPT" a commercial, three-step adhesive (Optibond FL, KERR, Bioggio, Switzerland) was used in

etch & rinse mode, while in group “UA-SE” the teeth were bonded using a universal adhesive (Prime & Bond Active, Dentsply Sirona, Konstanz, Germany), applied in SE mode. In group “RMGIC”, the teeth were restored using a resin-modified glass-ionomer cement (RIVA LC, SDI, Victoria, Australia). In group “EXP-SE” and group “EXP-ER” the specimens were bonded using the experimental adhesive applied in self-etching (SE) or in etch & rinse (ER) mode, respectively. All the information about the materials tested in this study, along with application mode, are depicted in Table 1. The specimens were submitted to different tests (μ TBS, SEM fractographic analysis and dye-enhanced confocal microscopy examination of the adhesive interface) at baseline (T0) and after 1 year (T1) under simulated pulpal pressure (deionised water) and immersed in DPBS (Dulbecco's Phosphate Buffered Saline, Merck Life Science SLU, Madrid, Spain); this solution was replaced every 15 days. A graphical representation of the experimental design is shown in Figure 1.

2.2. Specimen Preparation

The roots were removed under continuous water cooling 1 mm beneath the cemento–enamel junction using a diamond-embedded saw mounted on a low-speed microtome (Remet evolution, REMET, Bologna, Italy). Pulp tissue was removed from the exposed pulp chamber without altering the pre-dentine surface using tiny tweezers. A calliper was used to measure the remaining dentine thickness (RDT) that was between 0.5 and 0.7 mm. Each tooth was connected to a hydraulic pressure device (Figure 1) that delivered 20 cm water pressure [21].

An air turbine handpiece equipped with a round diamond bur (801-012, Intensive, Switzerland) was used to remove superficial demineralised enamel margins surrounding

the cavity and expose the entire lesion for subsequent selective dentine caries removal. Manual excavation of caries-infected dentine was performed using chemo-mechanical Carisolv™ gel (RLS Global AB, Mölndal, Sweden) [22]. This consists in two carboxymethylcellulose-based gels, one containing 0.1 M amino acids (glutamic acid, leucine and lysine), NaCl, NaOH, erythrosine (added in order to make the gel visible during use) and a second containing sodium hypochlorite (NaOCl — 0.5% w/v). The two were thoroughly mixed in equal parts at room temperature before use and then applied onto the exposed carious dentine and left undisturbed for 60 seconds. The softened infected dentine was removed a manual blunt excavator (RLS Global AB) avoiding direct exposure of the pulp chamber, retaining leathery caries-affected dentine (CAD) overlying the pulp [23–26].

Once completed, adhesions were performed as depicted in table 1 (see below) and the cavities restored using 4-mm increments of bulk-fill resin composite (SDR, Dentsply Sirona, Germany), excluding those in the group RMGIC, which were restored in bulk using the RMGIC RIVA LC (SDI, Australia) as per manufacturer's instructions.

2.3. Experimental Materials and Adhesive Procedures

After performing several pilot tests on stability and shelf-life, we formulated an experimental adhesive in three different parts: a dentine conditioner (45 %wt deionised water, 45 %wt absolute ethanol, 5 % wt polyacrylic acid (PAA, Mw 1800) and 5 %wt trimetaphosphate (TMP), pH 4.6. [10]); a self-etching primer (20 wt% glycerol-dimethacrylate-phosphate (GDMA-P, Yllor Biomateriais, Pelotas - RS, Brazil), 10 wt% hydroxyethyl-methacrylate (HEMA), 15 wt% urethane-dimethacrylate (UDMA), 5 wt% triethylene glycol dimethacrylate (TEGDMA), 20 wt% deionised water and 30 wt%

absolute ethanol) with pH adjusted to 2.1 using NaOH; a flowable resin bond [30 wt% UDMA, 25 wt% TEGDMA, 20 wt% GDMA-P, 17 wt% ethoxylated bisphenol-A-diglycidyl-dimethacrylate (Bis-EMA), 5 wt% bisphenol-AG, diglycidyl-dimethacrylate (Bis-GMA), 1 wt% ethyl 4-dimethylamine benzoate, 0.5 wt% camphorquinone and 1.5 wt% diphenylodonium hexafluorophosphate (20)]. Most of the monomers (excluding the GDMA-P), solvents and initiators/co-initiators were purchased from Merck Life Science SLU, Madrid, Spain. The flowable resin bond was mixed with 50 wt% microfilled (<30 μm) fluoride-doped bioglass (BAG-F), which was prepared as previously described by Tezvergil et al. [27]. The nominal composition of the bioglass used in this study was 42.7 mol% SiO_2 26.2 mol% CaO 26.1 mol% Na_2O 4.0 mol% P_2O_5 and 1 mol% CaF_2 [27].

The conditioner was applied on the CAD for 20 s followed by air-drying for 3s. The experimental self-etching primer was then brushed using a micro-pellet for 10s, and the excess was air-dried for 5s to allow solvent/water evaporation. A second coat of primer was utilised for 10 s and again air-dried for 5 s. Finally, the flowable resin bond was vigorously agitated on the dentine surface, air-dried for 5s to remove excess and light-cured for 30 s using a LED light source ($>1000 \text{ mW/cm}^2$) (Radii plus, SID Ltd., Bayswater VIC, Australia).

2.4. Micro-Tensile Bond Strength (μTBS) & Failure/Fractographic Analysis (FE-SEM)

After storage (T0 or T1), all specimens were sectioned using a hard-tissue microtome (Remet evolution, REMET, Bologna, Italy) in both X and Y planes across the resin-dentine interface, obtaining match-stick shaped specimens from each tooth (cross-sectional areas $\varnothing 0.9\text{mm}^2$; n= 7-12 from each tooth). These were submitted to a microtensile bond strength device, Luers LAM Technologies LMT 100 (Model: UU-K20 CAP) with a

stroke length of 50 mm, peak force of 500 N and a displacement resolution of 0.5 mm. Modes of failure were examined at 30× magnification using stereoscopic microscopy (SMZ161-BLED, Motic, Hong Kong, China) and classified as percentages of adhesive (A), mixed (M) or cohesive (C) failures. Five representative fractured specimens from each group were mounted on aluminium stubs with carbon glue after critical point drying. The specimens were gold-sputter-coated and analysed with field-emission scanning electron microscopy (FE-SEM S-4800; Hitachi, Wokingham, UK) at 10 kV and a working distance of 15 mm.

Data analysis was performed using statistical software (Statistical Program for Social Sciences, SPSS 11.5 Inc., Chicago, IL, USA). The normal distributions were determined using the Kolmogorov-Smirnov test. Homogeneity of variance was evaluated with the Levene test. A two-way Analysis of Variance (ANOVA, factors: material and time) was used to analyse the difference between the groups at a significance level of 5% ($\alpha = 0.05$). Post hoc Benferroni statistical analysis was also employed. The Chi-square (χ^2) test was used to compare failure mode among the experimental groups, considering the specimen as experimental unit. The significance level was set at $p < 0.05$.

2.5. Ultramorphology Bonded-Dentine Interfaces – Confocal Microscopy Evaluation

One dentine-bonded match stick specimen (\emptyset 0.9mm²) was selected from each experimental group during the specimen preparation for μ TBS testing at T0 and T1 storage times. These were coated with a fast-setting nail varnish, applied 1mm away from the bonded interface. The specimens were immersed for 24 h at 37 °C in a 0.1 wt% dye water/ethanol (80v%/20v%) solution of fluorescein isothiocyanate (FITC – Merck Life Science SLU, Spain) water solution (0.1 wt%) [28]. Subsequently, the specimens

were ultrasonicated in distilled water for 5 min and polished for 30 s using 500-grit, followed by 2400-grit SiC papers. The specimens were finally ultrasonicated in distilled water for further 5 min and immediately submitted for confocal microscopy analysis (CLSM - Olympus FV1000, Olympus Corp., Tokyo, Japan), using a 63X/1.4 NA oil-immersion lens and 543 nm LED illumination. Reflection and fluorescence images were obtained with a 1- μm z-step to section optically the specimens to a depth of up to 20 μm below the surface [29]. The z-axis scan of the interface surface was pseudo-coloured arbitrarily for improved visualisation and compiled into both single and topographic projections using the CLSM image-processing software (Fluoview Viewer, Olympus). The configuration of the system was standardised and used at constant settings for the entire imaging period. Each dentine interface was investigated completely and then five images were randomly captured and recorded; these represented the most common morphological features observed along the bonded interfaces [17,30].

