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ORIGINAL ARTICLE

Effects of three restorative techniques in the bond strength and nanoleakage at gingival wall of Class II restorations subjected to simulated aging

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Abstract
Objective To evaluate the effects of simulated aging in bond strength and nanoleakage of class II restorations using three different restorative techniques.
Materials and methods Class II preparations (n=12) were restored using: FS — composite resin Filtek Supreme Plus (3M/ESPE); RMGIC + FS — resin-modified glass ionomer cement Vitrebond Plus (3M/ESPE) + FS; and FFS + FS — flowable composite resin Filtek Supreme Plus Flowable (3M ESPE) + FS. The teeth were assigned into two groups: Control and Simulated Aging — Thermal/Mechanical cycling (3,000 cycles, 20–80 °C/500,000 cycles, 50 N). From each tooth, two slabs were assessed to microtensile bond strength test (μTBS) (MPa), and two slabs were prepared for nanoleakage assessment, calculated as penetration along the restoration margin considering the penetration length (%) and as the area of silver nitrate particle deposition (μm^2). Data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's post hoc test (p<0.05).
Results FS presented the highest μTBS to dentin (22.39± 7.55 MPa) after simulated aging, while the presence of flowable resin significantly decreased μTBS (14.53± 11.65 MPa) when compared to no aging condition. Both control and aging groups of RMGIC + FS presented the highest values of silver nitrate penetration (89.90±16.31 % and 97.14±5.76 %) and deposition area (33.05±12.49 and 28.08±9.76 μm^2). Nanoleakage was not affected by simulated aging.
Conclusions FS presented higher bond strength and lower nanoleakage and was not affected by simulated aging. Use of flowable resin compromised the bond strength after simulated aging.
Clinical relevance The use of an intermediate layer did not improve the dentin bond strength neither reduced nanoleakage at the gingival margins of class II restorations under simulated aging conditions.

Keywords Class II restorations · Simulated aging · Resin-modified glass ionomer cement · Flowable resin · Microtensile bond strength · Nanoleakage

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Introduction

The longevity of adhesive restorations and its clinical success are influenced by the mechanical and chemical properties of the materials and by the restorative technique; which directly affect hybrid layer quality and bond strength [1]. Although the immediate bond strength of most adhesive systems to dentin seems to be satisfactory, studies have shown in vitro [2, 3] and in vivo [4, 5] degradation of the interface components over time.

Successful restorative therapy relies on intact margins. The occurrence of leakage by fluids, bacterial products and bacteria may cause post-operative sensitivity, marginal staining and secondary caries [6, 7], and consequently decrease the longevity of the restoration. Adhesive restorations are exposed to harsh conditions that can affect the marginal sealing and reduce its durability. In the oral environment, teeth are subjected to challenges provided by mechanical stresses from mastication, parafunctional habits, temperature fluctuations and chemical substances [8]. Thermal and mechanical cycling have been widely used as potential aging methods that simulate challenges in vitro [9–11] mainly when associated with complex cavity preparations [12–18]. Polymerization shrinkage is the first stress induced at the adhesive interface and is high in Class II cavities due to its cavity configuration, especially in the absence of enamel in the gingival margin.

The use of restorative materials with different composition than that of composite resin has been proposed to reduce debonding failures of the restorations. Low-viscosity/flowable resins and glass ionomer cements (GIC) placed under hybrid resins have been indicated to overcome the polymerization shrinkage effects in gingival margins, therefore improving bond strength [13] and marginal sealing [19, 20]. Resin-modified glass ionomer cements (RMGIC) have better mechanical properties and lower solubility than conventional GIC. Due to their chemical bond to the tooth structure, they are considered the choice for open sandwich technique [15, 21]. The use of flowable resins has also been considered beneath composites mainly due to their low viscosity and low modulus of elasticity properties that could reduce the adverse effects of polymerization stress.

Studies have reported the effects of thermal and mechanical stresses on microleakage of different restorative materials [14, 15], but there is limited information about the effect of simulated aging methods on the microtensile bond strength [13, 16, 17] and nanoleakage. Although the comparison among studies using thermal and mechanical cycling procedures is difficult due to variations in methodologies, these aging procedures should be used in the in vitro assessment of restorative techniques and materials to provide valuable information under clinically relevant conditions. Therefore, the purpose of this study was to characterize the effect of simulated oral challenges in class II restorations using three different restorative techniques at the gingival wall. The null hypotheses tested were that simulated aging would not affect the (1) penetration and area of silver nitrate deposition (2) and bond strength of different restorative techniques.

