ABSTRACT

Objective: To evaluate the effect of load-cycle aging and/or 6 months artificial saliva (AS) storage on bond durability and interfacial ultramorphology of resin-modified glass ionomer cement (RMGIC) applied to dentine air-abraded using Bioglass 45S5 (BAG) with/without polyacrylic acid (PAA) conditioning.

Methods: RMGIC (Ionolux, VOCO) was applied onto human dentine specimens prepared with silicon-carbide abrasive paper or air-abraded with BAG with or without the use of PAA conditioning. Half of bonded-teeth were submitted to load cycling (150,000 cycles) and half immersed in deionised water for 24h. They were cut into matchsticks and submitted immediately to microtensile bond strength (μTBS) testing or 6 months in AS immersion and subsequently μTBS tested. Results were analysed statistically by two-way ANOVA and Student–Newman–Keuls test (α=0.05). Fractographic analysis was performed using FE-SEM, while further RMGIC-bonded specimens were surveyed for interfacial ultramorphology characterisation (dye-assisted nanoleakage) using confocal microscopy.

Results: RMGIC applied onto dentine air-abraded with BAG regardless PAA showed no significant μTBS reduction after 6 months of AS storage and/or load cycling (p>0.05). RMGIC–dentine interface showed no sign of degradation/nanoleakage after both aging regimens. Conversely, interfaces created in PAA-conditioned SiC-abraded specimens showed significant reduction in μTBS (p<0.05) after 6 months of storage and/or load cycling with evident porosities within bonding interface.

Conclusions: Dentine pre-treatment using BAG air-abrasion might be a suitable strategy to enhance the bonding performance and durability of RMGIC applied to dentine. The use of PAA conditioner in smear layer-covered dentine may increase the risk of degradation at the bonding interface.

Clinical Significance: A combined dentine pre-treatment using bioglass followed by PAA may increase the bond strength and maintain it stable over time. Conversely, the use of PAA conditioning alone may offer no significant contribute to the immediate and prolonged bonding performance of modern RMGICs.

Keywords: air-abrasion; bioactive glass; bonding; dentine pre-treatment; polyacrylic acid; resin-modified glass ionomer cements
1. INTRODUCTION

Conventional rotary instruments equipped with tungsten-carbide, carbon-steel or diamond burs are used routinely in clinical practice for dental cavity preparation. In minimally invasive dentistry (MID), the underlying tenet is to preserve sound dental hard tissues and minimise the unnecessary alteration of healthy tooth structure. Air-abrasion has been advocated to be a suitable approach to reduce the risk for unnecessary removal of sound dental tissues, although the choice of powders used may affect the quality and durability of the tooth-restoration interface. Bioglass 45S5 (BAG), a calcium/sodium phosphate-phyllosilicate glass, is used in air-abrasion with several advantages including the absence of pain during the operative procedure and the opportunity to leave cavities with rounded internal line angles, thus minimising the contraction stress of resin composites.

Moreover, BAG will embed into the dentine surface so creating a bioactive smear layer that can react with body fluids, encouraging mineral deposition through formation of hydroxyapatite. The stabilisation of the interface between tooth and restorative material, as well as the creation of in loco conditions that might protect and/or repair the retained demineralised dental hard tissues are of particular importance in MID. The use of fluoride-releasing restorative materials such as glass ionomer cements (GIC) or resin-modified glass ionomer cements (RMGIC) may contribute to interfacial protection because of their buffering ability and their fluoride ion release/re-charge. Moreover, since GIC-based materials have the aptitude to induce crystal growth within the interface of the restoration after long-term storage in water, with a chemical composition similar to that of dental hard tissues, it is hypothesised that the combination of dentine pre-treatment with BAG air-abrasion and subsequent restoration using GIC-based materials could be a suitable strategy to achieve longer-lasting bonding interfaces that can resist degradation over time.

