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An in-vitro study investigating the effect of air-abrasion bioactive glasses on dental adhesion, cytotoxicity and odontogenic gene expression

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ABSTRACT

Objective. To assess the microtensile bond strength (MTBS) and interfacial characteristics of universal adhesives applied on dentine air-abraded using different powders. The analysis includes the cytotoxicity of the powders and their effect on odontogenic gene expression.
Methods. Sound human dentine specimens were air-abraded using bioglass 45S5 (BAG), polycarboxylated zinc-doped bioglass (SEL), alumina (AL) and submitted to SEM analysis. Resin composite was bonded to air-abraded or smear layer-covered dentine (SML) using an experimental (EXP) or a commercial adhesive (ABU) in etch&rinse (ER) or self-etch (SE) modes. Specimens were stored in artificial saliva (AS) and subjected to MTBS testing after 24 h and 10 months. Interfacial nanoleakage assessment was accomplished using confocal microscopy. The cytotoxicity of the powders was assessed, also the total RNA was extracted and the expression of odontogenic genes was evaluated through RT-PCR.
Results. After prolonged AS storage, specimens in the control (SML) and AL groups showed a significant drop in MTBS ($p > 0.05$), with degradation evident within the bonding interface. Specimens in BAG or SEL air-abraded dentine groups showed no significant difference, with resin-dentine interfaces devoid of important degradation. The metabolic activity of pulp stem cells was not affected by the tested powders. SEL and BAG had no effect on the expression of odontoblast differentiation markers. However, AL particles interfered with the expression of the odontogenic markers.

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Significance. The use of bioactive glass air-abrasion may prevent severe degradation at the resin-dentine interface. Unlike alumina, bioactive glasses do not interfere with the normal metabolic activity of pulp stem cells and their differentiation to odontoblasts.

1. Introduction

Operative dentistry has embraced the minimally invasive philosophy with dental restorations based on the development and use of advanced adhesive materials and/or "bioactive" components that are able to remineralise the dental hard tissues [1–5]. Bioactive glasses, which were formerly intended for use as biocompatible materials for bone regeneration [6,7], have been advocated as suitable powders for air-polishing and/or selective caries removal with dental air-abrasion [8,9]. The most common technique for carious tissue removal is based on the use of non-selective rotary burs in air turbine or electric micromotor handpieces. Nevertheless, air-abrasion has been promoted as an alternative method for minimally invasive cavity preparation and/or caries removal [3,10]. Air-abrasion is a kinetic method using a stream of high-speed abrasive particles to remove sound and carious dental tissues via an end-cutting process [11–13]. It is able to create a smooth cavity with indistinct walls and margins [3]. Moreover, air-abrasion does not cause excessive vibrational stress on tooth structures, so resulting in increased comfort and less pain for patients [14,15]. Alumina (aluminium oxide) is the most common abrasive powder used to date [10,16]. Its drawbacks relate to its indiscriminate efficacy in selectively removing caries-affected dental hard tissues [16,17]. Moreover, there are some controversial issues about health and safety, in particular in those patients with severe dust allergies and asthma, chronic lung disease and other respiratory disorders. Studies evidenced early signs of mild fibrosis and emphysema associated to the use of alumina for air-abrasion procedures [18–20]. To the best of the authors' knowledge, there is no information about the potential cytotoxicity of alumina on dental pulp stem cells and on odontoblast metabolism. Although there may be no direct correlation between cytotoxic activity on dental pulp stem cells and respiratory diseases, it has been observed that during air-abrasion procedures, the abrasive powders can penetrate several microns into patent dentine tubules [2,8]. When used in deep dentine, in close proximity to the pulp, such abrasive particles penetrating the tubules may react with dentine fluid and release ions, which might induce a response on the pulp and/or stem cells. Unfortunately, there is no available information about this possible scenario, thus it is relevant to test if conventional powders such as alumina, or bioactive glasses may have an effect on the metabolism of such type of cells (odontogenic gene expression).

There is a clinical need for more biocompatible air-abrasion powders with greater selective ability in removing caries-infected dentine alongside their bioactive properties, but that do not interfere with the adhesion of modern universal adhesives [17]. Recently, an innovative polycarboxylated zinc-doped bioactive glass has been developed (SELECTA Kinetic, Velopex International, London, UK) as a potential remineralising air-abrasion powder for selective caries removal in dentine and enamel. To date, there is limited information in the literature apart from its capability to induce the formation of apatite-like crystallites when used as a microfiller for resin adhesives and cements [2,5,21]. Carvalho et al. [22] reported that the use of air-abrasion with an experimental niobo-phosphate bioactive glass on dentine had no negative impact on the immediate bond strength of both self-etching and self-adhesive resin-based systems. Moreover, the use of bioglass 45S5 (Sylc^{TM}, OSspray Ltd., London, UK) or experimental PAA-doped bioglass 45S5 in air-abrasion procedures was demonstrated to produce a bioactive smear layer characterised by protective/reparative properties, preserving the integrity of the dentine-bonded interface and the bond strength created using self-etch adhesives (SEAs) or resin-modified glass-ionomer cements (RMGICs) [23,24]. Conversely, it was hypothesised that the residual presence of alkaline air-abrasion powders within the smear layer, such as bioglass 45S5 [2,23] or other biomaterials, may affect negatively the bonding performance of simplified self-etching adhesives [22]. Thus, it is necessary to evaluate the residual alkalinity in dentine interferes with the bonding performance of modern universal adhesives (UAS).

Progress in adhesive dental biomaterials development is represented by UAS, which are based on an "all-in-one" approach [21,24], but can be also used in self-etch, etch-and-rinse or selective-enamel etch mode for direct and indirect restorations [25–27]. This is due to the incorporation of mild and ultra-mild functional acidic monomers within the UAS composition, along with conventional cross-linking and non-acidic emulsifying monomers, light- or dual-curing catalysts. Moreover, definite solvents are also employed to enhance monomer diffusion and infiltration in substrates such as alloys and polycrystalline ceramics. In some cases, silanes are also incorporated within the composition of UAS to enhance their chemical interaction with glass-ceramic materials [28–30]. Thus, considering the important roles of UAS in minimally invasive operative adhesive dentistry, further examination of their bonding performance is required, especially when they are applied on dental substrates air-abraded using conventional and innovative powders.

The objective of this study was to evaluate the pH and scanning electron microscopy (SEM) ultramorphology of dentine after air-abrasion performed using Bioglass 45S5 (BAG), a novel polycarboxylated zinc-doped bioactive glass (SEL) or aluminium oxide (AL). The dentine microtensile bond strength (MTBS) at 24 h and after 10 months of storage in artificial saliva (AS), of two universal adhesives applied in self-etch (SE) or etch&rinse (ER) mode to air-abraded dentine, was assessed. The interfacial dye-assisted nanoleakage of the bonded inter-

faces was analysed using confocal laser-scanning microscopy (CLSM). The biocompatibility and the expression of genes related to odontoblast differentiation (ocn, dspp, dmp1, mepe) were also evaluated.

The first hypothesis was that the air-abrasion powders would not affect the bonding performance of the adhesive applied in SE or ER mode at 24 h. The second hypothesis was that the air-abrasion powders would not affect the bonding performance of the adhesive applied in SE or ER mode after 10-month aging in artificial saliva. The third hypothesis was that there would be no difference in biocompatibility and influence on gene expression between the tested air-abrasion powders.

2. Materials and methods

2.1. Preparation of dentine specimens and experimental design

One hundred twenty eight sound human molars extracted for periodontal or orthodontic reasons were collected according to the guidelines of the local ethics committee, under protocol number (CEI20/097) and stored in distilled water at 5 °C for no longer than 3 months. The roots were removed 1 mm beneath the cemento–enamel junction using a diamond blade mounted on a low-speed microtome under continuous water cooling (Remet evolution, REMET, Bologna, Italy). A second parallel cut was made to remove the occlusal enamel and mid-coronal dentine was exposed using 320-grit SiC papers. Four main groups (n = 32 specimens/group) were created based on the dentine air-abrasion pre-treatments performed prior to the bonding procedures: **SML**: no treatment-smear layer (320-grit SiC papers); **AL**: air-abrasion using aluminium oxide abrasive (29 μm); **BAG**: air-abrasion using Sylc Bioglass 45S5 (particle size 30–90 μm); **SEL**: SELECTA Kinetic, a polycarboxylated zinc-doped bioglass (particle size 20–60 μm). All powders were purchased from Velopex International, Harlesden, London, UK.