Materials and methods

A total of 72 human pre-molars were collected, cleaned and stored in 0.1 % thymol solution for no longer than 1 month. Study protocol was approved by the IRB Committee of Araçatuba Dental School, UNESP (protocol # 2007–01119). The roots were embedded in acrylic resin and the occlusal surfaces were ground flat at the level of marginal ridges under water refrigeration to obtain a flat surface, 5 mm above the cementum–enamel junction and perpendicular to the long axis of the tooth.

Class II slots were prepared on the mesial and distal surfaces using a #245 carbide bur (Brasseler USA Dental Instrumentation, Savannah, GA, USA) mounted in a high-speed water-cooled handpiece. The cavity preparation dimensions were: 3 mm width, 6 mm height (1.0 mm below the cementum–enamel junction) and 1.5 mm depth.

The preparations were randomly divided into three groups (n=12): FS (composite resin); RMGIC + FS (resin-modified glass ionomer cement + composite resin); and FFS + FS (flowable resin + composite resin). The RMGIC and flowable resin were inserted as an intermediate layer between the tooth and the resin composite. The composition, batch number and manufacturers are shown in Table 1.

Preparations for the FS group were restored with Adper Single Bond Plus adhesive system (3M/ESPE; Dental Products, St. Paul, MN, USA) according to the manufacturer's instructions: acid etching using phosphoric acid 35 % (3M/ESPE) for 15 s, rinse for 10 s and dry maintaining wet dentin, apply two consecutive coats of the adhesive with gentle

Table 1 Composition, batch number and manufacturer of the restorative materials used in the present study

Material	Composition (batch number)	Manufacturer
Adper Single Bond Plus	Scotchbond Etchant: phosphoric acid 35 % (7KE) Adhesive: Bis-GMA, HEMA, dimethacrylates, ethanol, water, novel photoinitiator system, methacrylate functional copolymer of polyacrylic and polyitaconic acids (7MK)	3M ESPE
Filtek Supreme Plus Universal Restorative	Bis-GMA, Bis-EMA, UDMA, TEGDMA, photoinitiator, inorganic filler (7LP)	3M ESPE
Filtek Supreme Plus Flowable Restorative	Bis-GMA, TEGDMA, Bis-EMA, dimethacrylate polymer, photoinitiator, inorganic filler (7FB)	3M ESPE
Vitrebond Plus	Liquid: resin-modified polyalkenoic acid, HEMA, water, initiators Paste: HEMA, Bis-GMA, water, initiators and radiopaque FAS (BL7AL)	3M ESPE

Bis-GMA bisphenol-A glicidyl dimethacrylate, *HEMA* 2-hydroxyethymethacrylate, *Bis-EMA* ethoxylated bisphenol-A dimethacrylate, *UDMA* urethane dimethacrylate, *TEGDMA* tri-ethylene glycol dimethacrylate, *FAS* fluoraluminosilicate agitation, gently air dry and light-cure for 10 s. A mylar band (JR Rand Corp., Deer Park, NY, USA) was placed around the teeth using a Tofflemire retainer, the preparation was filled with three horizontal increments of Filtek Supreme Plus resin composite (3M/ESPE) and each increment was light-cured for 20 s (Optilux 501; Kerr, Orange, CA, USA). A 1.0-mm overfill was left on the occlusal surface to perform the mechanical cycling [9, 22].

The preparations for the RMGIC + FS group were restored in the same manner described for the FS group except that a 1.5-mm layer of resin-modified glass ionomer cement Vitrebond Plus (3M/ESPE) was placed prior to the etching procedures. The resin-modified glass ionomer cement was placed using tips (Centrix C-R Syringe System; Centrix Incorporated, Shelton, USA) and light-cured for 20 s (Optilux 501, Kerr).

The FFS + FS group were also restored in the same manner described for the FS group except for a 1.5-mm increment of flowable resin Filtek Supreme Plus Flowable (3M/ESPE) placed and light-cured for 20 s (Optilux 501, Kerr) prior to composite resin build-up.

After all restorative procedures, the teeth were stored in distilled water for 24 h at 37 °C and then finished and polished using Al_2O_3 abrasive discs Sof-Lex Pop-On (3M/ESPE). The restorations were subdivided into two groups: Simulated Aging: thermal cycling (3,000 cycles, 20–80 °C, dwell time 60 s) and mechanical cycling (500,000 cycles, load=50 N) (Fatigue tester; Proto-tech, Portland, OR, USA) or control (no thermal and mechanical cycling, kept in distilled water).