RMGICs combine the therapeutic properties of GICs with the mechanical properties of resin polymers. The setting process of RMGIC is based on free-radical polymerisation as well as the acid–base reaction between polyalkenoic acids and fluoroaluminosilicate glass. The self-adhesive...
mechanism of GIC-based materials to dentine is the micromechanical interlocking achieved by shallow hybridisation of the micro-porous collagen network. There is a chemical reaction that occurs through the formation of ionic bonds between the carboxyl groups of the polyalkenoic acids and calcium of hydroxyapatite-coated collagen fibrils \(^{29,30}\). Polyacrylic acid (PAA) is the most common conditioner used in enamel/dentine pre-treatment to remove the smear layer prior to the application of GIC-based restorative materials onto dentine and enamel. However, concerns exist regarding its use, application times and concentrations as these factors may interfere with the overall bonding performance. Indeed, a high number of adhesive failures between a RMGIC and resin composite have been reported when a polyalkenoic conditioner was used on smear-layer covered dentine \(^{30}\).

Milly et al., \(^{31}\) used a combination of PAA-BAG powder in air-abrasion as a pre-treatment of white spot lesions in enamel (WSL). It was showed that surface pre-conditioning was able to enhance the remineralisation of WSL. Indeed, there were reported increased mineral content, improved mechanical properties and alterations in the enamel ultrastructure. To the present authors’ knowledge, there are no previous published studies assessing the effect of dentine pre-treatments using BAG air-abrasion and/or by PAA conditioning on the interfacial characteristics of resin-modified glass ionomer cements submitted to different aging regimes.

The aim of this study was to test the microtensile bond strength (MTBS), after load-cycle aging and/or 6 months storage in artificial saliva (AS), of RMGIC applied to dentine air-abraded using Bioglass 45S5 (BAG) with or without subsequent surface pre-conditioning using 10% polyacrylic acid (PAA). Fractographic analysis and interfacial dye-assisted nanoleakage assessment of the bonded interfaces were evaluated using field-emission scanning electron microscopy (FE-SEM) and confocal laser-scanning microscopy (CLSM), respectively. The tested null hypotheses were that the durability of RMGIC applied with or without the use of PAA conditioner onto a BAG air-abraded dentine surface would not be affected by: (i) 6-month aging in artificial saliva (AS); (ii) load-cycle aging only; (iii) load cycling followed by 6-month AS aging.
2. MATERIAL AND METHODS

2.1. Preparation of dentine specimens

Sound human molars (from 20- to 40-yr-old subjects) extracted for periodontal or orthodontic reasons under a protocol approved by an Institutional Review Board (nºCEI16/020), were used in this study. The teeth were stored in deionised water at 4°C for no longer than 1 month. The roots were removed 1 mm beneath the cemento–enamel junction (CEJ) using a diamond-embedded blade (high concentration XL 12205; Benetec, London, UK) mounted on a hard-tissue microtome (Remet evolution, REMET, Casalecchio di Reno, Italy). A subsequent parallel cut was performed to remove the occlusal enamel and expose mid-coronal dentine. This flat dentine surface was polished with silicon-carbide paper (SiC #320-grit) for 1 min under continuous water irrigation to simulate the creation of a smear layer that would be created clinically after rotary dentine preparation. The specimens were divided into experimental groups and subgroups as shown in Table 1.

2.2 Experimental design: dentine pre-treatments and aging protocols

The experimental design required that half of the dentine specimens were air-abraded with BAG (Sylc, VELOPEX International, London, UK) under water irrigation. An Aquacare air-abrasion unit (VELOPEX International) was used with an air pressure of 4 bar (400 MPa) for 1 min at a distance of 1 cm from the dentine surface and with continuous mesio-distal and bucco-lingual movements. Subsequently, the air-abraded dentine surface were conditioned with 10% PAA gel (GC Fuji conditioner, Newport Pagnell, UK) for 20 s and rinsed with water for 20 s, or left unconditioned.