3. RESULTS

The results of microtensile bond strength in MPa (\pm SD), percentage of failure mode and percentage of pre-test failures in each experimental group are depicted in table 2.

The results of the two-way ANOVA demonstrated that the factors material and storage time, as well as the interaction between the factors, were significant ($p < 0.05$). All the materials tested at baseline (T0) showed bond strength values between 8.7 and 10.6 MPa; there was no significant difference between them both at baseline (T0) and after storage for 1 year (T1 - $P > 0.05$). The only important difference was observed after 1 year storage with OPT and AU-SE, which both exhibited a significant drop in bond strength ($P < 0.05$) compared to their baseline values.

In terms of failure mode, the RMGIC showed no significant difference in the number of adhesive failures both at T0 and T1 ($P>0.05$), but at T1 it was detected a higher number of cohesive failures ($P<0.05$) and lower number of mixed failures ($P<0.05$). The two commercial adhesives showed increased cohesive failures in dentine at T1 ($P<0.05$) with reduced failure in adhesive mode ($P<0.05$). Conversely, the experimental adhesive showed at T1 a lower number of adhesive failures ($P<0.05$), but an increase in mixed mode failures ($P<0.05$).

The most representative features observed during the SEM fractographic analysis performed at T0 were the presence of exposed collagen fibrils in the specimens bonded with the adhesives OPT and EXP-ER (Figure 2A and 2B). However, the same group of specimens showed different characteristics at T1. The OPT specimens showed degradation in dentine (Figure 2C), while the EXP-ER specimens showed a dentine surface covered by mineral deposits, with little exposed dentine tubules (Figure 2D). Likewise, the UA-SE specimens were characterised by signs of degradation in dentine and absence of sound collagen fibrils at T1 (Figure 2E). Conversely, the EXP-SE specimens showed a dentine surface characterised by the presence of mineral deposits and few patent dentine tubules (Figure 2F). The RMGIC specimens depicted a dentine surface covered by residual cement after debonding, both at T0 (Figure 2G) and at T1 (Figure 2H).

The ultramorphological analysis performed using confocal microscopy at T0 (Figures 3) showed that in all groups, most specimens failed cohesively within the dentine just beneath the resin-dentine interfaces. Moreover, when the specimens were analysed in fluorescence mode, it was also observed that the dentine substrate was porous and therefore more easily infiltrated by the fluorescent dye (Figures 3a1, 3b1, 3D and 3e1).

However, the EXP-SE specimens presented most frequently with no cohesive failures within dentine (Figures 4C) and also the RMGIC specimens (Figures 4D). In these latter groups, a bonding interface characterised by infiltration of the fluorescent dye within the interdiffusion layer, was often observed.

The results of the ultramorphological analysis performed at T1 are depicted in Figure 4. It was possible to observe during the analysis that none of the tested materials was able to significantly reduce the porosity of the caries-affected dentine underneath the bonding interface (Figures 4a1, 4b1, 4c1 and 4d1). Moreover, in all groups, many specimens showed evident gaps at the bonding interface, in particular with the two commercial adhesives (Figures 4A, a1) and the RMGIC (Figures 4B, b1). Conversely, the specimens created with the EXP-SE (Figure 4C) and EXP-ER (Figure 4D) were characterised by presence of minerals precipitated within the gaps at the resin-dentine interfaces.

4. DISCUSSION

Dentine caries comprises of an outer layer of a bacterially contaminated zone, also known as caries-infected dentine (CID), and of an inner, less contaminated, demineralised zone identified as caries-affected dentine (CAD) [31]. CID is mainly characterised by a highly demineralised and irreversible denatured collagen fibrils impossible to repair and/or remineralise. Conversely, CAD consists in a layer of dentine characterised by partially demineralised collagen fibrils, which can support remineralisation. It is also important to mention that underlying the CAD there can be a further layer of dentine that undergoes structural changes during the caries process. This is known as transparent or reactive dentine, submitted to a continuous deposition

of minerals within the tubules in presence of the caries process. These tubules are often obliterated by large mineral rhombohedral plate-like crystals [32] of Mg-substituted orthophosphate (whitlockite) [33], which alters the optical refractive index of dentine and generates the transparent / translucent appearance.

Traditional hand and rotary instrumentation are conventionally employed in clinical practice to excavate the carious tissue from dental cavities. Minimally invasive operative approaches advocate selective caries excavation that may allow the operator to remove selectively the heavily bacteria-contaminated CID and preserve as much CAD as possible for potential remineralisation [34–37]. Several methods have been advocated as appropriate clinically for selective removal of CID [38,39]. Chemo-mechanical caries removal (CMCR) is based on such biological principles in form of gels that dissolve the infected and denatured collagen once applied on the dentine caries, which is then removed using special abrasive hand instruments [35,40–42].

However, several studies demonstrated that the bond strengths of adhesive systems applied in CAD are significantly lower than when they are bonded in sound dentine, so potentially reducing the longevity of the tooth-restoration complex [15, 43–48] .

The results of the current study are in agreement with those observations and showed that all the tested materials presented low bond strength values at baseline (T0) and the commercial adhesives systems (OPT; AU-SE) and the RMGIC exhibited a significant drop in bond strength after 1 year of storage with a significant ($P>0.05$) increase number of cohesive failures in dentine (Table 2). It has been widely demonstrated that both simplified and multi-step etch and rinse or self-etch systems result in an increase of cohesive failures within dentine [45,46,49,50].

One of the main reasons may be correlated to the reduced mechanical properties of caries-affected dentine. Indeed, Pugach *et al.* [51] demonstrated that the elastic modulus and hardness [52] of the outer layer of caries-affected dentine was significantly lower than that of sound and transparent dentine, especially when measured in wet conditions. Hybrid layers created by adhesive systems within caries-affected dentine are thicker than those generated in sound dentine and therefore impossible to infiltrate completely with any adhesive system [44,47,49,53]. The ultramorphological analysis performed using confocal microscopy at T0 showed the entire bonding interface and the underlying dentine totally porous and, thereby, infiltrated with the fluorescent dye (Figure 3).

Therefore, a porous bonding interface in caries-affected dentine affected by occlusal, thermal and chemical challenges in the oral cavity, along with the impact of proteolytic enzymes on exposed collagen fibrils, result in an adhesive restoration with compromised durability. A previous study of Erhardt *et al.*[6] demonstrated that regardless of the adhesive systems bonded to caries-affected dentine, there was always a significant drop in bond strength after direct exposure of the interface to water for a period of 6 months. Pashley *et al.* [54], demonstrated that incompletely resin-infiltrated caries-affected dentine is more prone to hydrolytic degradation of demineralised collagen due to the presence of host-derived matrix metalloproteinases (MMPs) enzymes. In agreement with this latter study, the specimens created using the universal adhesive (UA) applied in SE mode presented signs of degradation in dentine and absence of intact collagen fibrils after prolonged storage (Figure 2E). Moreover, the results of the confocal ultramorphological analysis performed after 1 year storage showed that in all groups it was possible to observe gaps as a possible consequence of collagen degradation within

the bonding interface and the demineralised dentine, in particular in those created using the two commercial adhesives (Figures 4A, 4a1).

The SEM analysis performed after bond strength testing (fractographic analysis) indicated that the specimens bonded using the EXP-ER (Figure 2D) and those bonded using EXP-SE (Figure 2F) were characterised by a consistent presence of minerals deposited on the dentine surfaces. These results were also confirmed during the confocal analysis performed in reflection mode after 1 year storage, which showed the presence of minerals precipitated within the resin-dentine interfaces both in the specimens created with the EXP-SE (Figure 4C) and those made with EXP-ER (Figure 4D).