After thermal and mechanical cycling, the teeth were assigned to either microtensile bond strength or nanoleakage analyses.

Microtensile bond strength evaluation (μTBS)

A 3-mm-thick resin block was built on the surface of the restorations to facilitate the microtensile bond test (Fig. 1). The restorations were sectioned longitudinally at the center using a slow speed diamond saw under water irrigation (Isomet-Buehler Ltd., Lake Bluff, IL, USA) to obtain two 0.7±0.2-mm thick slabs sections perpendicular to the bonded interface [9]. A 1-mm^2 cross-sectional surface area was produced by trimming the interface with a diamond bur 557D (Brasseler USA Dental Instrumentation, Savannah, GA, USA) (Fig. 1). The slabs were fixed to a jig with Super Glue Gel (Loctite, Henkel North America, Rocky Hill, CT, USA) and tested in tension using a microtensile tester (Bisco, Inc., Schaumburg, IL, USA) at a cross-head speed of 1 mm/min. The μTBS means and standard deviations for each group were calculated and expressed in MPa. The specimens that prematurely de-bonded during the microtensile bond strength test were included in the statistical analysis with zero as bond strength value (six specimens from the RMGIC control group, six specimens from the FFS aging group, and 19 specimens from the RMGIC aging group).

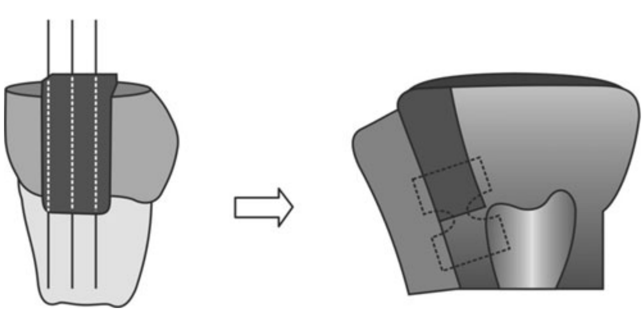

Fig. 1 Schematic representation of the insertion of composite in the outermost surface of the restorations and attainment of the hourglass slabs

Nanoleakage evaluation

Teeth were entirely coated with two layers of nail varnish except for a 1-mm width around the cervical margin. The teeth were immersed in a 50 % ammoniacal silver nitrate solution (pH=9.5) for 24 h [23], thoroughly rinsed in distilled water and immersed in a photodeveloper solution (Kodak, Rochester, NY, USA) for 8 h under fluorescent light. The teeth were sectioned longitudinally, in a mesio-distal direction through the center of the restoration using a slow speed diamond saw under water irrigation (Isomet, Buehler Ltd.). The sections were embedded in epoxy resin and gloss polished using silicon carbide papers (grits #600, 800, 1200; Buehler Ltd.) and diamond pastes (9, 6, 3, 1 μm; Buehler Ltd.). Specimens were cleaned using a ultrasonic cleaner after the use of each polishing paste, mounted on stubs, left to dry for 24 h, gold sputter coated (E5150; Polaron Equipment Ltd, USA) and examined under a scanning electron microscope (S-3000 N; Hitachi Science System Ltd., Japan) using backscattered electron mode. The length of silver nitrate penetration along the gingival wall was measured using various magnifications (×150, ×500, ×1,000, ×2,500) for localization of the silver deposits. The extension of leakage was calculated as the percentage of the total length of cut dentin surface that was penetrated by silver nitrate, i.e., the ratio of the length of silver nitrate penetration along the gingival margin dentin–restoration interface and the total length of the gingival restoration wall. Also, the analysis of the area of silver nitrate deposition was performed using image analysis software (Image J; NIH, Frederick, MD, USA). The total area of silver nitrate deposition at the gingival margin was calculated in square micrometers.

Data were analyzed by two-way analysis of variance (ANOVA) and Tukey's post hoc test ($p<0.05$) using SPSS software (Statistical Package for Social Sciences; SSPS, Chicago, IL, USA).

Results

The microtensile bond strength results are shown in Table 2. Two-way ANOVA test showed statistically significant differences among the restorative materials ($p<0.0001$) and between simulated aging and control groups ($p=0.002$). Tukey's test showed that the group restored with RMGIC+ FS presented the lowest bond strength values and the bond strength was not affected by the simulated aging ($p=0.714$). The thermal and mechanical load cycling significantly reduced the bond strength values only when the flowable composite was used as an intermediate layer ($p=0.023$).