Overall, four primary groups (n=32 specimens/group) were created for this experimental design based on the preparation of the dentine substrate:

- **Group 1.** Specimens abraded using 320-grit SiC abrasive paper (1 min) under continuous irrigation, followed by a water rinse (20 s), air-drying (2 s) and restored with a light-cured RMGIC (no PAA conditioning).
Group 2. Specimens abraded with 320-grit SiC abrasive paper (1 min), conditioned with 10% PAA gel for 20 s rinsed with water (20 s), dried, and restored with a light-cured RMGIC (PAA conditioning).

Group 3. Specimens abraded using 320-grit SiC abrasive paper (1 min) under continuous irrigation and then air-abraded with BAG particles under a continuous water shroud (1 min), rinsed with water (20 s), dried, and restored with a light-cured RMGIC (BAG-no conditioning).

Group 4. Specimens abraded using 320-grit SiC abrasive paper (1 min) under continuous irrigation, air-abraded with BAG particles under a continuous water shroud (1 min), rinsed with water (20 s), conditioned with 10% PAA (20 s), rinsed with water (20 s), dried, and restored with a light-cured RMGIC (BAG-PAA conditioning).

The restorative procedure was performed by applying the content of two mono-dose capsules of a commercial RMGIC (Ionolux; Voco GmbH, Cuxhaven, Germany), mixed for 10 s in a trituration unit and applied in bulk on to the dentine surface and light-cured for 30 s with a light-curing unit (Radii plus, SID Ltd, Bayswater VIC, Australia) with a LED light source (>1000 mW/cm²).

Each main group was subsequently subdivided in four sub-groups (n=8 specimens) based on the aging protocol: 1) CRT: no aging (control, 24 h in deionised water); 2) LC: Load cycling (150,000 cycles in artificial saliva); 3) AS: prolonged water storage (6 months in artificial saliva); 4) LC+AS: Load cycling (150,000 cycles) + AS storage (6 months).

The composition of the artificial saliva was (AS: 0.103 g l⁻¹ of CaCl₂, 0.019 g l⁻¹ of MgCl₂.6H₂O, 0.544 g l⁻¹ of KH₂PO₄, 30 g l⁻¹ of KCl, and 4.77 g l⁻¹ HEPES (acid) buffer, pH 7.4] at 37ºC for 24 h or 6 months) ₃². The subgroup specimens LC and LC+AS were mounted in plastic rings with dental stone for load cycle testing (150,000 cycles; 3 Hz; 70 N). A compressive load was applied to the flat surface of the RMGIC using a 5-mm diameter spherical stainless steel plunger attached to a cyclic loading machine (model S-MMT-250NB; Shimadzu, Tokyo, Japan) while immersed in AS ₃³.
2.3 Micro-tensile bond strength (MTBS) and fracture analysis (FE-SEM)

The specimens were sectioned using a hard-tissue microtome (Remet evolution, REMET) in both X and Y planes across the dentine-RMGIC interface, obtaining approx. 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 6 months and then tested for their MTBS. The latter 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 classified as a percentage of adhesive (A), mixed (M) or cohesive (C) failures when the failed interfaces were examined at 30X magnification by stereoscopic microscopy. Five representative fractured specimens from each sub-group were critical-point dried and mounted on aluminium stubs with carbon cement. The specimens were gold-sputter-coated and imaged using field-emission scanning electron microscopy (FE-SEM S-4100; Hitachi, Wokingham, UK) at 10 kV and a working distance of 15 mm.

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 analysed statistically by two-way ANOVA including interactions between factors, using MTBS as a dependent variable. Dentine surface treatment and aging method were considered as independent variables. Post-hoc multiple comparisons were performed using the Student–Newman–Keuls test. Statistical significance was set at $\alpha = 0.05$.