The presence of minerals is easily detected during confocal microscopy when imaging the interface in reflection mode, as previously describe in literature. [...]

Such a mineral precipitation induced by the EXP adhesive may be a possible explanation to the absence of a significant drop in bond strength observed at T1. A possible explanation may be found in recent studies [17,18] that demonstrated an improved longevity of dentine bonded with ion-releasing adhesive materials doped with biomimetic analogues of dentine phosphoproteins, since these may remineralise the mineral-depleted dentine collagen within the hybrid layer and reduce their hydrolytic degradation sue to fossilisation and/or inhibition of dentine proteases [19,20].

Moreover, in a study of Abuna *et al.* [10], it was proved showed that the ion-releasing resins used in combination with the primer containing both biomimetic analogues such as PAA and TPM, were able to induce consistent mineral precipitation both at an intra-fibrillar and extra-fibrillar level after ageing for 6 months under simulated pulpal pressure. Unfortunately, it was not possible to ascertain in this study the composition of such mineral precipitation, but we can only hypothesize that there was the same

apatite precipitation as described in the study of Tezvergil-Mutluay A et al. [...], since the same fluoride-doped bioglass was used as filler for their experimental resin-based materials

It is important to consider that the presence of remnant apatite crystallites as centres for heterogeneous nucleation within caries-affected dentine, can play an important role in producing a more thermodynamically favourable remineralisation process [55,56]. Conversely, it is hypothesised the acid-etching procedure in dentine using orthophosphoric acid, may remove part of these residual minerals from the already partially demineralised caries-affected dentine, so jeopardising further the remineralisation via deposition of apatite-like crystallites.

The RMGIC-treated specimens were characterised by a significant drop in bond strength after prolonged storage (1 year). The ultramorphological analysis at T0 showed a bonding interface characterised by an infiltration of the fluorescent dye as a sign of low maturation of the reactive interaction zone [30]. This may explain the high number of pre-test failures attained in this group of specimens at baseline. After 1 year storage, the presence of a gap between the RMGIC and dentine was observed (Figures 4B, 4b1). However, this group of specimens presented a significant increase in the number of cohesive failures within the cement after storage ($P < 0.05$) and during SEM fractographic analysis it was clear that those specimens never presented with an exposed dentine after failure, both at baseline (Figure 2G) and after prolonged storage (Figure 2H). These results are in agreement with some recent articles where it was shown the potential of glass ionomer cements in increasing the mineral density in artificial caries dentine created via a microbial protocol [...]

However, GICs can induce a diffusion of ions specific such as strontium and silicon within the demineralised dentine, but with no sign of apatite deposition within the demineralised collagen fibrils even in presence of biomimetic analogues in different remineralisation storage media [...]

It is hypothesised that the gap observed during confocal analysis was a consequence of an artefact created during specimen preparation due to the cohesive fracture of a brittle interface created after prolonged storage [57]. Such a brittle interface may be also the reason for the significant reduction in bond strength observed after 1 year storage in the specimens made with the RMGIC. This hypothesis may be also applied to the gaps observed in the interfaces produced with the EXP-SE (Figure 4C and 4c1) and EXP-ER (Figure 4D and 4d1). Conversely, the specimens created with the two commercial adhesives showed signs of degradation within a caries-affected dentine even when demineralised and infiltrated by the fluorescent dye (Figures 4A, 4a1).

The results of this study may corroborate previous scientific reports that concluded that GIC-based materials may be indicated for the restoration of dental cavities after selective caries removal, owing to their remineralisation ability through fluoride release [58,59]. It is well known that conventional adhesive systems have no remineralisation properties and usually result in lower bond strengths when applied to caries-affected dentine compared to sound dentine. Conversely, RMGICs have similar bond strengths to both sound and caries-affected dentine, although they may show a significant drop in bond strength over time as in the case of the RMGIC tested in this study (Table 2). Such differences in bonding stability mainly depend on the chemical formulation of each glass-ionomer and resin-modified glass-ionomer cements [60,61]. The hypothesis that the experimental adhesive applied in ER and SE mode after selective caries removal

would have protective effect on the bonding longevity after 1 year storage compared to the commercial products used as control groups in this study must be accepted.

One of the main limitations of the current study is associated to the vast variability of caries dentine in a real clinical scenario, thus further *in vitro* and *in vivo* investigation must be performed to validate the results the results obtained in this study. Moreover, our ongoing studies are assessing the ability of the materials tested in the current project to remineralise simulated and natural caries-affected dentine through nano-indentation and Raman spectroscopy analysis.

5. CONCLUSIONS

Although both the adhesive systems and the RMGIC used in this study showed similar bonding performance at baseline and a significant drop of the bond strength after 1-year storage, the specimens bonded with the GI cement never presented with an exposed dentine after failure, even after prolonged storage. Conversely, the specimens bonded with conventional adhesives often presented an exposed dentine with signs of degradation of collagen fibrils after prolonged storage. Therefore, considering the limitations of the current *in-vitro* study, we consider that GIC-based materials may remain an appropriate choice for application in cavities after selective chemo-mechanical caries removal rather than conventional adhesive systems. However, innovative ion-releasing resin-based bonding materials need to be developed, along with self-etching primers containing biomimetic analogues of phosphoproteins, in order to achieve a greater level of remineralisation of CAD and a more stable bonding to caries-affected dentine over time.

Declaration of interests.

All authors gave their final approval and agree to be accountable for all aspects of the work. 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.

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Table 1

Code	Adhesives	Main components	pH	Manufacturer
UA-SE	Prime & Bond Active	HEMA, Bis-GMA, UDMA, PENTA, MDP, ACTIVE GUARD™, isopropanol, water, initiator, stabilizer.	2.5	Dentsply Sirona, Konstanz, Germany
OPT	Optibond FL	Primer: HEMA, GPDM, PAMM, ethanol, water, CQ. Adhesive: Bis-GMA, HEMA, GDMA, CQ, glycerol, dimethacrylate resins. Filler: sodium hexafluorosilicate.	1.9	Kerr, Bioggio, Switzerland
RMGIC	RIVA LC	HEMA, APA, acid monomer, dimethacrylate cross-linker, tartaric acid, glass powder.	2.5-3	SDI, Victoria, Australia
EXP-SE EXP-ER	Experimental Adhesive	Biomimetic conditioner: APA, TMP, ethanol, water. Adhesive: GPDM, HEMA, UDMA, TEGDMA, water, ethanol Flow resin: Bis-EMA, Bis-GMA, CQ, ethyl 4-dimethylamine benzoate and diphenylodonium hexafluorophosphate. Filler: fluoride-doped bioglass (BAG-F)	4.6 2.1 6.8	Experimental
Resin composite		Main components	Manufacturer	
SDR		SDR patented urethane di-methacrylate resin, di-methacrylate resin, di-functional diluents, barium and strontium alumino-fluoro-silicate glasses Photo-initiating system and colorants.	Dentsply Sirona, Konstanz, Germany	
Dentine conditioning mode		Procedure		
SE (self-etch)		<p>UA: phosphoric acid pre-etching was not performed. CAD air-dried up to remove all the excess of water from the surface. The adhesive was brushed for 20 seconds and air dried for 5 seconds to evaporate the solvent until the adhesive no longer moves on the surface. Light cure for 10 seconds.</p> <p>EXP: the biomimetic primer was applied on the specimens for 20 seconds followed by air-drying for 3 seconds. The SE primer was applied for 10 seconds, and the excess was removed with strong air-blow hard 5 seconds to allow a proper solvent/water evaporation. A second coat of primer was applied for 10 seconds and again blow gently for 5 seconds. Finally, the bioactive flowable resin was applied (1 mm) and finally light-cured for 30 seconds.</p>		
ER (etch-&-rinse) <i>(DeTrey conditioner, 36%, phosphoric acid. Dentsply Sirona, Konstanz, Germany)</i>		<p>OPT: CAD surface was phosphoric acid etched for 15 seconds. Etched surface was rinsed with water for 15 seconds (three-way dental syringe) and air-dried up to remove all the excess of water from the surface. Then primer for 15 seconds; evaporation of the solvent; followed by adhesive for 20 seconds; and light-curing.</p> <p>EXP: after CAD etching, we followed the same procedure as in SE.</p>		