2.2. Air-abrasion, pH assessment and SEM ultramorphology

Air-abrasion was performed using an Aquacare air-abrasion unit (Velopex International, London, UK) under continuous distilled water irrigation at a pressure of 4 bar (400 MPa). The unit was equipped with a nozzle tip (Ø 0.6 mm), which was positioned at 1 cm and 90° to the dentine surface and operated for 1 min with continuous mesio-distal and bucco-lingual movements [31]; a plastic nozzle for water/powder delivery was used and regularly replaced. The reservoir of the abrasive powder was always filled up to a quarter of its capacity throughout the entire procedure [32]. After each air-abrasion treatment, specimens were rinsed with deionised water to remove from the dentine surface the remaining excess of debris. No further treatment was performed in order to simulate a clinical scenario where the powder may remain embedded on the dentine surface within the smear layer [2,5].

A further twelve sound third human molars were selected (n = 3 teeth/group) and a standardised, box-shaped occlusal Class I preparation (4 mm mesio-distal width ×1 mm bucco-lingual width ×3 mm deep), with margins located in the occlusal enamel and the cavity floor located in dentine, was prepared using a diamond bur mounted in a high-speed air turbine handpiece with water cooling. These cavity specimens were left untreated (control) or air-abraded using the test powders as described previously. These were then rinsed with distilled water and desiccated overnight in a hermetic chamber containing absorbent silica. The specimens were subsequently critical-point dried and mounted on aluminium stubs with carbon cement, gold-sputter-coated and analysed using field-emission scanning electron microscopy (FE-SEM S-4100; Hitachi, Wokingham, UK) at 10 kV and at different magnifications in order to qualitatively study the ultramorphological features resulting from the air-abrasion procedures.

Two sound extracted human molars per group (SML, AL, BAG, SEL) were used to create dentine discs (1 mm thickness), which were prepared and air-abraded (3 discs/treatment) as previously described in Section 2.1. The specimens were rinsed thoroughly with distilled water for 10 s, immersed individually in 15 mL of distilled water (pH 6.8) and stored for 24 h at 37 °C in polypropylene sealed containers. The pH of the media was then evaluated in triplicate for each specimen using a professional pH electrode (Mettler-Toledo, Leicester, UK) at room temperature in order to evaluate the alkalinisation ability of the tested powders.

2.3. Bonding procedures and resin composite build-up

A resin blend was prepared by mixing 25 wt% urethane-dimethacrylate (UDMA), 10 wt% bisphenol-A-diglycidyl-methacrylate (BisGMA), 7 wt% decandiol dimethacrylate (DECMA), 6.5 wt% hydroxyethyl-methacrylate (HEMA), 20 wt% deionised water, 30 wt% absolute ethanol and 3 wt% photoinitiation system. The photosensitive activator used in this resin was camphoroquinone (CQ, 0.5 wt%) and ethyl 4-dimethylaminebenzoate (EDAB, 1 wt%) was used as the co-initiator. An acidic functional monomer (glycerol-dimethacrylate-phosphate (GDMA-P; Yller Biomaterials, Pelotas - RS, Brazil), was added in 15 mol% [32] to finally create an experimental universal adhesive (**EXP**), which was used a control material with a known composition to be used in self-etching (**SE**) and in etch-and-rinse (**ER**) mode.

A commercial universal adhesive system was also used, All Bond Universal (ABU; Bisco, Schaumburg, IL, USA) and applied both in SE and ER mode. In groups **ABU–ER** and **EXP-ER** (8 teeth/each group), the dentine was etched with 35% orthophosphoric acid (Ultra-Etch, Ultradent, South Jordan, UT, USA) for 15 s and subsequently rinsed with distilled water (15 s) and blotted, leaving the dentine substrate moist. The adhesive was applied on the surface and agitated for 10 s, followed by gently air-drying for 5 s to evaporate the solvent. In groups **ABU–SE** and **EXP-SE** (8 teeth/each group), the adhesive was firstly agitated for 20 s on the dentine surface and then air-dried for 5 s to evaporate the solvent. A final light-curing procedure was performed for 10 s using a LED light source (>1000 mW/cm^2) (Radii plus, SID Ltd., Bayswater VIC, Australia). The specimens were finally restored using a micro-

Table 1 – Materials used in this study.

Code	Adhesives	Main components	pH	Manufacturer
ABU	All-Bond Universal	10-MDP, HEMA, BisGMA, ethanol, water, photoinitiator	Ultra-mild 3.1	Bisco, Schaumburg, IL, USA
EXP	Experimental adhesive	UDMA, BisGMA, GDMA-P, DECMA, HEMA, CQ, EDAB, deionised water, absolute ethanol,	Mild 2.4	Experimental

Resin composite	Main components	Manufacturer
Clearfil AP-X	bis-GMA, TEGDMA, silane barium glass filler, silane silica filler, silanated colloidal silica, CQ, pigments, others	Kuraray Noritake Dental

Dentine conditioning mode	Pre-etching procedure
SE (self-etch)	Phosphoric acid pre-etching was not performed. The dentine surface air-dried up to remove all the excess of water from the surface. The adhesive was brushed for 20 s and air dried for 5 s to evaporate the solvent until the adhesive no longer moves on the surface
ER (etch-&-rinse) *(Ultra-Etch, 35% phosphoric acid Ultradent, South Jordan, UT, USA)*	Dentin surface was phosphoric acid etched for 15 s. Etched surface was rinsed with water for 15 s (three-way dental syringe) and air-dried up to remove all the excess of water from the surface. The adhesive was brushed for 10 s and air dried for 5 s to evaporate the solvent until the adhesive no longer moves on the surface

UDMA: urethane-dimethacrylate, bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate, DECMA: Decandiol dimethacrylate, CQ: *dl*-camphoroquinone, EDAB: ethyl 4-dimethylaminebenzoate.

hybrid dental resin composite (Clearfil AP-X, Kuraray Noritake, Tokyo, Japan) in 2 mm increments up to 6 mm and light-cured as per manufacturer's instructions. All the materials used in this study along with their mode of application are listed in Table 1.

At this point, the specimens in each adhesive sub-group were further divided into two sub-groups (n = 4 specimens/group) based on the aging protocol: **T0** - control, (24 h in deionised water); **T10** - water storage (10 months in artificial saliva). The composition of the artificial saliva (AS) was 0.103 g L^{-1} of $CaCl_2$, 0.019 g L^{-1} of $MgCl_2 \times 6H_2O$, 0.544 g L^{-1} of KH_2PO_4, 30 g L^{-1} of KCl, and 4.77 g L^{-1} HEPES (acid) buffer, pH 7.4 [33].

2.4. *Micro-tensile bond strength and failure modes*

All the specimens created as previously described were sectioned using a precision diamond low speed saw (Remet evolution, REMET, Bologna, Italy) in both X and Y planes across the resin-dentine interface, obtaining approximately 20 matchstick-shaped specimens from each tooth, with cross-sectional areas of 0.9 mm^2. These were stored in AS for 24 h or 10 months as previously mentioned and subsequently subjected to MTBS to evaluate their bonding performance. This was performed using a microtensile bond strength device 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 and classified as a percentage of adhesive (A), mixed (M) or cohesive (C) failures.

The normality of MTBS data was evaluated using Shapiro-Wilk test ($p > 0.05$). Homogeneity of variance was calculated using the Brown–Forsythe test. For all tests, the variances were homoscedastic ($p > 0.05$). Data were then analysed using a three-way Analysis of Variance (ANOVA Factors: bonding system, dentine treatment and aging protocol) and Newman–Keuls multiple-comparison test ($\alpha = 0.05$). SPSS V16 for Windows (SPSS Inc., Chicago, IL, USA) was used.

2.5. *Ultramorphology of bonded-dentine interfaces – confocal microscopy*

One dentine-bonded slab specimen (Ø 0.9 mm^2) was selected from each experimental sub-group (n = 8) during the cutting procedures to obtain the match-sticks for MTBS testing. These were coated with a fast-setting nail varnish, applied 1 mm from the bonded interface. They were immersed in a rhodamine-B (Sigma Chemicals) water solution (0.1 wt%) for 24 h. Subsequently, the specimens were ultrasonicated with distilled water for 5 min and then polished for 30 s each side with a 500-grit and subsequently with 2400-grit SiC paper. The specimens were ultrasonicated again in distilled water for 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 optically section the specimens to a depth of up to 20 μm below the surface [34]. 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 investigation. 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 [35,36].