2.4 Ultramorphology of the bonded-dentine interfaces - confocal microscopy evaluation

One dentine-bonded slab sample (Ø 0.9 mm$^2$) was selected from each experimental sub-group (n=8) during the cutting procedures of match-sticks. 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 per side with a 2400-grit SiC paper. The specimens were finally ultrasonicated again with distilled water for 5 min and submitted for confocal microscopy.
Using a confocal scanning microscope (Olympus FV1000, Olympus Corp., Tokyo, Japan), equipped with a 63X/1.4 NA oil-immersion lens and a 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. 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 optical images were randomly captured. Micrographs representing the most common morphological features observed along the bonded interfaces were captured and recorded.

3. RESULTS

3.1 Micro-tensile bond strength (MTBS) and failure mode analysis

Microtensile bond strength means and standard deviations are expressed in MPa in table 2. Dentine surface treatments and aging in AS influenced the MTBS results ($P < 0.01$). Interactions between factors were also significant ($F = 58.15; P < 0.05$). In brief, at 24 h (no load cycling) the use of air-abrasion and/or PAA as dentine pre-conditioners caused an increase in the microtensile bond strength of RMGIC compared to those created without the use of PAA and/or air-abrasion pre-conditioning. However, there was no significant difference among all groups ($p>0.05$). After load cycling only, the lowest results ($p<0.05$) were observed with the specimens created by applying the RMGIC onto the dentine that received no PAA conditioning and BAG air-abrasion. After 6 months of AS storage, the specimens created in dentine air-abraded with BAG and subsequently conditioned with or without PAA presented higher values compared to the specimens that received no air-abrasion. Again, no significant difference was found between the main groups ($p>0.05$). The specimens with the lowest
(p<0.05) bonding after load cycling and subsequent immersion in AS for 6 months were those created by applying the RMGIC onto the dentine pre-conditioned with PAA.

The RMGIC applied onto dentine surfaces without PAA conditioning showed no significant drop (p>0.05) in bond strength after any aging challenge (e.g. load cycling, AS 6 months or load cycling + AS 6 months). However, after aging in AS for 6 months or load cycling + AS 6 months there was no significant difference (p>0.05) between the specimens created with the RMGIC applied onto dentine with or without PAA.

The RMGIC applied onto PAA-conditioned dentine surfaces showed a significant drop (p<0.05) in bond strength after 6 months of AS storage as well as after load cycling followed by prolonged AS storage (6 months). However, the aging protocol induced no significant difference (p>0.05) between groups 3 and 4 after aging (table 2). Indeed, the specimens tested after 24 h, load cycling, AS for 6 months and after load cycling followed by prolonged AS storage (6 months) showed comparable results (p>0.05).

Regarding the mode of failure, most of the specimens failed predominantly in cohesive mode within RMGIC (range: 73-95%) after 24h and load cycling aging (Table 2). Most of the specimens tested after 6 months of storage in AS and those firstly load-cycled and then immersed in AS for 6 months failed prevalently in mixed mode (range: 38-80%), a part the group of specimens treated with BAG air-and PAA that still maintained a mode of failure prevalently in cohesive mode. The number of adhesive failures in the specimens after AS storage was higher (range: 3-12%) compared to those tested after 24h or load cycling. However, the load-cycled specimens presented no adhesive failures, apart from those created with the RMGIC applied onto dentine without PAA conditioning.

3.2 Fractographic FE-SEM analysis

The fractographic analysis showed the specimens created without PAA conditioning and air-abraded with or without BAG presented a fractured surface constantly devoid of any exposed collagen fibrils and/or dentine tubules, even when samples failed in mixed mode (Fig. 1A and 1B). The specimens created with RMGIC applied onto dentine air-abraded with or without BAG and subsequently
conditioned with PAA presented some exposed collagen fibrils still protected by apatite (Fig. 1C) and patent dentin tubules (1C-1).