Abbreviations: ACTIVE GUARD™ (bifunctional acryl crosslinker containing N-allyl), APA (polyacrylic acid), BAG-F (fluoride-doped bioglass 45S5), Bis-EMA (dimethacrylate), Bis-GMA (bisphenol-AG, diglycidyl-dimethacrylate), CAD (caries-affected dentine), CQ (*dl*-camphoroquinone), EXP-SE (Experimental adhesive self-etch), EXP-ER (Experimental adhesive etch and rinse). GPDM (glycerol phosphate dimethacrylate), GDMA (glycerol dimethacrylate) HEMA (2-hydroxyethyl methacrylate), MDP (10-methacryloyloxydecyl dihydrogen phosphate), PAMM (phthalic acid monomethacrylate, PENTA (dipentaerythritol pentacrylate phosphate), TEGDMA (triethylene glycol dimethacrylate), TMP (trimetaphosphate), UDMA (urethane-dimethacrylate).

Table 2.- Adhesive strength: Average (\pm standard deviation) in MPa, cases percentage according to failure mode (adhesive / cohesive / mixed) and pre-test failures [%].

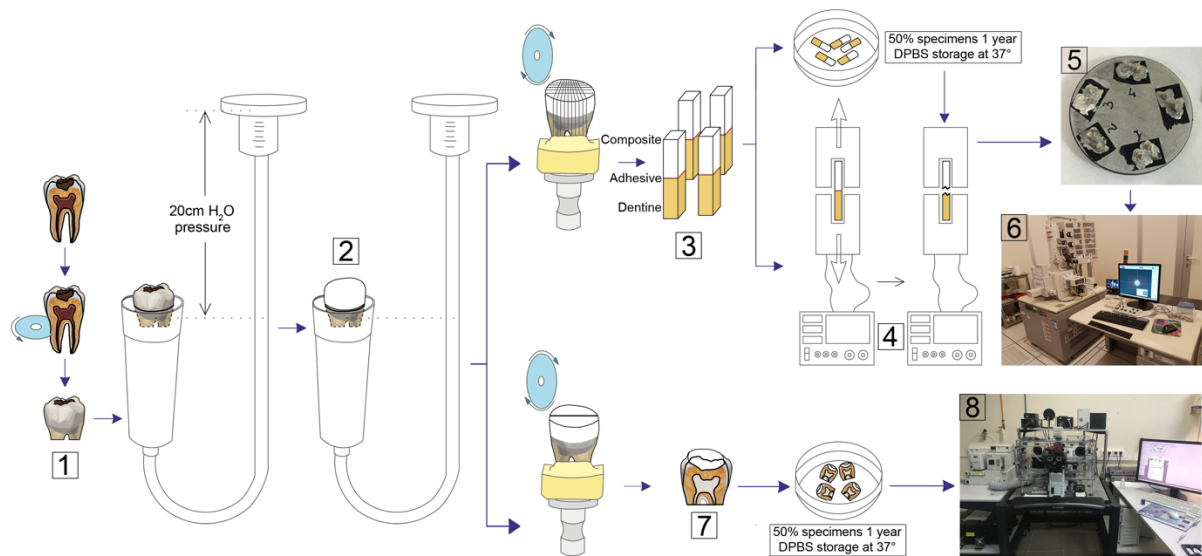
	T0 (24h)	pre-test failures	T1 (1 year)
UA-SE	9.4 (\pm 7.7) ^{a1} (38 / 14 / 48)	[0.0%]	4.8 (\pm 4.2) ^{a2} (5 / 45 / 50)
OPT	10.6 (\pm 8.1) ^{a1} (42 / 21 / 37)	[2.4%]	5.2 (\pm 6.4) ^{a2} (9/50 / 41)
RMGIC	8.7 (\pm 5.9) ^{a1} (20 / 20 / 60)	[52.5%]	3.9 (\pm 5.1) ^{a2} (22 / 45 / 33)
EXP-SE	7.9 (\pm 4.9) ^{a1} (64 / 7 / 29)	[22.5%]	6.6 (\pm 7.3) ^{a1} (24 / 5 / 71)
EXP-ER	6.2 (\pm 5.4) ^{a1} (59 / 5 / 32)	[0.0%]	4.3 (\pm 4.0) ^{a1} (20 / 20 / 60)

The same letter indicates no significant differences between the tested materials at the same time ($p > 0.05$)

The same number indicates no significant differences between the tested materials before and after prolonged storage ($p > 0.05$).

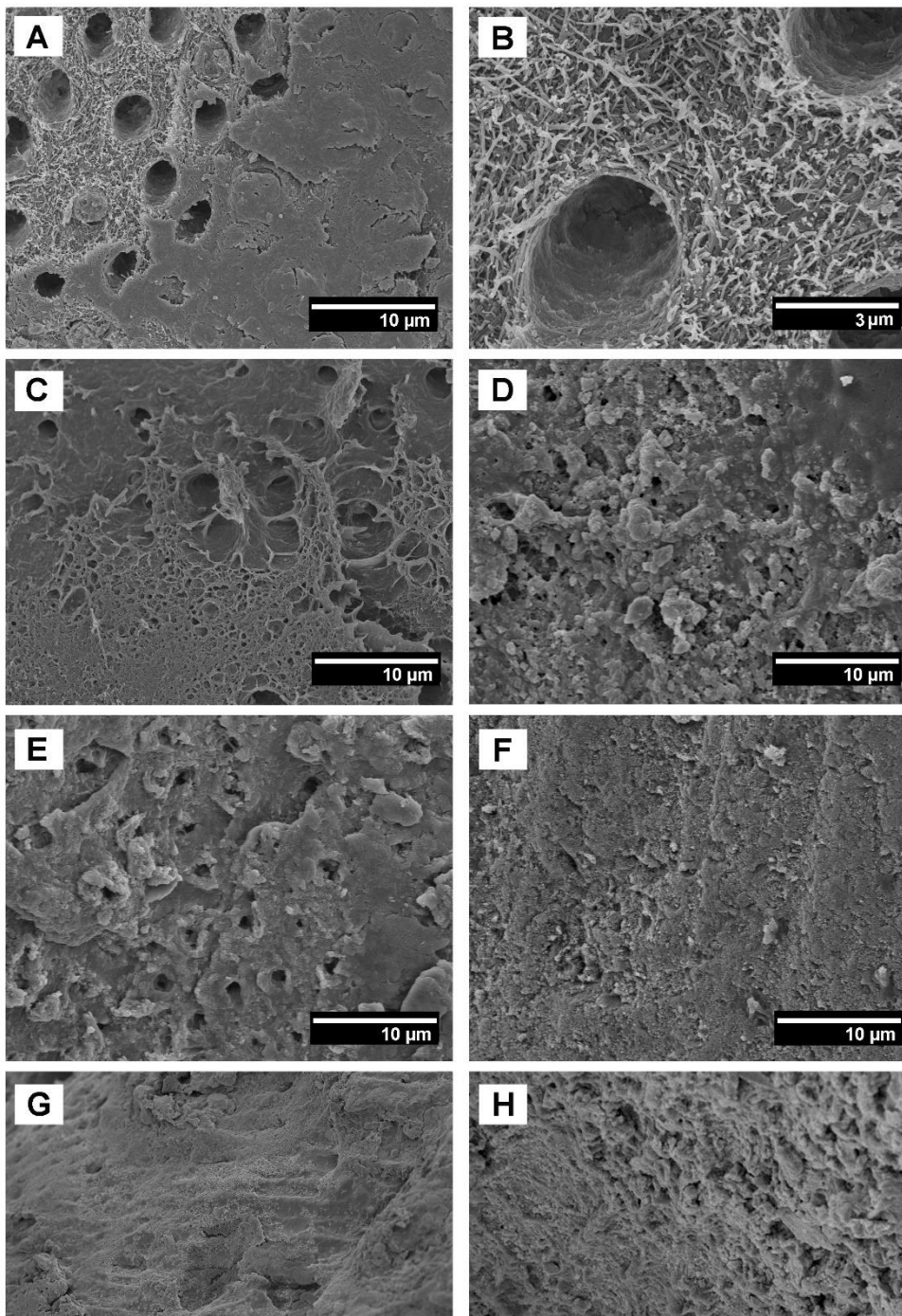
OPT and UA exhibited the highest bond strength at 24h, while after 1 year, EXP SE was the only adhesive to show no significant reduction in bond strength ($p < 0.05$).