2.6. Cell isolation and magnetic-activated cell sorting

Human dental pulp cells (hDPSC) were enzymatically isolated from unerupted third molars (adults 18–22 years of old) as described in the literature [37,38]. The teeth were obtained in accordance with the local ethics legislation (including informed consent and institutional review board approval of the protocol number 7413). In order to obtain STRO-1$^+$ stem cells, hDPSCs were directly sorted from pulp cell cultures at passage 3 with mouse anti–human STRO-1 IgM (Life Technologies, Milan, Italy) with immune magnetic beads, according to the manufacturer's protocol (Dynabeads; Life Technologies). After cell sorting, each of the following experiments was performed in triplicate on pooled STRO-1–sorted cells (STRO-1$^+$ cells).

2.7. Cell viability

Prior to performing the viability assay, particles were immersed for 24 h in α-MEM serum-free at a concentration of 5 mg/mL at 37 °C. Following centrifugation, the tested air-abrasion particles (AL, BAG and SEL) were re-suspended in cell expansion media to 2.5 w/v %, vortexed to break the agglomerates and further diluted in expansion medium to 0.1, 0.5, 1, and 2.5 w/v %. Each dilution of 0.5 mL was added on a Transwell® insert (6.5 mm Transwell® with 0.4 μm pore polycarbonate membrane; Corning) and placed into a 24-well plate. Cell proliferation was evaluated at 1, 4, and 7 days using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) proliferation assay according to the manufacturer's instructions (Sigma-Aldrich). Briefly, at each time point, MTT solution was added to each well and incubated for 4 h at 37 °C and 5% CO_2. Absorbance was measured at 570 nm using a microplate reader (Cytation 3; AHSI, Milan, Italy). Cells cultured on tissue culture polystyrene were used as the control. The experiment was repeated 3 times and the mean value calculated.

2.8. Odontogenic-related gene expression of STRO-1$^+$ cells

Total RNA was extracted from STRO-1$^+$ cells seeded in the presence of the tested air-abrasion particles for 28 days, using TRIzol reagent (Invitrogen, Milan, Italy) according to Toledano et al. [37] Total RNA (0.2 μg) was first treated at 37 °C for 30 min with DNase (Promega, Milan, Italy) and then subjected to reverse transcription (RT) with 0.4 μg random hexamers and 20 U AMV reverse transcriptase (Promega) in a 25-μL reaction mixture at 42 °C for 1 h. The resulting mixture was amplified by real-time polymerase chain reaction (PCR) using specific primers for osteocalcin (*ocn*), matrix extracellular phosphoglycoprotein (*mepe*), dentine sialophosphoprotein (*dspp*), dentine matrix protein 1 (*dmp1*), and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) (Table 2).

Real-time polymerase chain reaction (RT-PCR) assays were achieved using an Opticon-4 machine (Bio-Rad, Milan, Italy). The reactions were performed according to the manufacturer's instructions using SYBR Green PCR Master mix (Invitrogen). The PCR conditions were as follows: AmpliTaq Gold DNA Polymerase (Life Technologies) activation for 10 min at 95 °C and 40 cycles at 95 °C (denaturation) for 15 s and 60 °C (annealing/extension) for 1 min. All reactions were executed in triplicate and were normalized to the control gene, *gapdh*. Relative differences in the PCR results were calculated using the comparative cycle threshold (CT) method. The variations in gene expression are given as arbitrary units.

All quantitative data were presented as the mean ± SD. Each experiment was performed at least 3 times. Student's *t* test was used for the fluoride release. Statistical analyses for the cytotoxicity test, cell migration assay, and quantitative real-time PCR were performed by 1-way analysis of variance (ANOVA) with Bonferroni's post hoc test.

3. Results

3.1. Micro-tensile bond strength, failure mode and SEM analysis

The SEM ultramorphological analysis performed on the specimens air-abraded with the tested powders showed that the dentine and enamel surfaces were always characterised by smoother walls and indistinct margins compared to the specimens prepared only with burs (Fig. 1). The storage media of specimens treated with AL and those untreated (SML) of the control group had, after 24 h, an average pH of 6.6 (±0.33) and 7.1 (±0.29), respectively. Conversely, the storage media of the specimens treated with BAG or SEL had an average pH of 8.6 (±0.37) and 7.9 (±0.31), respectively.

The results of the microtensile bond strength tests (mean and ±SD) and failure mode analysis are presented in Table 3. There were no pre-test failures before MTBS in most of the groups, excluding those created with the experimental adhesive (EXP) applied in SE mode (range: 5–9%). Three-way ANOVA revealed a significant effect on the bond strength of the adhesive system ($F = 16.58$, $p < 0.001$), dentine treatment ($F = 21.35$, $p < 0.001$) and aging protocol ($F = 18.27$; $p < 0.001$). The interactions between the three variables were significant ($p < 0.001$).

The overall bonding performance at 24 h of the adhesives applied in ER mode depended on the type of the adhesive rather than the dentine pre-treatments. Indeed, ABU showed always the greatest bond strength results (MPa) at 24 h (t0) in all types of dentine pre-treatments (SML, BAG, AL, SEL ($p < 0.05$)). Also the number of adhesive failures detected with EXP ER as well as EXP SE, both at 24 h (t0) and 10 months (t10) in AS storage was always numerically higher than those of ABU ER and ABU SE. However, the only specimens created in ER mode using both ABU and EXP to show no significant drop in bond strength after 10 months of storage in AS ($p > 0.05$) were those created in dentine air-abraded with the two bioactive powders (BAG and SEL). Conversely, those created in SML dentine or AL showed a significant drop in MTBS ($p > 0.05$). Likewise, all the groups of specimens created with EXP applied in ER mode showed at 24 h (t0) no significant difference ($p > 0.05$) between the groups of different dentine pre-treatments. Also in this case, the specimens that showed no significant drop ($p > 0.05$) in bond strength after 10 months of storage in AS were those created in dentine air-abraded with BAG and SEL. Both adhesives applied in ER mode failed primarily in adhesive and mixed mode in all pre-treatment groups at 24 h, but with an

Table 2 – Sequence of primers used in Real time-polymerase chain reaction.

Genes	Forward primers (5′-3′)	Reverse primers (5′-3′)
ocn	CATTGCAGGTCTCCTGGAACAA	TTAGCATCGGTGGTTTCCGTTC
mepe	GTCTGTTGGACTGCTCCTCTT	CACCGTGGGATCAGGATACA
dspp	AATGGGACTAAGGAAGCTG	AAGAAGCATCTCCTCGGC
dmp1	TGGGGATTATCCTGTGCTCT	TACTTCTGGGGTCACTGTCG
gapdh	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