The PAA-conditioned specimens that received no air-abrasion (BAG), which failed in mixed (Fig 1D) or in adhesive mode (Fig. 1E) after prolonged AS storage with or without load cycling, were characterised by the presence of exposed collagen fibrils and patent dentin tubules (Fig. 1F). However, such fibrils were less abundant compared to the specimens that were stored in AS for 24h (Fig. 1F-1). The PAA-conditioned specimens air-abraded with BAG, which failed in mixed or adhesive mode (Fig 1G) after prolonged AS storage (6 months) with or without load cycling, showed a fractured dentine surface protected by mineral with no sign of patent tubules and / or degraded exposed collagen fibrils (Fig. 1H), but with some residual degraded resin present (Fig. 1I).

3.3 Ultramorphology of the bonded-dentine interfaces - confocal microscopy evaluation

The results of the ultramorphology and nanoleakage analysis of the RMGIC-dentine interfaces performed through dye-assisted confocal microscopy are shown in figures 2. In brief, it was possible to see at 24 h of AS storage a gap-free interface characterised by the presence of a thin interdiffusion layer (IDF) in all the specimens created by applying RMGIC onto dentine air-abraded with (Fig. 2A) or without BAG (Fig. 2B), but without PAA conditioning. An IDF was not clearly distinguishable in the load-cycled specimens; the overall morphology of the interface remained unaltered both in the specimens created in dentine air-abraded (Fig. 2C) or not, with BAG (Fig. 2D).

After prolonged storage in AS (6 months), with or without load-cycle aging, the IDF was absent and the area subjacent to the RMGIC appeared subjectively less permeable to the fluorescent dye (low nanoleakage) in both groups of specimens created in dentine air-abraded (Fig. 2E) or not, with BAG (Fig. 2F).

As opposed to an IDF layer, a porous hybrid layer-like structure was observed at the interface of those specimens created by applying the RMGIC onto the dentine air-abraded with (Fig. 3A) and without BAG (Fig. 3B) and subsequently conditioned with 10% PAA gel. Such a hybrid zone remained affected
by nanoleakage in those specimens created with RMGIC applied on dentine not air-abraded with BAG, but PAA-conditioned only and subsequently submitted to aging protocols (load cycling, 6 months in AS and load cycling + 6 months in AS) (Fig. 3C). The specimens created by applying RMGIC onto air-abraded dentine and subsequently PAA-conditioned showed after all aging protocols, no nanoleakage at the bonding interface as well as subjacent to the RMGIC and dentine tubules (Fig. 3D).

DISCUSSION

The two null hypotheses tested in this study were partially rejected as only the specimens created using RMGIC applied onto PAA-conditioned dentine, without air-abrasion with BAG showed a significant reduction in bond strength after AS aging for 6 months and after load cycling and subsequent AS aging for 6 months. Conversely, this group of specimens showed no significant reduction in MTBS after load-cycle aging alone.

SEM ultramorphology analysis performed on the specimens after microtensile testing highlighted the ability of PAA to remove the smear layer without widening the dentine tubules and demineralising the collagen fibrils completely (Fig 1C). The results from the specimens tested at 24h showed that the use of PAA conditioner induced no significant increase of the bond strength compared to the specimens created without PAA conditioning (Table 1). These results are contrary to those observed by De Munck et al., who showed that PAA-conditioned dentine yielded higher µTBS values compared to the specimens that received no PAA pre-treatment. On the other hand, current results confirm those of Inoue et al., who showed that bonding of the GIC-based materials to dentine can be achieved without the separate use of a polyalkenoic acid conditioner, even with the interposition of a smear layer within the GIC-dentine interface. Moreover, no significant difference was observed in those specimens created without PAA pre-treatment before and after 6 months of storage in AS. It is believed that the difference between the current results and those reported by De Munck et al., may be due to the use of different type of GIC (contemporary restorative RMGIC vs. RMGIC Bond) as well as to the
storage time variations (i.e. 6 months vs. 4 years) and type of media for the aging protocols (i.e. artificial saliva vs. deionised water).