Figure 1. Graphical representation of the experimental design.



1. Freshly extracted human molars n=50. Mid-root dentine preparation and pulp tissue were removed.
2. Bonding procedures and composite build-up.
3. Sectioning specimens for μ TBS.
4. Test machine LMT100.
5. Template with metalized sticks for fractographic analysis.
6. FE-SEM Hitachi S4800.
7. Sectioning specimens for confocal microscopy analysis.
8. CLSM Olympus FV1000.

Figure 2 SEM fractographic analysis of the specimens tested at baseline and after 1 year storage



Representative SEM images of the most characteristic morphological features observed during the fractographic analysis.

At baseline storage time the specimens created using the tested adhesives Optibond FL (OPT) [A] and the experimental adhesive applied in ER mode [B] show, in those zones of the bonding interface that failed in adhesive mode, the presence of poorly resin-infiltrated exposed collagen fibrils and patent dentine tubules.

[C] This image shows the representative morphological features of the specimens made with OPT after prolonged storage. It is visible a clear sign of degradation in dentine with very few remaining collagen fibrils.

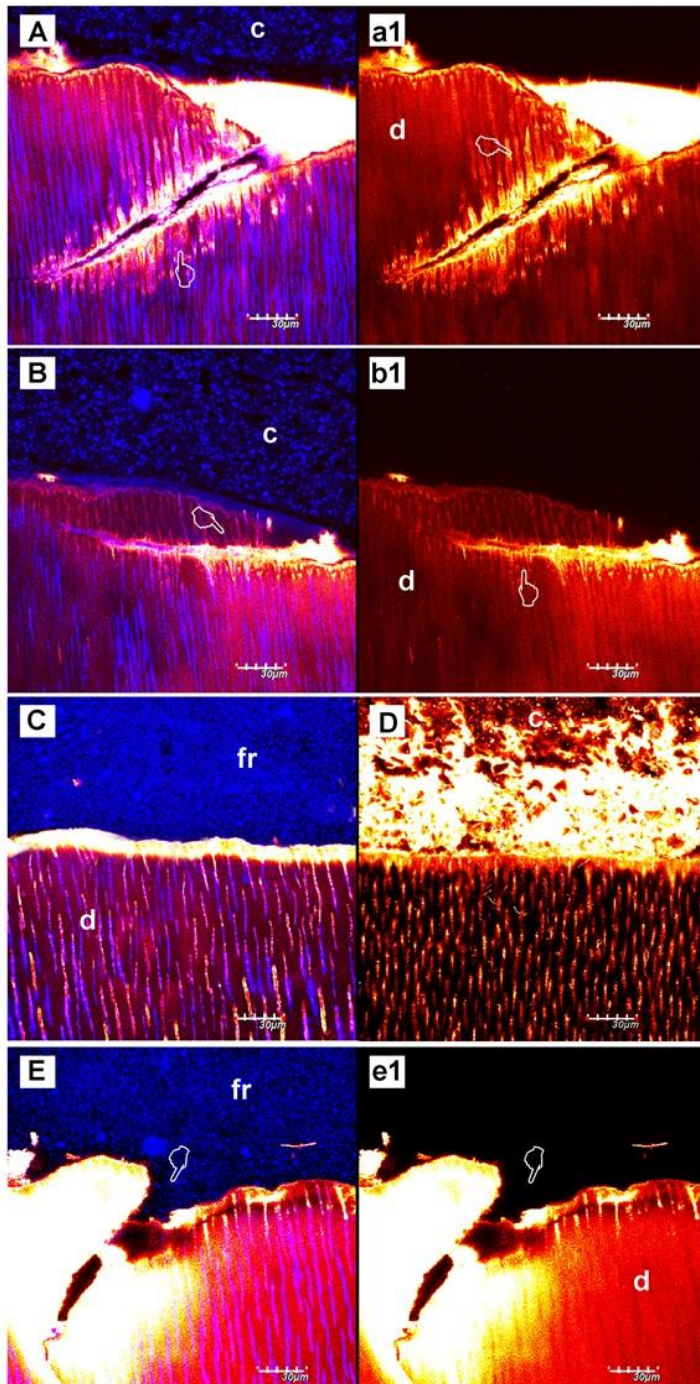
[D] This is a representative image of the specimens produced with the experimental system applied in ER mode (EXP-ER) where a dentine surface covered by minerals and with very little presence of exposed dentine tubules is clearly visible.

[E] The specimens made using the universal adhesive (UA) applied in SE mode showed clear sign of degradation in dentine after prolonged storage, as it is shown in this representative SEM fractography image

[F] This is a representative image of the specimens produced with the experimental system applied in SE mode (EXP-SE). Please note also in this case a dentine surface characterised by the presence of minerals and very little presence of exposed dentine tubules.

The specimens created using the RMGIC present a fractured surface characterised by a dentine always covered by residual material or by minerals both at baseline [G] and after prolonged storage [H].

Figure 3. Confocal microscopy images of the bonded-dentine interfaces tested at 24h storage



[A] CLSM reflection/fluorescence projection image exemplifying the interfacial characteristics of the resin-dentine interface produced by application of Prime & Bond Active (UA) in SE mode. Note the presence of a cohesive fracture (pointer) within the dentine (d); a demineralised substrate clearly porous and infiltrated by the fluorescein [a1: single projection in fluorescence mode].