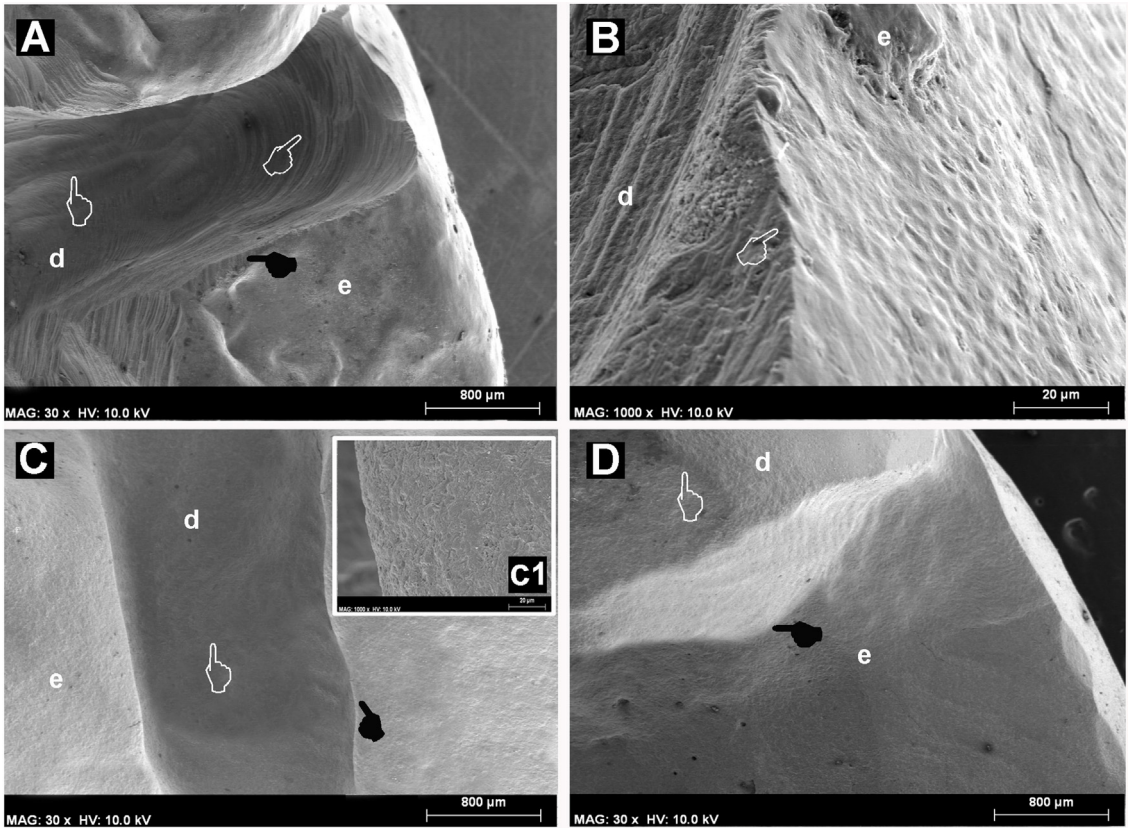

Fig. 1 – SEM micrographs of the dental cavities prepared with diamond burs and subsequently air-abraded with the tested powders. (A) it is possible to see that the control specimens created with diamond burs only presented a rough dentine (d) surface characterised by the presence of several neat margins and scratch-like irregularities (white pointer), while the enamel presented edges clearly irregular (black pointer). At higher magnification those enamel margins appeared visibly jagged (B). (C) This is a representative image of the specimens air-abraded with the bioglasses employed in this study; in this specific case, the use of zinc-doped polycarboxylated bioglass (SEL) left the both dentine (open pointer) and in enamel (black pointer) surfaces smooth and devoid of clear irregularities. At higher magnification it is possible to see that an edge-free enamel, totally rounded and smooth (C1). (D) Also in this specimen air-abraded with alumina, it is possible to detect dentine (white pointer) and enamel (black pointer) walls with smooth margins.

increase in number of adhesive failures after 10 months of storage in AS.

A similar trend of results was observed in the specimens created with ABU in SE in all pre-treatment groups both at 24 h (t0) and after prolonged storage (t10). However, the only exception was encountered in the group ABU SE in dentine pre-treated with BAG at t0; a significant lower bond strength ($p < 0.05$) was detected in this group (BAG) when compared to the other pre-treatment groups (SML, AL, SEL). After prolonged storage in AS, none of the ABU SE groups showed a significant drop in MTBS ($p > 0.05$). The EXP adhesive applied in SE mode onto dentine air-abraded with BAG or SEL showed significantly lower bond strength values at 24 h ($p < 0.05$) compared to all the other groups created with the same adhesive. However, these specimens created in dentine air-abraded with the bioactive glasses BAG or SEL, showed no significant difference after storage in AS for 10 months, while those specimens created in dentine air-abraded with AL or created in smear layer-covered dentine had a significant drop in bond strength values ($p < 0.05$) after storage in AS for 10 months. The EXP adhesive applied in ER mode failed prevalently both in adhesive and mixed mode in all pre-treatment groups, with a numerical increase in adhesive failures after prolonged storage in AS (t10). Conversely, The EXP adhesive applied in SE failed prevalent in adhesive mode both at baseline (t0) and after prolonged storage (t10) in all pre-treatment groups, although the num-

Table 3 – The results show the mean (SD) of the MTBS (MPa) to dentine and the percentage (%) of the failure mode analysis and pre-test fail.

	SML t0	SML t10	BAG t0	BAG t10	Al t0	Al t10	SEL t0	SEL t10
ABU ER	40.2(5.4)^{a1} [33/42/25] (0%)	31.8(4.9)^{a2} [54/38/8]	39.4(4.7)^{a1} [29/58/13] (0%)	35.1(5.7)^{a1} [46/54/0]	40.3(4.3)^{a1} [33/40/22] (0%)	32.3(5.9)^{a2} [48/45/7]	39.7(4.9)^{a1} [28/59/13] (0%)	38.6(5.2)^{a1} [44/56/0]
ABU SE	30.8(5.6)^{b1} [41/54/5] (0%)	27.4(6.3)^{a1,2} [58/42/0]	24.8(4.9)^{b2} [55/45/0] (0%)	22.2(5.8)^{b2} [62/38/0]	31.8(6.1)^{b1} [39/53/8] (0%)	32.4 ± 6.6^{a1} [49/51/0]	34.1(5.2)^{a1} [41/54/5] (0%)	33.2(6.4)^{a1} [52/46/0]
EXP ER	31.1(5.3)^{b1} [58/35/7] (0%)	22.8(5.9)^{b2} [74/26/0]	27.1(5.5)^{b1,2} [60/38/2] (0%)	24.4(6.9)^{b2} [69/31/0]	33.7(6.2)^{b1} [51/46/3] (0%)	21.9(6.7)^{b2} [72/28/0]	28.7(6.1)^{b1,2} [55/41/4] (0%)	26.9(7.1)^{b1,2} [61/39/0]
EXP SE	26.2(5.8)^{b1,3} [78/22/0] (7%)	14.9(6.5)^{c2} [95/5/0]	16.2(5.9)^{c2} [83/17/0] (9%)	15.1(6.2)^{c2} [91/9/0]	30.2(7.1)^{b1} [58/35/7] (3%)	23.1(7.8)^{b3} [75/25/0]	18.9(6.8)^{c2} [76/24/0] (5%)	15.8(7.3)^{c2} [78/22/0]

Smear layer (SML); Bioglass 45S5 (BAG); Alumina 29 μm (Al); Polycarboxylated zinc-doped bioglass (SEL); All Bond Universal (BISCO) (ABU); Experimental adhesive (EXP); Etch and rinse (ER); Self-etching (SE).
Pre-test failure (%). Since the number of pre-failure in all groups was <10%, these 0 values were not included in the statistical analysis.
% Failure mode [A/M/C].
The same lowercase letter indicates no differences in columns ($p > 0.05$).
The same number indicates no significance in rows ($p > 0.05$).

ber of specimens failed in adhesive mode after 10 months was numerically higher compared to those observed at 24 h.

3.2. *Ultramorphology of the bonded-dentine interfaces – confocal microscopy*

The baseline (t0) confocal reflection/fluorescence images of the resin-dentine interfaces created using the tested adhesives (ABU, EXP) applied in etch and rinse (ER) or self-etch (SE) mode on dentine pre-treated with different methods (SML, AL, BAG, SEL) are depicted in Fig. 2

.

Overall, results indicated that both ABU and EXP applied in ER mode on the control smear layer-covered dentine (SML) created a resin-dentine interface often characterised by a porous hybrid layer, totally infiltrated by the fluorescent rhodamine-B dye (Fig. 2A). Likewise, both ABU and EXP applied in SE mode onto the SML dentine created a bonding interface characterised by an interdiffusion layer slightly infiltrated by the fluorescent dye (Fig. 2B). A different scenario was obtained when applying the adhesives in ER or SE mode on dentine air-abraded with the different air-abrasion powders used in this study. Such treatment caused a total or partial obliteration of dentine tubules, reducing the porosity within the hybrid layer. For instance, the resin-dentine interface created with the ABU adhesive applied in ER mode on the dentine air-abraded with alumina was characterised by a hybrid layer only slightly infiltrated by the rhodamine-B (Fig. 2C). This absence of fluorescence within the resin-dentine interface was even more evident in the specimens created using both adhesives in SE mode applied on the dentine air-abraded with BAG 45S5 (Fig. 1D) or with bioglass SEL (Fig. 2F). Likewise in the case of EXP adhesive applied in ER mode on the dentine air-abraded with bioglass SEL, it was possible to observe a resin-dentine interface with a hybrid layer completely devoid of dye infiltration (Fig. 2E).