Current results are in agreement with previous published results\textsuperscript{19,30} regarding the ultramorphology of the specimens that received no PAA pre-treatment; failures in adhesive mode occurred just above the dentine surface (Fig 1A and 1B). The PAA-conditioned specimens that did not receive BAG air-abrasion were characterised by the presence of exposed collagen fibrils, before and after prolonged AS storage with or without load cycling. However, such fibrils were less abundant after prolonged AS storage compared to those observed in the specimens aged in AS for 24h (Fig. 1F-1). These specimens also showed an increase in the number of adhesive failures. Such an outcome was possibly due to hydrolytic degradation processes that occur over time within the collagen. Indeed, within this specific bonding interface it is hypothesised that an enzyme-mediated degradation process may occur due to exposure and activation of endogenous matrix collagenolytic (MMP-1, MMP-8, MMP-13) and gelatinolytic (MMP-2 and MMP-9) metalloproteinases\textsuperscript{34} as a result of PAA accumulation within the conditioned dentine surface and inside the dentine tubules. Es-Souni et al.,\textsuperscript{35} showed using X-ray photoelectron spectroscopy and staining experiments, a higher concentration of carbon products at the PAA-treated dental surfaces along with deconvolution patterns suggesting that carboxylic groups in PAA acid conditioner were involved in a reaction with residual calcium and formation of a PAA-based polymeric gel layer. The ionised hydrogen of the carboxylic acid and the non-ionised groups in PAA may interact with the negative charge of the polymer chain in RMGIC forming intermolecular bonds, thus limiting the availability of the carboxylic groups. This prevents them reacting with the metals in the RMGIC and causes more water sorption at the interface. The RMGIC itself may also have degraded and become more porous over time in AS, thus facilitating diffusion of water towards the glass-ionomer–dentine interface and causing acceleration of the degradation processes\textsuperscript{36}.

Based on the results of this study, it is possible to confirm that modern RMGICs developed for restorative purposes can be applied onto a representative smear layer-covered dentine (no air-abrasion) without the use of PAA conditioning as there was no significant difference in bond strength before and after 6 months of AS storage and with or without load cycling (Table 2)\textsuperscript{31}. However, the
clinical decision of using PAA conditioner should depend upon the histological quality of the dentine retained after cavity preparation (e.g. sound / caries-affected dentine) rather than the immediate bonding performance such materials can achieve in vitro when bonded to standardised smear layer-covered sound dentine specimens. Further tests are ongoing to ascertain if the use of PAA dentine conditioner may affect the longevity of GIC-based materials when applied on caries-affected dentine or sound dentine prepared with conventional burs or chemo-mechanical hand excavation.