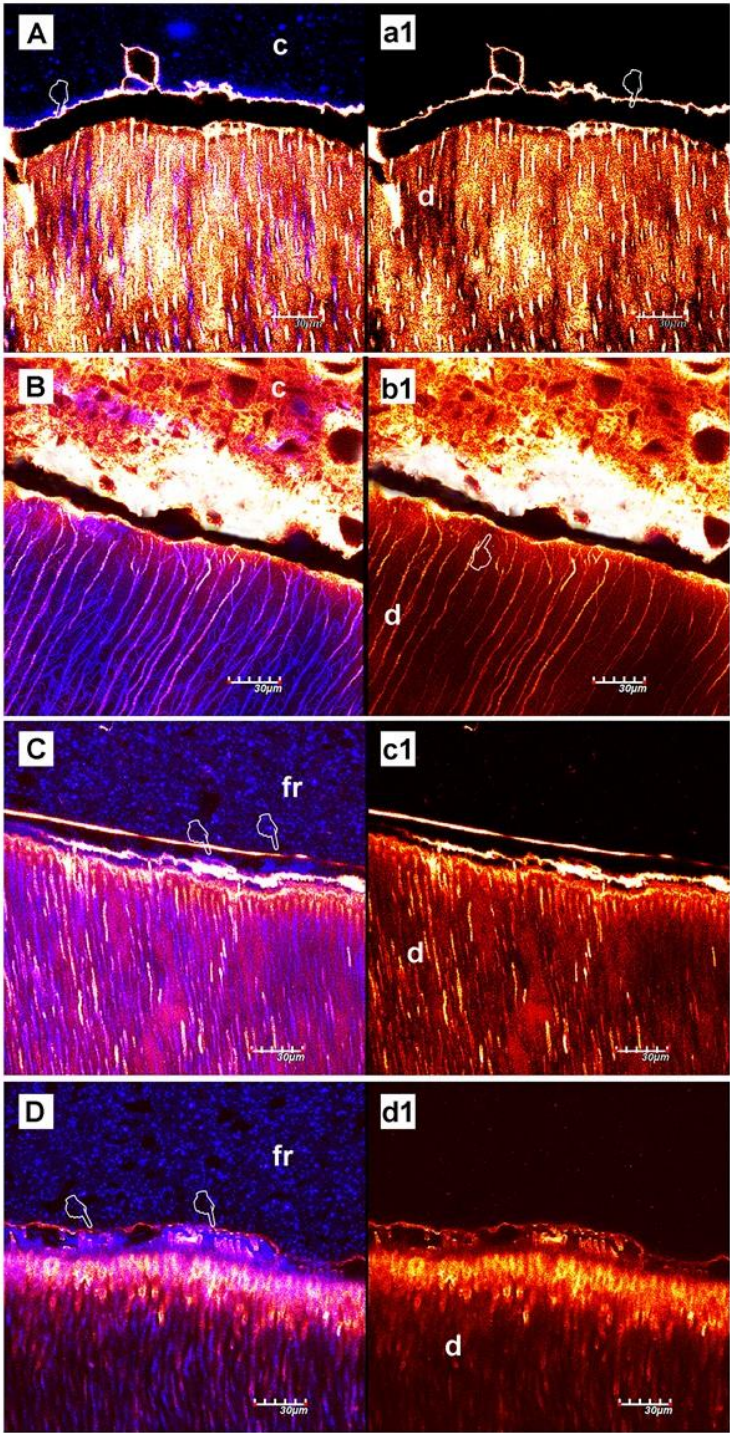
[B] Similarly, the resin-dentine interface created with Optibond FL (OPT) in ER mode presents a cohesive fracture (pointer) within a demineralised dentine (d) porous and also in this case infiltrated by the fluorescein [b1: single projection in fluorescence mode].

[C] CLSM reflection/fluorescence projection image showing the interfacial characteristics of the resin-dentine interface created by application of the experimental bonding system (EXP-SE) in SE mode. In this case it is visible a clear accumulation of fluorescein at the bonding interface (pointer) and within demineralised dentine (d).

[D] This CLSM single projection image obtained in fluorescence mode shows the bonded-dentine interface created using the resin-modified glass ionomer cement RIVA (RMGIC) characterised by a clear accumulation of fluoresceine at the bonding interface (pointer) and within the demineralised dentine (d).

[E] CLSM reflection/fluorescence projection image of the resin-dentine interface created by application of the experimental bonding system (EXP-ER) in ER mode. Note the presence of a cohesive fracture (pointer) within the demineralised dentine (d), which clearly porous and infiltrated by the fluorescein [e1: single projection in fluorescence mode].

Figure 4. Confocal microscopy images of the bonded-dentine interfaces tested after 1 year aging



[A] CLSM reflection/fluorescence projection image exemplifying the interfacial characteristics after prolonged aging under pulpal pressure for 1 year of the resin-dentine interface created using the Optibond FL (OPT). The presence of a gap/degradation (pointer) within a dentine (d) still demineralised and infiltrated by the fluorescein [a1: single projection in fluorescence mode] can be seen in this image.

[D] This CLSM single projection image obtained in fluorescence mode shows the bonded-dentine interface made by using the resin-modified glass ionomer cement RIVA (RMGIC). Also in this case a clear gap (pointer) within a dentine (d) still demineralised and infiltrated by the fluorescein [d1: single projection in fluorescence mode] was detected.

[C] CLSM reflection/fluorescence projection image showing the interfacial characteristics after 1 year ageing of the resin-dentine interface created using the experimental bonding system (EXP-SE) applied in SE mode. In this case it is possible to see a clear mineral precipitation (pointer) within the gap at the bonding interface dentine (d) - flowable composite (fc). Also in this case it is possible to detect still a clear accumulation of fluorescein within a demineralised dentine (d) infiltrated by fluorescein [c1: single projection in fluorescence mode].

[D] CLSM reflection/fluorescence projection image of the resin-dentine interface after 1 year aging produced using the experimental bonding system (EXP-ER) applied in ER mode. Note a clear mineral precipitation (pointer) between the dentine (d) and the experimental flowable composite (fc). Also in this case it is possible to see a clear accumulation of fluorescein within a demineralised dentine (d) infiltrated by fluorescein [d1: single projection in fluorescence mode].

REFERENCES

- [1] I. Nedeljkovic, W. Teughels, J. De Munck, B. Van Meerbeek, K.L. Van Landuyt, Is secondary caries with composites a material-based problem?, *Dent. Mater.* 31 (2015) 247–77. <https://doi.org/10.1016/j.dental.2015.09.001>.
- [2] F.F. Demarco, K. Collares, M.B. Correa, M.S. Cenci, R.R. de Moraes, N.J. Opdam, Should my composite restorations last forever? Why are they failing?, *Braz. Oral Res.* 31 (2017) 56. <https://doi.org/10.1590/1807-3107BOR-2017.vol31.0056>.
- [3] Y. Liu, L. Tjäderhane, L. Breschi, A. Mazzoni, N. Li, J. Mao, D.H. Pashley, F.R. Tay, Limitations in bonding to dentin and experimental strategies to prevent bond degradation, *J. Dent. Res.* 90 (2011) 953–968. <https://doi.org/10.1177/0022034510391799>.
- [4] H. Sano, M. Yoshiyama, S. Ebisu, M.F. Burrow, T. Takatsu, B. Ciucchi, R. Carvalho, D.H. Pashley, Comparative SEM and TEM observations of nanoleakage within the hybrid layer, *Oper. Dent.* 20 (1995) 160–167.
- [5] A.D. Loguercio, R. Stanislawczuk, L.G. Polli, J.A. Costa, M.D. Michel, A. Reis, Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability, *Eur. J. Oral Sci.* 117 (2009) 587–596. <https://doi.org/10.1111/j.1600-0722.2009.00663.x>.
- [6] M.C.G. Erhardt, M. Toledano, R. Osorio, L.A. Pimenta, Histomorphologic characterization and bond strength evaluation of caries-affected dentin/resin interfaces: effects of long-term water exposure, *Dent. Mater.* 24 (2008) 786–798. <https://doi.org/10.1016/j.dental.2007.09.007>.
- [7] K.L. Van Landuyt, S. Krifka, K.-A. Hiller, C. Bolay, C. Waha, B. Van Meerbeek, G.