Different outcomes were observed with the resin-dentine interface created with the ABU and EXP applied on smear layer-covered dentine (SML) in specimens stored in AS for 10 months (Fig. 3

). The two tested adhesives applied in ER mode showed the presence of a gap at the bonding interface (Fig. 3A1); dye uptake within the entire adhesive layer of the EXP adhesive was detected both in ER or SE mode (Fig. 3A2). Conversely, the ABU adhesive applied in SE mode showed no sign of degradation, but only some dye accumulation at the interface (Fig. 3A3). The resin-dentine interfaces created with the universal adhesives applied in ER mode on dentine pre-treated with air-abrasion and BAG 45S5 were characterised by the presence of a slight dye infiltration within the hybrid layer, but no sign of evident degradation for the ABU group (Fig. 3B1). Conversely, the specimens in the EXP group often showed gaps and evident dye uptake within adhesive layer (Fig. 3B2). The resin-dentine interface created with the tested adhesives applied in SE mode presented no presence of gaps or degradation at the bonding interface, although the experimental (EXP) adhesive was fully infiltrated by the fluorescent dye (Fig. 3B2).

The resin-dentine interface created with the tested adhesives applied in etch and rinse mode (ER) on the dentine air-abraded with alumina (AL) were characterised by porous hybrid layers infiltrated by fluorescent dye (Fig. 3C1), while the EXP adhesive presented gaps, with degradation of the hybrid layer and dye uptake within the adhesive layer (Fig. 3C2). Conversely, the tested adhesives applied in SE mode on the dentine air-abraded with AL showed no sign of degradation and/or gaps at the interface, but clear dye infiltration within the EXP adhesive (Fig. 3C3).

Similar to the specimens created in dentine air-abraded with BAG, those created in dentine pre-treated with the innovative polycarboxylated zinc-doped bioglass (SEL) and bonded with the ABU adhesives in ER mode, showed a resin-dentine interface with low level dye infiltration at the hybrid layer with no sign of degradation (Fig. 3D1). However, the resin-dentine interface created with the EXP adhesives applied in ER mode showed a gap-free interface, although there was dye infiltration within the adhesive layer (Fig. 3D2) observed in the

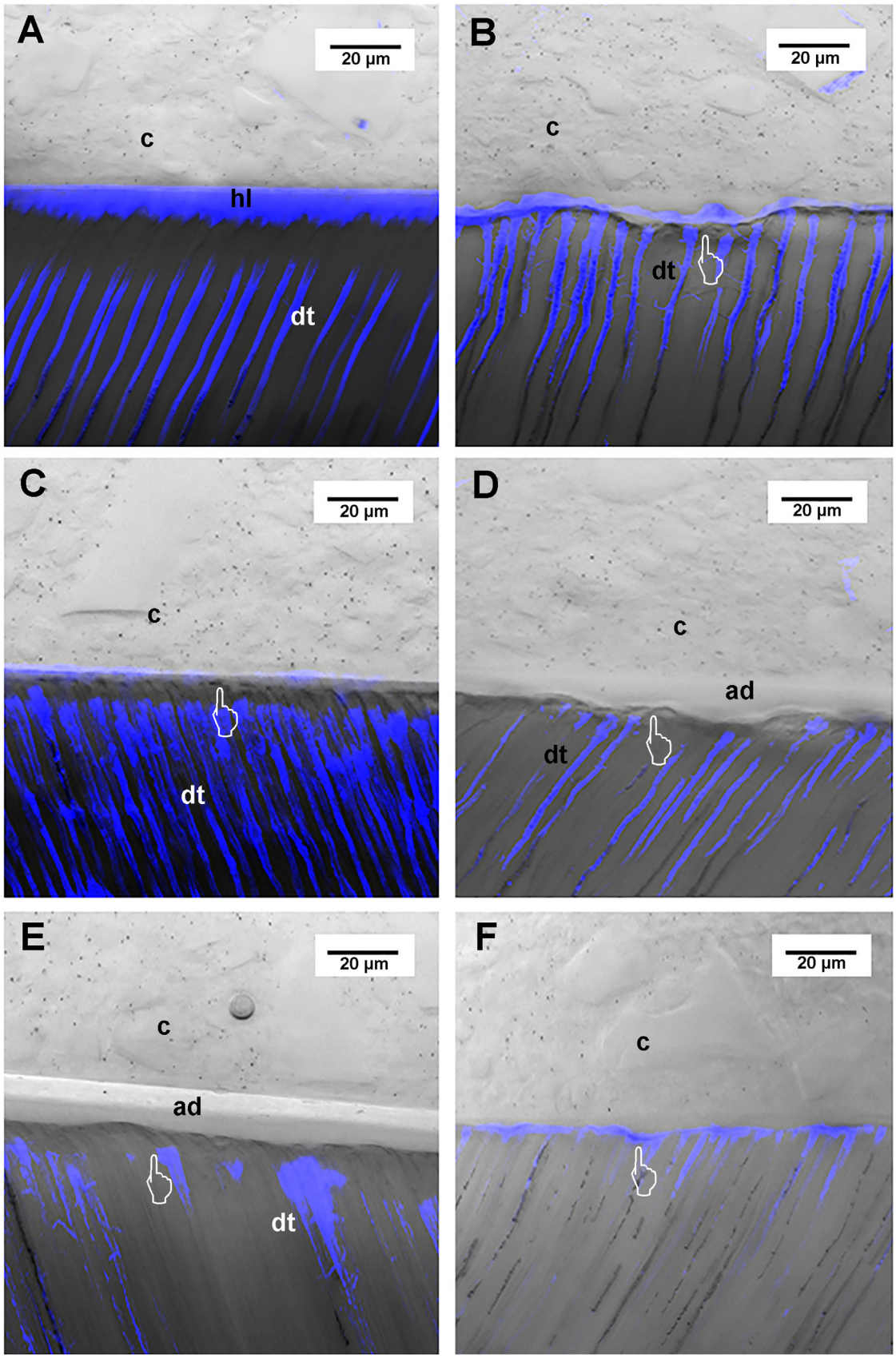

Fig. 2 – Confocal reflection/fluorescence single projection images (Baseline t0) of the bonding interfaces created using the tested universal bonding systems applied in etch and rinse or self-etch mode on dentine pre-treated with different methods. (A) It is possible to see the resin-dentine interface created with the universal adhesive ABU in etch and rinse

other groups. Once more, the resin-dentine interface created using the two tested adhesives applied in SE mode on SEL air-abraded dentine was characterised by reduced dye permeability at the interface, with no gap or signs of degradation (Fig. 3D3).

3.3. *Cell viability and odontogenic-related gene expression of STRO-1⁺ cells*

The metabolic activity of hDPSCs was not jeopardised by the presence of any concentration of SEL, AL or BAG particles, at each time point evaluated in this study ($P > 0.05$) (Fig. 4); in other words, none of the tested air-abrasion powders resulted in cytotoxicity.

The results evaluating the expression of genes related to odontoblast differentiation (*ocn*, *dspp*, *dmp1*, *mepe*), where real-time PCR was performed on STRO-1⁺ cells cultured in presence of SEL, AL or BAG over a period of 28 d, are depicted in Fig. 5. It was possible to observe that the presence of SEL or BAG particles had no negative affect on the early expression of *mepe* and late odontoblast differentiation markers (*ocn*, *dspp*, and *dmp1*). In contrast, AL particles interfered with the correct modulation of genes involved in the odontoblast differentiation. Indeed, *mepe* was upregulated ($p < 0.001$) throughout the experiment disrupting STRO-1⁺ proper transition between immature and mature odontoblasts. In addition, the level of *ocn*, *dmp1*, and *dspp* inadequately increased ($p < 0.001$) over the 28 days inhibiting STRO-1⁺ dentine matrix formation.

4. Discussion

The ultramorphological data of all the specimens air-abraded using AL, BAG or SEL, characterised by the presence of smoother dentine walls and indistinct margins compared to the those prepared with burs, are in accordance with those presented by Banerjee A. in 2013 [3], who reported that the most relevant morphological characteristic of a cavity prepared by air-abrasion is a rounded-shape and halo contour; saucer-shaped cavities with indistinct walls and margins were also previously identified [39,40]. It has been reported that cavities characterised by rounded internal line angles would have less stress concentration on the bonding interface, as a result of a reduced C-factor [3,41]. Conversely, cavities prepared with conventional burs are characterised by greater surface roughness, so when they are restored using traditional resin composites, fatigue failure might occur as a result of the masticatory load, especially if the magnitude of the stress at the interface is adequate to trigger crack propagation [42,43].