A possible explanation as to why the specimens prepared with or without PAA on BAG-abraded dentine showed no significant drop in microtensile bond strength along with no clear signs of nanoleakage after load cycling and/or prolonged AS storage, may be the synergic therapeutic properties of RMGIC to induce growth of mineral crystals and the bioactivity of BAG retained on the dentine surface during the air-abrasion procedure. This has the potential to induce therapeutic remineralisation within the bonded-dentine interface, which protects it against the action of endogenous dentine proteases. It is documented that the presence of bioglass particles (i.e. 45S5) within resin-dentine interfaces may induce the release of a hydrated silica Si(OH)$_4$ which polymerises into a porous SiO$_2$-rich layer, acting as a template for precipitation of amorphous calcium phosphate. This subsequently converts into biomimetic nonstoichiometric apatite in an alkaline environment. Such an alkaline environment is attained through a rapid exchange of sodium (Na$^+$) and hydrogen ions (H$^+$) or hydronium ion (H$_3$O$^+$), and along with Si(OH)$_4$ condensation and precipitation of Ca$^{2+}$ and PO$_4^{3-}$ ions, contributes to fossilisation of proteolytic enzymes, thereby reducing their degradation activity. It has been advocated that mineral precipitation and apatite crystallisation might immobilise proteases through the formation of [Ca/PO-MMP] complexes. However, it is currently hypothesised that the alkalinity of the BAG may buffer the acidity of residual PAA gel within the dentine tubules as well as that of the polyalkenoic acid present within the composition of GIC-based materials. It is believed that such an alkalinisation effect may reduce the potential “retard” demineralisation effect of such acids on the collagen fibrils with consequential late activation of dentine proteases during prolonged aging in AS. Holman et al., reported that an optimum pH 7 is required for several MMPs to function at near-maximum rates, while to degrade
telopeptides at same rate of MMPs, cathepsin K works efficiently at pH 5.5. Tezvergil-Mutluay et al., showed that BAG 45S5 and fluoride-doped bioactive glasses are able to alkalinise the incubation media and reduce the enzymatic degradation of dentine induced by MMPs and Cathepsin K. Teeth are subjected to stresses during mastication, swallowing and parafunctional habits. Maximum biting force in molars is approx. 0.4-0.9 kN, which can challenge the long-term durability of resin–dentine restorative interfaces in teeth. Indeed, it is documented that load cycling produces increased collagen degradation in dentine etched with phosphoric acid (35-40%) and subsequently bonded to using dental adhesives. Nevertheless, the current study showed a slight, but non-significant increase of bond strength values along with reduction of interfacial nanoleakage, in those specimens created with application of the RMGIC on BAG air-abraded dentine. It is believed that load cycling may have contributed to enhance the bioactive synergic effect of BAG and the RMGIC, which protected and remineralised the bonded-dentine interface due to mineral crystallites remaining within the collagen after partial demineralisation, acting as seed sites for apatite growth. Indeed, Toledano at al., showed that mechanical loading may promote dentine mineralisation at 24h and 21 days of storage in distilled water, with increase of the mineral–matrix ratio, lack of nanoleakage and permeability at the resin–dentine interface of specimens created through self-etching and EDTA-conditioned bonding approaches.

CONCLUSIONS
Modern RMGICs can be applied onto dentine covered with smear layer with or without the use of PAA conditioning, although the latter may increase the risk of interface degradation after prolonged aging. However, the durability of modern resin-modified glass ionomer cements applied with or without the use of polyacrylic acid conditioner onto dentine surface air-abraded with bioactive glass is not affected by load cycling and/or prolonged aging in AS. Considering the limitation of this in-vitro study, it is possible to affirm that the synergic combination of the therapeutic properties of RMGIC to induce fluoride release and the bioactivity of BAG to induce mineral growth may represent an alternative restoration approach to achieve a long-lasting restoration.
REFERENCES


CAPTIONS OF THE FIGURES

FIGURE 1: SEM micrographs
[A]: Representative SEM micrograph of specimen created with the use of no PAA applied on dentine air-abraded with or without air-abrasion (BAG) failed in mixed mode where it is possible to note a surface partially covered by residual RMGIC. At higher magnification it is possible to see that the area failed in adhesive mode is characterised by the presence of residual smear layer with a bark-tree appearance [B].
[C]: Representative SEM micrograph at 24h of specimens created with the RMGIC applied onto dentine air-abraded with or without BAG and subsequently etched with PAA where it is possible to see several exposed collagen fibrils still protected by apatite and patent dentinal tubules [C-1]
[D]: Representative SEM fractographic analysis of specimens created with the use of PAA applied on dentine that received no air-abrasion (BAG) that failed in mixed mode after prolonged AS storage with or without load cycling. At higher magnification it is possible to see the presence of unprotected dentinal tubules totally exposed (open pointer), and the presence of some residual collagen fibrils partially demineralised (white pointer) [F and F-1].
[G]: Representative SEM micrograph of specimens created with the use of PAA applied on dentine air-abraded with BAG, which failed or mixed after prolonged AS storage (6 months) with or without load cycling. At higher magnification it is possible to see that the dentine surface is still protected by minerals (white pointer) and residual RMGIC, but with no sign of patent tubules and/or exposed collagen fibrils [H]. Conversely, it is possible to observe some residual resin that was probably affected by hydrolytic degraded [I].