- Schmalz, H. Schweikl, Evaluation of cell responses toward adhesives with different photoinitiating systems, *Dent. Mater.* 31 (2015) 916–927.
<https://doi.org/10.1016/j.dental.2015.04.016>.
- [8] J. De Munck, K. Van Landuyt, M. Peumans, A. Poitevin, P. Lambrechts, M. Braem, B. Van Meerbeek, A critical review of the durability of adhesion to tooth tissue: Methods and results, *J. Dent. Res.* 84 (2005) 118–132.
<https://doi.org/10.1177/154405910508400204>.
- [9] P.M. Pires, A. de A. Neves, I.M. Makeeva, F. Schwendicke, V. Faus-Matoses, K. Yoshihara, A. Banerjee, S. Sauro, Contemporary restorative ion-releasing materials: current status, interfacial properties and operative approaches, *Br. Dent. J.* 229 (2020) 450–458. <https://doi.org/10.1038/s41415-020-2169-3>.
- [10] G. Abuna, V.P. Feitosa, A.B. Correr, G. Cama, M. Giannini, M.A. Sinhoreti, D.H. Pashley, S. Sauro, Bonding performance of experimental bioactive/biomimetic self-etch adhesives doped with calcium-phosphate fillers and biomimetic analogs of phosphoproteins, *J. Dent.* 52 (2016) 79–86.
<https://doi.org/10.1016/j.jdent.2016.07.016>.
- [11] R.M. Carvalho, L. Tjäderhane, A.P. Manso, M.R. Carrilho, C.A.R. Carvalho, Dentin as a bonding substrate, *Endod. Top.* 21 (2009) 62–88.
- [12] L. Tjäderhane, M.A.R. Buzalaf, M. Carrilho, C. Chaussain, Matrix metalloproteinases and other matrix proteinases in relation to cariology: The era of “dentin degradomics,” *Caries Res.* 49 (2015) 193–208.
<https://doi.org/10.1159/000363582>.
- [13] H. Sonoda, A. Banerjee, M. Sherriff, J. Tagami, T.F. Watson, An in vitro investigation of microtensile bond strengths of two dentine adhesives to caries-affected dentine, *J. Dent.* 33 (2005) 335–342.
<https://doi.org/10.1016/j.jdent.2004.09.009>.
- [14] A. Banerjee, S. Kellow, F. Mannocci, R.J. Cook, T.F. Watson, An in vitro evaluation of microtensile bond strengths of two adhesive bonding agents to residual dentine after caries removal using three excavation techniques, *J. Dent.* 38 (2010) 480–489. <https://doi.org/10.1016/j.jdent.2010.03.002>.
- [15] M. Nakajima, H. Sano, M.F. Burrow, J. Tagami, M. Yoshiyama, S. Ebisu, B. Ciucchi, C.M. Russell, D.H. Pashley, Tensile Bond Strength and SEM Evaluation of

- Caries-affected Dentin Using Dentin Adhesives, *J. Dent. Res.* 74 (1995) 1679–1688. <https://doi.org/10.1177/00220345950740100901>.
- [16] A. de Almeida Neves, E. Coutinho, M.V. Cardoso, P. Lambrechts, B. Van Meerbeek, Current concepts and techniques for caries excavation and adhesion to residual dentin, *J. Adhes. Dent.* 13 (2011) 7–22. <https://doi.org/10.3290/j.jad.a18443>.
- [17] S. Sauro, R. Osorio, T.F. Watson, M. Toledano, Influence of phosphoproteins' biomimetic analogs on remineralization of mineral-depleted resin-dentin interfaces created with ion-releasing resin-based systems, *Dent. Mater.* 31 (2015) 759–777. <https://doi.org/10.1016/j.dental.2015.03.013>.
- [18] Y. Liu, Y.-K. Kim, L. Dai, N. Li, S.O. Khan, D.H. Pashley, F.R. Tay, Hierarchical and non-hierarchical mineralisation of collagen, *Biomaterials.* 32 (2011) 1291–1300. <https://doi.org/10.1016/J.BIOMATERIALS.2010.10.018>.
- [19] Y. Qi, N. Li, L. Niu, C.M. Primus, J.-Q. Ling, D.H. Pashley, F.R. Tay, Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate, *Acta Biomater.* 8 (2012) 836–842. <https://doi.org/10.1016/j.actbio.2011.10.033>.
- [20] L. Niu, W. Zhang, D.H. Pashley, L. Breschi, J. Mao, J. Chen, F.R. Tay, Biomimetic remineralization of dentin, *Dent. Mater.* 30 (2014) 77–96. <https://doi.org/10.1016/J.DENTAL.2013.07.013>.
- [21] S. Sauro, D.H. Pashley, M. Montanari, S. Chersoni, R.M. Carvalho, M. Toledano, R. Osorio, F.R. Tay, C. Prati, Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives, *Dent. Mater.* 23 (2007) 705–713. <https://doi.org/https://doi.org/10.1016/j.dental.2006.06.010>.
- [22] N. Meraji, M.H. Nekoofar, K.A. Yazdi, M.R. Sharifian, N. Fakhari, J. Camilleri, Bonding to caries affected dentine, *Dent. Mater.* 34 (2018) 236–245. <https://doi.org/10.1016/j.dental.2018.05.017>.
- [23] F. Schwendicke, J.E. Frencken, L. Bjørndal, M. Maltz, D.J. Manton, D. Ricketts, K. Van Landuyt, A. Banerjee, G. Campus, S. Doméjean, M. Fontana, S. Leal, E. Lo, V. Machiulskiene, A. Schulte, C. Splieth, A.F. Zandona, N.P.T. Innes, Managing Carious Lesions: Consensus Recommendations on Carious Tissue Removal, *Adv. Dent. Res.* 28 (2016) 58–67. <https://doi.org/10.1177/0022034516639271>.

- [24] A. Banerjee, J.E. Frencken, F. Schwendicke, N.P.T. Innes, Contemporary operative caries management: consensus recommendations on minimally invasive caries removal, *Br. Dent. J.* 223 (2017) 215–222.
<https://doi.org/10.1038/sj.bdj.2017.672>.
- [25] N.P.T. Innes, J.E. Frencken, L. Bjørndal, M. Maltz, D.J. Manton, D. Ricketts, K. Van Landuyt, A. Banerjee, G. Campus, S. Doméjean, M. Fontana, S. Leal, E. Lo, V. Machiulskiene, A. Schulte, C. Splieth, A. Zandona, F. Schwendicke, Managing Carious Lesions: Consensus Recommendations on Terminology, *Adv. Dent. Res.* 28 (2016) 49–57. <https://doi.org/10.1177/0022034516639276>.
- [26] F. Schwendicke, C. Splieth, L. Breschi, A. Banerjee, M. Fontana, S. Paris, M.F. Burrow, F. Crombie, L.F. Page, P. Gatón-Hernández, R. Giacaman, N. Gugnani, R. Hickel, R.A. Jordan, S. Leal, E. Lo, H. Tassery, W.M. Thomson, D.J. Manton, When to intervene in the caries process? An expert Delphi consensus statement, *Clin. Oral Investig.* 23 (2019) 3691–3703. <https://doi.org/10.1007/s00784-019-03058-w>.
- [27] A. Tezvergil-Mutluay, R. Seseogullari-Dirihan, V.P. Feitosa, G. Cama, D.S. Brauer, S. Sauro, Effects of Composites Containing Bioactive Glasses on Demineralized Dentin, *J. Dent. Res.* 96 (2017) 999–1005.
<https://doi.org/10.1177/0022034517709464>.
- [28] V.P. Feitosa, M.G. Bazzocchi, A. Putignano, G. Orsini, A.L. Luzi, M.A.C. Sinhoreti, T.F. Watson, S. Sauro, Dicalcium phosphate (CaHPO₄·2H₂O) precipitation through ortho- or meta-phosphoric acid-etching: Effects on the durability and nanoleakage/ultra-morphology of resin–dentine interfaces, *J. Dent.* 41 (2013) 1068–1080. [https://doi.org/https://doi.org/10.1016/j.jdent.2013.08.014](https://doi.org/10.1016/j.jdent.2013.08.014).
- [29] S. Sauro, D.H. Pashley, F. Mannocci, F.R. Tay, P. Pilecki, M. Sherriff, T.F. Watson, Micropermeability of current self-etching and etch-and-rinse adhesives bonded to deep dentine: a comparison study using a double-staining/confocal microscopy technique., *Eur. J. Oral Sci.* 116 (2008) 184–193.
<https://doi.org/10.1111/j.1600-0722.2007.00518.x>.
- [30] S. Sauro, T. Watson, A.P. Moscardó, A. Luzi, V.P. Feitosa, A. Banerjee, The effect of dentine pre-treatment using bioglass and/or polyacrylic acid on the interfacial characteristics of resin-modified glass ionomer cements, *J. Dent.* 73 (2018) 32–