The results of this study showed that although the ABU system had a greater bonding performance, both adhesives employed in this study exhibited failure patterns mainly in mixed and cohesive mode with no significant difference in bond strengths between the groups (t0 - baseline). An interesting observation during confocal interfacial analysis in those specimens pre-treated with air-abrasion, was the presence of dentine tubules still occluded by the powders in those specimens bonded in self-etching mode (Fig. 2). The ability of bioactive glasses to occlude dentine tubules and resist acid attack has been reported in previous studies [22,23,44]. However, the tested adhesives applied in SE mode produced a significantly lower bond strength in those specimens that were air-abraded with BAG, while the experimental (EXP) adhesive applied on dentine air-abraded with SEL also had a lower bond strength ($p > 0.05$) compared to the groups EXP-SML and EXP-AL. It is hypothesised that in such a scenario, the adhesive performance may have been influenced by the alkalinity of BAG and SEL, which interfered with the bonding ability of the acidic functional monomers of the tested adhesives. Indeed, the current results showed that the storage media of the specimens treated with BAG or SEL had an average pH of 8.6 (±0.37) and 7.9 (±0.31), respectively. Although none of the treatments generated resin-dentine interfaces characterised by gaps, a possible explanation of the fact that SEL had no effect on the bond strength of ABU-SE compared to EXP-SE may be related to the composition of the two adhesives, in particular on the type of functional monomer within their formulations. It is important to anticipate that the reason why an experimental adhesive was formulated and used in this study was to have a control material with a well-known composition, which could allow a better understanding of the main factors influencing the results of the study. Indeed, it was formulated, through pilot investigations, with a specific composition to perform as a control "lower-performance" system due to its relatively high hydrophilicity and due to the use of the functional monomer glycerol-dimethacrylate-phosphate (GDMA-P) [45] rather than 10-methacryloyloxydecyl dihydrogen phosphate (MDP). The latter has been advocated as a chief constituent to achieve a reliable bond in dentine and enamel [45,46]. However, the bonding performance of MDP depends

mode (ER) applied in control smear layer-covered dentine (SML) is characterised by an hybrid layer (HL) and dentinal tubules (dt) totally permeable to fluorescent dye. (B) This is the interface created with the experimental universal adhesive EXP applied in in self-etch mode on the control smear layer-covered dentine (SM) where it is possible to see an interdiffusion layer (pointer) as well as the dentinal tubules (dt) permeable to fluorescent dye. (C) The resin-dentine interface created with ABU applied in ER mode on the dentine air-abraded with alumina (AL) is characterised by a hybrid layer slightly permeable to the fluorescent dye; this fills the tubules (dt) only up to certain level, several microns away from the HL (pointer). (D) Same situation for the resin-dentine interface created with EXP adhesive applied in SE mode on the dentine air-abraded with BAG 45S5, which is characterised by a bonding interface free from dye infiltration (pointer) and with few tubules (dt) filled with fluorescent dye. (E) The resin-dentine interface created with the EXP adhesive applied in ER mode on the dentine air-abraded with the bioglass SEL is characterised by a hybrid layer totally free from the fluorescent dye (pointer), but only very few tubules (dt) are filled with fluorescent dye. (F) the interdiffusion area of the interface created with the EXP adhesive applied in SE mode onto dentine pre-treated with bioglass SEL is partially permeable, although only few tubules were filled by the fluorescent dye(pointer).

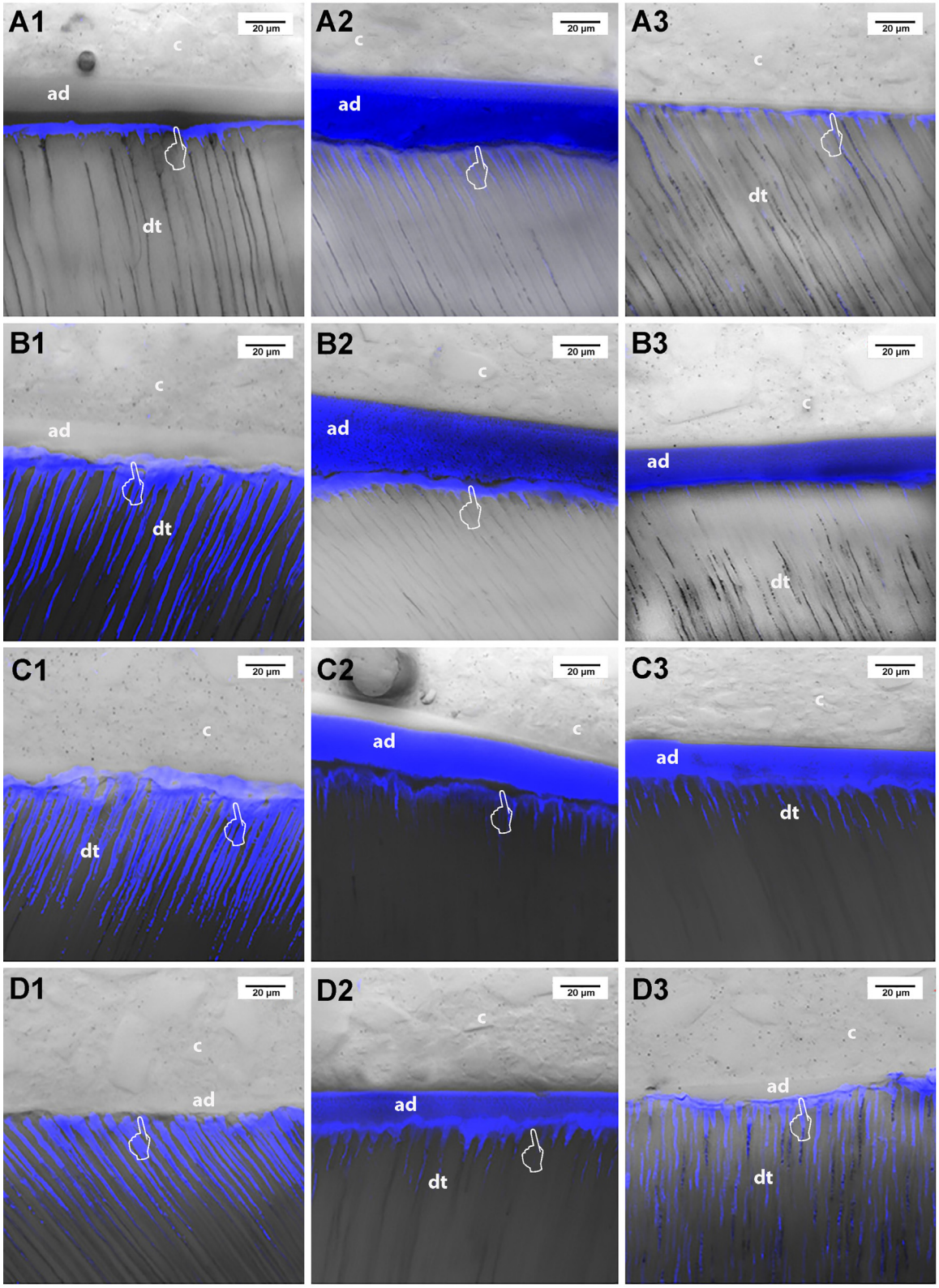

Fig. 3 – Confocal reflection/fluorescence single projection images (10 months of AS storage) of the bonding interfaces created using the tested universal bonding systems applied in etch and rinse or self-etch mode on dentine pre-treated with different methods. (A1) It is possible to see the resin-dentine interface created with the universal adhesive ABU in etch and rinse mode (ER) applied in control smear layer-covered dentine (SML) characterised by the presence of a clear gap between the adhesive (ad) and the dentine (dt), as a consequence of a severe degradation of the hybrid layer (pointer). (A2) The resin-dentine interface created with the experimental (EXP) adhesive applied in ER mode on SML dentine is characterised also in this case by the presence of a gap at the interface (pointer) and an evident dye uptake within the adhesive layer (ad).

on its degree of purity [47] and on the type of solvents and other additives employed within the adhesive's composition [48–50]. Moreover, the pH of the adhesive is also an important aspect to consider, as it needs to oscillate in a range of 2.5–3 in order to achieve the most effective chemical interaction with the dentine [51].