Figure 2: Confocal images of interfaces created with or without BAG air-abrasion followed by no use of PAA conditioner
[A]: CLSM projection image exemplifies the interfacial characteristics at 24 h of the bond–dentine interface created by application of the resin-modified glass ionomer cement (RMGIC) onto dentine air-abraded with bioactive glass (BAG) and with the use of no PAA etchant. It was possible to see a permeable gap-free interface that absorbed the fluorescein solution thought the dentinal tubules (dt) and, in particular, highlighted the existence of a thin interdiffusion layer (pointer). Also note the presence of fluoro-alumino silicate (FAS) filler (*) through the entire RMGIC layer.
[B]: The bond–dentine interface created by application of RMGIC onto dentine that received no air-abrasion and with the use of no PAA gel shows a permeable gap-free interface characterised by the presence of a thin interdiffusion layer (pointer) and fluoro-alumino silicate (FAS) filler (*) through the entire RMGIC layer.
[C]: CLSM projection image showing the interfacial characteristics of the bond–dentine interface created by application of the resin-modified glass ionomer cement (RMGIC) onto dentine air-abraded with bioactive glass (BAG) and with the use of no PAA etchant. It is possible to see that after 150,000 load cycles, the interdiffusion layer seems to be “fused” with the RMGIC layer and it is not clearly distinguished (pointer). Similar features were observed in the interface created by using the RMGIC in dentine that received no air-abrasion and no PAA conditioning; the interdiffusion layer was rarely seen within the RMGIC-dentine interface (pointer) [D]. However, the bond–dentine interfaces created by application of the RMGIC onto dentine air-abraded with bioactive glass (BAG) and with the use of no PAA etchant [E] and those created by application of the RMGIC onto dentine that received no air-abrasion and no PAA [F] showed after prolonged storage in AS (6 months), with or without load cycling aging, the total absence of the interdiffusion layer with an area underneath the RMGIC layer less permeable to the fluorescein solution (pointer).
Figure 3: Confocal images of interfaces created with or without BAG air-abrasion followed by PAA conditioning

[A]: CLSM projection image exemplifies the interface of the specimens created by applying the RMGIC onto the dentine air-abraded with bioglass Sylc (BAG) and subsequently conditioned with PAA gel. It is possible to note that a hybrid layer-like structure and dentinal tubules (dt) totally infiltrated by the fluorescent dye solution (pointer). Similar features can be observed within the interface of a specimen created in dentine air-abraded that received no air-abrasion, but conditioned with PAA gel; the hybrid layer-like structure and dentinal tubules (dt) appear totally infiltrated by the fluorescent dye solution (pointer) [B].

[C]: A representative CLSM projection of the specimens created by applying the RMGIC onto a dentine that received no air-abrasion, but conditioned with 10% PAA gel. It is possible to observe that the aging protocols (load cycling, 6 months in AS and load cycling + 6 months in AS) had no effect on the overall morphology of the interface, and the hybrid zone is still evidently infiltrated by rhodamine, although its thickness resulted slightly reduced compared to the same specimens that received no aging (pointer). Please also note the presence of several fluoroaluminosilicate fillers within the RMGIC layer (*).

[D]: A representative CLSM projection of the specimens created by applying the RMGIC onto a dentine air-abraded with BAG and subsequently conditioned with 10% PAA gel. In this case it is possible to see that the aging protocols (load cycling, 6 months in AS and load cycling + 6 months in AS) reduced presence of rhodamine at the hybrid zone and inside the dentine tubules underneath the RMGIC layer (pointer).