39. <https://doi.org/10.1016/j.jdent.2018.03.014>.
- [31] T. Fusayama, Two layers of carious dentin; diagnosis and treatment, *Oper. Dent.* 4 (1979) 63–70.
- [32] K. Ogawa, Y. Yamashita, T. Ichijo, T. Fusayama, The ultrastructure and hardness of the transparent layer of human carious dentin, *J. Dent. Res.* 62 (1983) 7–10. <https://doi.org/10.1177/00220345830620011701>.
- [33] G. Daculsi, R.Z. LeGeros, A. Jean, B. Kerebel, Possible physico-chemical processes in human dentin caries, *J. Dent. Res.* 66 (1987) 1356–1359. <https://doi.org/10.1177/00220345870660081401>.
- [34] A. Banerjee, I.D. Thompson, T.F. Watson, Minimally invasive caries removal using bio-active glass air-abrasion, *J. Dent.* 39 (2011) 2–7. <https://doi.org/10.1016/j.jdent.2010.09.004>.
- [35] A. Banerjee, E.A. Kidd, T.F. Watson, In vitro evaluation of five alternative methods of carious dentine excavation, *Caries Res.* 34 (2000) 144–150. <https://doi.org/10.1159/000016582>.
- [36] A. Banerjee, The contemporary practice of minimally invasive dentistry, *Fac. Dent. J.* 6 (2015) 78–85.
- [37] L. Mackenzie, A. Banerjee, Minimally invasive direct restorations: a practical guide, *Br. Dent. J.* 223 (2017) 163–171. <https://doi.org/10.1038/sj.bdj.2017.661>.
- [38] A. de A. Neves, E. Coutinho, J. De Munck, B. Van Meerbeek, Caries-removal effectiveness and minimal-invasiveness potential of caries-excitation techniques: a micro-CT investigation, *J. Dent.* 39 (2011) 154–162. <https://doi.org/10.1016/j.jdent.2010.11.006>.
- [39] A. Banerjee, H.M. Pickard, T.F. Watson, *Pickard's manual of operative dentistry*, Oxford university press, 2011.
- [40] A. Banerjee, E.A. Kidd, T.F. Watson, Scanning electron microscopic observations of human dentine after mechanical caries excavation, *J. Dent.* 28 (2000) 179–186. [https://doi.org/10.1016/s0300-5712\(99\)00064-0](https://doi.org/10.1016/s0300-5712(99)00064-0).
- [41] A. Banerjee, T.F. Watson, E.A.M. Kidd, Dentine Caries: Take It or Leave It?, *Dent. Update.* 27 (2000) 272–276. <https://doi.org/10.12968/denu.2000.27.6.272>.
- [42] A. Banerjee, E.A.M. Kidd, T.F. Watson, In vitro validation of carious dentin removed using different excavation criteria., *Am. J. Dent.* 16 (2003) 228–30.

- [43] M. Nakajima, M. Ogata, M. Okuda, J. Tagami, H. Sano, D.H. Pashley, Bonding to caries-affected dentin using self-etching primers, *Am. J. Dent.* 12 (1999) 309–314.
- [44] M. Yoshiyama, A. Urayama, T. Kimochi, T. Matsuo, D.H. Pashley, Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin, *Oper. Dent.* 25 (2000) 163–169.
- [45] L. Ceballos, D.G. Camejo, M. Victoria Fuentes, R. Osorio, M. Toledano, R.M. Carvalho, D.H. Pashley, Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine, *J. Dent.* 31 (2003) 469–477.
[https://doi.org/10.1016/s0300-5712\(03\)00088-5](https://doi.org/10.1016/s0300-5712(03)00088-5).
- [46] J. Doi, T. Itota, Y. Torii, S. Nakabo, M. Yoshiyama, Micro-tensile bond strength of self-etching primer adhesive systems to human coronal carious dentin, *J. Oral Rehabil.* 31 (2004) 1023–1028. <https://doi.org/10.1111/j.1365-2842.2004.01339.x>.
- [47] Y. Wang, P. Spencer, M.P. Walker, Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy, *J. Biomed. Mater. Res. Part A.* 81A (2007) 279–286. <https://doi.org/10.1002/jbm.a.30981>.
- [48] M. Nakajima, S. Kunawarote, T. Prasansuttiorn, J. Tagami, Bonding to caries-affected dentin, *Jpn. Dent. Sci. Rev.* 47 (2011) 102–114.
<https://doi.org/10.1016/J.JDSR.2011.03.002>.
- [49] M. Nakajima, H. Sano, I. Urabe, J. Tagami, D.H. Pashley, Bond strengths of single-bottle dentin adhesives to caries-affected dentin, *Oper. Dent.* 25 (2000) 2–10.
- [50] C.A.G. Arrais, M. Giannini, M. Nakajima, J. Tagami, Effects of additional and extended acid etching on bonding to caries-affected dentine, *Eur. J. Oral Sci.* 112 (2004) 458–464. <https://doi.org/10.1111/j.1600-0722.2004.00159.x>.
- [51] M.K. Pugach, J. Strother, C.L. Darling, D. Fried, S.A. Gansky, S.J. Marshall, G.W. Marshall, Dentin caries zones: mineral, structure, and properties, *J. Dent. Res.* 88 (2009) 71–76. <https://doi.org/10.1177/0022034508327552>.
- [52] A. Banerjee, M. Sherriff, E.A.M. Kidd, T.F. Watson, A confocal microscopic study relating the autofluorescence of carious dentine to its microhardness, *Br. Dent. J.* 187 (1999) 206–210. <https://doi.org/10.1038/sj.bdj.4800241>.
- [53] E.C. Say, M. Nakajima, P. Senawongse, M. Soyman, F. Ozer, J. Tagami, Bonding

- to sound vs caries-affected dentin using photo and dual-cure adhesives, *Oper. Dent.* 30 (2005) 90–98.
- [54] D.H. Pashley, F.R. Tay, C. Yiu, M. Hashimoto, L. Breschi, R.M. Carvalho, S. Ito, Collagen degradation by host-derived enzymes during aging, *J. Dent. Res.* 83 (2004) 216–221. <https://doi.org/10.1177/154405910408300306>.
- [55] S. Gajjerman, K. Narayanan, J. Hao, C. Qin, A. George, Matrix macromolecules in hard tissues control the nucleation and hierarchical assembly of hydroxyapatite, *J. Biol. Chem.* 282 (2007) 1193–1204. <https://doi.org/10.1074/jbc.M604732200>.
- [56] Y. Liu, N. Li, Y. Qi, L. Niu, S. Elshafiy, J. Mao, L. Breschi, D.H. Pashley, F.R. Tay, The use of sodium trimetaphosphate as a biomimetic analog of matrix phosphoproteins for remineralization of artificial caries-like dentin, *Dent. Mater.* 27 (2011) 465–477. <https://doi.org/10.1016/j.dental.2011.01.008>.
- [57] S. Sauro, T.F. Watson, I. Thompson, M. Toledano, C. Nucci, A. Banerjee, Influence of air-abrasion executed with polyacrylic acid-Bioglass 45S5 on the bonding performance of a resin-modified glass ionomer cement, *Eur. J. Oral Sci.* 120 (2012) 168–177. <https://doi.org/10.1111/j.1600-0722.2012.00939.x>.
- [58] H.A. El-Deeb, E.H. Mobarak, Microshear Bond Strength of High-viscosity Glass-ionomer to Normal and Caries-affected Dentin Under Simulated Intrapulpal Pressure., *Oper. Dent.* 43 (2018) 665–673. <https://doi.org/10.2341/17-154-L>.
- [59] S. Mickenautsch, G. Mount, V. Yengopal, Therapeutic effect of glass-ionomers: an overview of evidence, *Aust. Dent. J.* 56 (2011) 10–5; quiz 103. <https://doi.org/10.1111/j.1834-7819.2010.01304.x>.
- [60] L. Tjäderhane, A. Tezvergil-Mutluay, Performance of Adhesives and Restorative Materials After Selective Removal of Carious Lesions: Restorative Materials with Anticaries Properties, *Dent. Clin. North Am.* 63 (2019) 715–729. <https://doi.org/10.1016/j.cden.2019.05.001>.
- [61] R.G. Palma-Dibb, C.G. de Castro, R.P. Ramos, D.T. Chimello, M.A. Chinelatti, Bond strength of glass-ionomer cements to caries-affected dentin, *J. Adhes. Dent.* 5 (2003) 57–62.