Although the pH of the bioactive glasses used in this study may have influenced the immediate (t0) bond strength of the adhesives applied in SE at t0, after prolonged storage (t10) in artificial saliva (AS), it is hypothesised that their residual presence in dentine tubules and within the smear layer may have a protective effect on the degradation of the resin-dentine interface [2]. Indeed, the current study showed that both adhesives applied in ER mode on dentine air-abraded with BAG or SEL had no significant drop in bond strength compared to baseline (t0). Conversely, the EXP adhesive presented signs of degradation at the interface probably induced by severe water sorption [44,52,53]. In SE mode, the degradation of the hybrid layer is usually less drastic [53,54] compared to ER mode. ABU showed no significant drop in bond strength and no degradation was detected at the resin-dentine interface after prolonged storage in AS (t10) (Fig. 2). EXP showed no significant drop in bond strength at t10 only when applied on dentine air-abraded with BAG or SEL. A possible explanation for such results obtained in dentine air-abraded using BAG or SEL may be related to their "bioactive" effects on the resin-dentine interface. However, it is important to consider that the two main mechanisms involved in the degradation of the resin-dentine hybrid layer are: (i) intrinsic proteolytic degradation of the organic matrix by matrix metalloproteinases (MMPs) and cysteine cathepsins; (ii) hydrolytic extrinsic degradation of the resin matrix. Such mechanisms occur simultaneously, jeopardising the longevity of resin-dentine bonds [55,56]. However, there is general consent that collagen degradation is more evident in adhesives applied in ER mode due to phosphoric acid etching, which totally demineralises dentine collagen, making it more susceptible to proteolytic degradation [57–59]. Conversely, milder SEAs cause degradation to a lesser extent compared to ER adhesives as they do not totally expose dentine collagen fibrils or the small smear plugs within the dentine tubule orifices [55,60]. Unfortunately, no dental dimethacrylate in simplified adhesives can completely infiltrate the water-filled spaces within acid-etched dentine and create a "high quality" and durable hybrid layer [55,61–63]. Furthermore, the water within the demineralised collagen fibrils, in particular in acid-etched dentine, is primarily responsible for phase separation [56,63], nanoleakage [55,64,65] and the poor degree of polymer conversion [66,67]. Unlike intrinsic collagen degradation of the hybrid layer mediated by proteases, the hydrolysis of a poorly polymerised resin matrix is also related to the degree of water sorption in simplified SE and ER adhesives [67], which induces polymer swelling and plasticisation of the resin matrix [68], as well as to the infiltration of salivary esterase [69].

It was assumed previously that when performing dentine air-abrasion using BAG, it is possible to generate a "bioactive" smear layer [2] which can be incorporated into the interface created with glass-ionomer cements or SE adhesives, so helping to reduce the degradation processes over time [23,24]. In accordance with the results of the current study, it seems that air-abrasion performed with alumina has no negative effect on the immediate bonding performance of SE adhesives, but its use may compromise the bonding longevity after prolonged water storage [70]. A possible mechanism for protection of the hybrid layer offered by BAG can be associated with the hydrated silica $Si(OH)_4$ produced once in contact with fluids (e.g. water, saliva, blood), which subsequently may diffuse and bind non-specifically to the demineralised, poorly resin-impregnated collagen [71]. It condenses via polymerisa-

(A3) The resin-dentine interface created with the ABU adhesive applied SE mode in SML dentine present no sign of degradation, but only some dye accumulation at the bonding interface (pointer) between the composite (c) and dentine (dt). (B1) It is possible to see the resin-dentine interface created with the universal adhesive ABU in etch and rinse mode (ER) applied on the dentine air-abraded with BAG 45S5, which is characterised by the presence of a slight dye within the hybrid layer, but with no sign of evident degradation (pointer) between the adhesive (ad) and dentine (dt). (B2) The resin-dentine interface created with the experimental (EXP) adhesive applied in ER mode on BAG air-abraded dentine is characterised in this case by the presence of a gap at the interface and an evident dye uptake within the hybrid layer (pointer) and the adhesive layer (ad). (B3) The resin-dentine interface created with the experimental (EXP) adhesive applied in SE mode on BAG air-abraded dentine is characterised by the presence of a clear dye permeability within the adhesive (ad), but with no sign of gap or evident degradation at the bonding interface (pointer). (C1) It is possible to see the resin-dentine interface created with the universal adhesive ABU in etch and rinse mode (ER) applied on the dentine air-abraded with alumina (AL), which is characterised by the presence of a clear dye infiltration at the hybrid layer (pointer) and at the adhesive (ad) layer. (C2) The resin-dentine interface created with the experimental (EXP) adhesive applied ER mode on the dentine air-abraded with alumina (AL) is characterised by the presence of a gap degradation (pointer) at the interface with an evident dye uptake within the adhesive layer (ad). (C3) The resin-dentine interface created with the experimental (EXP) adhesive applied in SE mode on AL air-abraded dentine is characterised by the presence of a clear dye permeability within the adhesive (ad), but with no sign of gap or evident degradation at the bonding interface (pointer). (D1) It is possible to see the resin-dentine interface created with the universal adhesive ABU in etch and rinse mode (ER) applied on the dentine air-abraded with SEL bioglass, which characterised no dye infiltration at the hybrid layer and no sign of evident degradation (pointer) between the adhesive (ad) and dentine (dt). (C2) The resin-dentine interface created with the experimental (EXP) adhesive applied in ER mode on the dentine air-abraded with SEL bioglass is characterised a gap-free interface (pointer), but with an evident dye uptake within the adhesive layer (ad). (C3) The resin-dentine interface created with the ABU adhesive applied in SE mode on SEL air-abraded dentine is characterised by little dye permeability the bonding interface, but with no presence of gap or any sign evident degradation at the bonding interface (pointer).

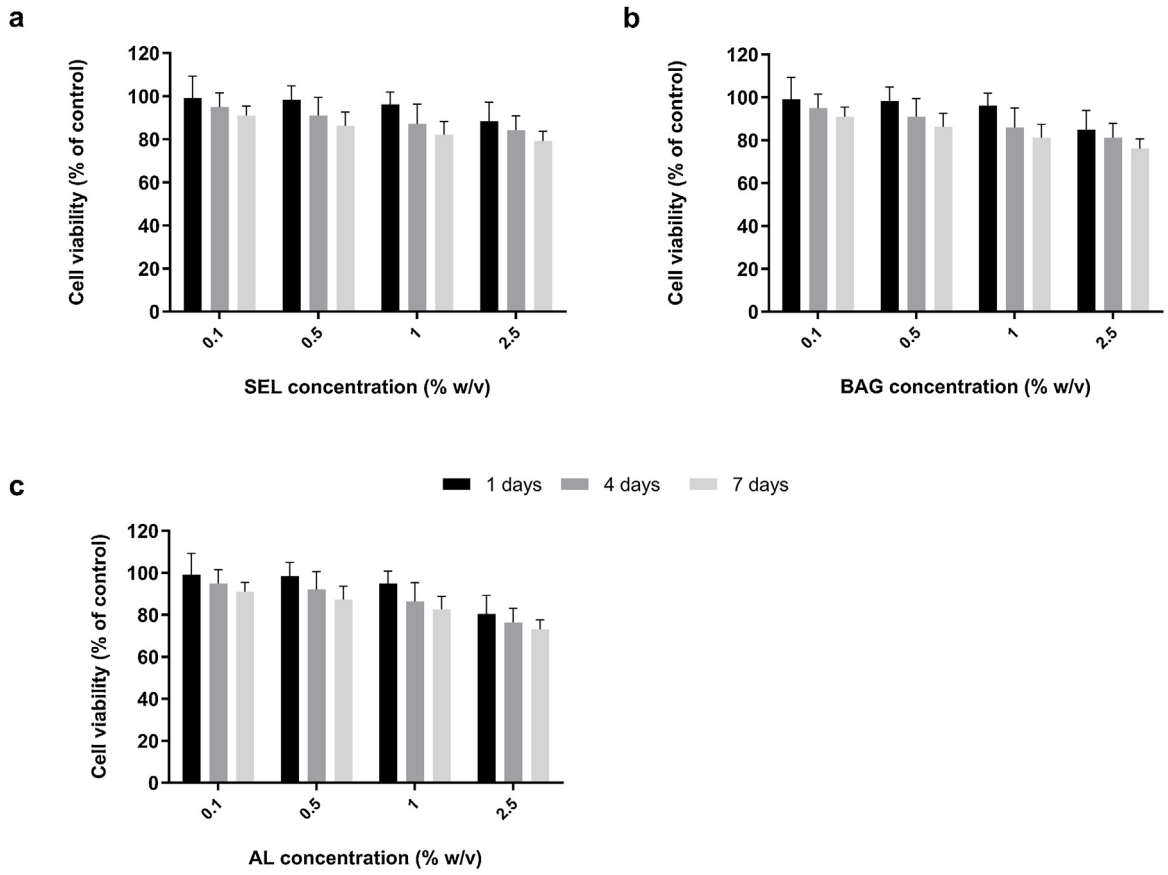

Fig. 4 – Cytotoxicity evaluation of particles assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in dental pulp stem cells (DPSCs). Cells were incubated with increasing concentrations of particles from 0.1 to 2.5 % w/v for 1, 4, and 7 days. In each experiment, four replicates per concentration were tested. The experiment was repeated three times. The data are mean values of three independent experiments (± SEM).

tion reactions into a porous SiO_2 layer and serves as template for apatite precipitation [6,72]. This helps remineralise and protect the resin-dentine interface, as well as reduce the proteolytic action of endogenous MMPs [73,74]. MMP inhibition may be also correlated to the alkaline pH generated by bioactive glasses [75–77], as well as to specific ions, such as copper and zinc that may deactivate dentine MMPs by a direct chelating reaction [78,79]. On the other hand, the different effects of BAG and SEL on the results obtained in this study in terms of bonding performance may be correlated also to their different bioactive mechanisms. For instance, the polycarboxylated zinc-doped bioglass was demonstrated previously to have a more attenuated alkalinising activity compared to BAG [80–83]. This aspect may be responsible for less interference in the interaction of the acidic functional monomers with calcium ions in dental hydroxyapatite of the dentine air-abraded with SEL rather than BAG.

In view of the results and the above discussion, the first hypothesis that the air-abrasion powders would not affect the bonding performance of the tested adhesives applied in SE or ER mode at 24 h is partially accepted. Moreover, the second hypothesis that air-abrasion would not affect the bonding performance of the tested adhesive after prolonged aging in artificial saliva must also be partially accepted.

Regarding the biocompatibility and cytotoxicity of the air-abrasion powders tested in this study, it is well known that bioglass 45S5 (BAG) shows biocompatibility and low cytotoxicity [84]. Unfortunately, there is no information about the gene expression for odontoblast differentiation (*ocn*, *dspp*, *dmp1*, *mepe*) and the biocompatibility of alumina and the innovative zinc-doped polycarboxylated bioglass used in this study. The results of the current study showed the air-abrasion powders SEL, AL or BAG were biocompatible since they had no negative cytotoxic effect on hDPSCs (Fig. 3). However, the presence of AL powder interfered with the expression of the *mepe* gene, which resulted in upregulation. Moreover, it also inhibited dentine matrix formation; the normal level of *ocn*, *dmp1*, and *dspp* increased minimally over a period of 28 days. Conversely, BAG and SEL had no negative effect on the expression of the genes *mepe ocn*, *dspp*, and *dmp1*.

The matrix extracellular phosphoglycoprotein (*mepe*) expression by dental pulp stem cells (DPSC) is a marker for early odontogenic differentiation [85–87] and when the odontoblasts are mature, this phosphoglycoprotein becomes downregulated [88,89]. The *dspp* belongs to the family of small integrin-binding ligand N-linked glycoproteins (SIBLINGs) and its expression also indicates early odontogenic differentiation [90]; *dmp1* is involved in the regulation of dentine collagen

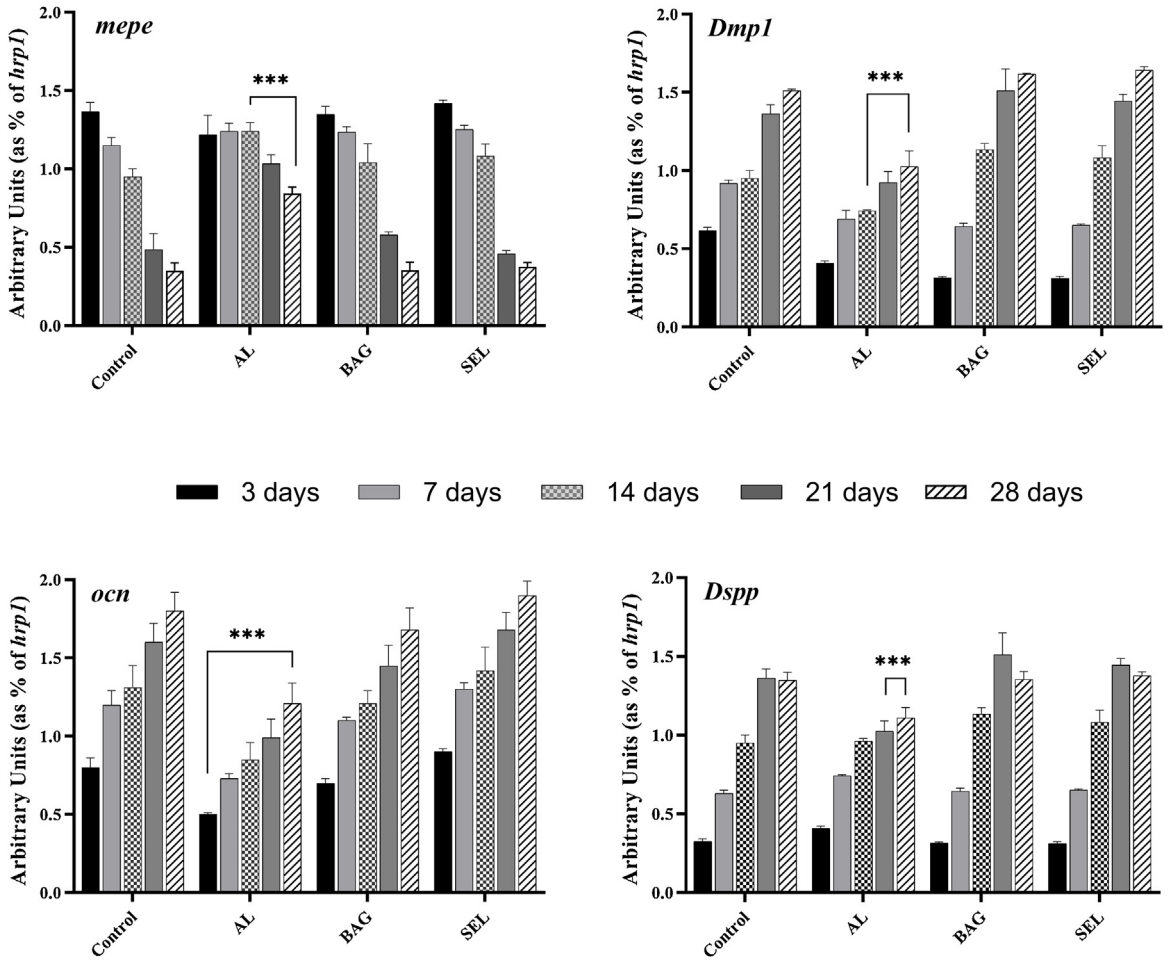

Fig. 5 – Quantitative RT-PCR analysis of *mepe*, *dspp*, *ocn*, and *dmp1* in STRO-1$^+$ cells cultured in presence of control, AL, BAG or SEL for 3, 7, 14, 21 and 28 days. Cell cultured on tissue culture polystyrene was used as control. The target gene expression was normalised to the house-keeping gene *gapdh*. Relative differences in PCR results were calculated using the comparative cycle threshold (CT) method. The bars represent means ± standard deviation (n = 3). *** $p < 0.001$ vs CTL, BAG and SEL.

matrix organisation and mineralisation. It is mainly expressed during early odontoblast differentiation [91] prior to the *dspp* expression [92,93]. Conversely, the *ocn,* a -γ-carboxyglutamic acid containing protein, is an indicator of late period of osteoblast differentiation [94]. Thus, it is possible to assume that the presence of alumina inside the dentine tubules might interfere with the normal differentiation of pulp stem cells to odontoblasts and its use should be avoided at least in deep dentine, especially in close proximity to the pulp chamber. The third hypothesis was that there would be no difference in biocompatibility and influence on gene expression between the tested air-abrasion powders must be partially accepted.

In conclusion, the use of such bioactive powders does not interfere with the immediate bonding performance of universal adhesives applied in ER mode, but the high alkalinity of Bioglass 45S5 might affect the immediate bonding of some universal adhesives applied in SE mode. However, the use of such bioactive glasses in air-abrasion procedures may prevent excessive degradation at the resin-dentine interface and reduction of bonding performance over time of universal adhesives applied on dentine in ER and SE mode. Moreover, keeping in consideration the limitations of this in-vitro study, it is possible to affirm that air-abrasion procedures performed with conventional Bioglass 45S5 or with the innovative polycarboxylated zinc-doped bioactive glass are potentially safe and would not interfere with the physiological metabolism of stem cells and their differentiations to odontoblasts.

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