The extent of silver nitrate penetration and the area of silver particle deposition are shown in Tables 3 and 4. The RMGIC + FS aging group presented the highest value of extent of silver nitrate penetration ($p<0.05$), but no differences were observed between RMGIC + FS and FFS + FS aging groups. Gingival margins restored with RMGIC + FS presented statistically significant higher area of silver particles deposition when compared to FS ($p<0.0001$) and FFS + FS ($p<0.0001$) groups. Even though the FFS + FS control group presented the lowest values of silver nitrate deposition area, it was not statistically different compared to FS. Simulated aging did not significantly affect the nanoleakage results. The SEM images (Fig. 2) showed similar patterns of silver nitrate deposition for FS and FFS + FS groups, which were mainly present at the bottom of the hybrid layer and within the hybrid layer. Deposits of silver particles were located at the RMGIC + FS interface and also in the material itself. A great number of specimens from RMGIC + FS both in control and aged groups showed gap in the interface or within the material.

Table 2 Microtensile bond strength mean values of the dentin–restoration interface

	Microtensile bond strength (MPa) − mean (SD)		
	FS	RMGIC + FS	FFS + FS
Control	25.03 (8.24)^{A,a}	5.41 (4.35)^{B,a}	21.71 (7.93)^{A,a}
Artificial aging	22.39 (7.55)^{A,a}	2.15 (5.06)^{C,a}	14.53 (11.65)^{B,b}

Same upper case letters within rows and lower case letters within columns represent no statistically significant differences between groups ($p>0.05$)

FS composite resin Filtek Supreme Plus (3M/ESPE), *RMGIC + FS* resin-modified glass ionomer cement Vitrebond Plus (3M/ESPE) + composite resin Filtek Supreme Plus (3M/ESPE), *FFS + FS* Flowable resin Filtek Supreme Plus Flowable (3M/ESPE) + composite resin Filtek Supreme Plus (3M/ESPE)

Table 3 Results of the extent of silver nitrate penetration on the gingival margins of the groups evaluated

	Nanoleakage − mean (SD) (%)		
	FS	RMGIC + FS	FFS + FS
Control	71.07 (29.60)$^{A, B, a}$	89.90 (16.31)^{B,a}	58.93 (32.44)^{A,a}
Artificial aging	73.80 (23.72)^{A,a}	97.14 (5.76)^{B,a}	78.18 (23.34)$^{A, B, a}$

Same lower case letters within columns and upper case letters within rows indicate no statistical significant differences ($p>0.05$)

FS composite resin Filtek Supreme Plus (3M/ESPE), *RMGIC + FS* resin-modified glass ionomer cement Vitrebond Plus (3M/ESPE) + composite resin Filtek Supreme Plus (3M/ESPE), *FFS + FS* flowable resin Filtek Supreme Plus Flowable (3M/ESPE) + Composite resin Filtek Supreme Plus (3M/ESPE)

Discussion

The permanent seal of restorations placed in cavities with gingival margins in dentin remains a challenge. The results of this study showed the nanoleakage for all restorative techniques tested, in which the use of RMGIC resulted in the highest extent of penetration and area of silver deposition. However, simulated aging did not affect the nanoleakage; therefore the first null hypothesis was accepted. The μTBS values were affected by the restorative material placed on the gingival wall and also by the simulated aging; therefore, the second hypothesis was rejected.

Flowable resins present thin consistency, which can make the application easy, especially in deep and/or posterior restorations. The use of a flowable resin at the gingival margin did not improve the bond strength when compared to the use of a composite resin alone, but compromised the bond strength under simulated aging. While few studies have reported higher bond strength [24] and improved marginal adaptation [25], the great majority showed no benefits

Table 4 Results of the area of silver deposition on the gingival margins of the groups evaluated

	Nanoleakage − mean (SD) (μm^2)		
	FS	RMGIC + FS	FFS + FS
Control	17.08 (14.64)^{A,a}	33.05 (12.49)^{B,a}	11.06 (7.74)^{A,a}
Artificial aging	16.70 (11.06)^{A,a}	28.08 (9.76)^{B,a}	17.50 (11.36)^{A,a}

Same lower case letters within columns and uppercase within rows indicate no statistical significant differences ($p>0.05$)

FS composite resin Filtek Supreme Plus (3M/ESPE), *RMGIC + FS* resin-modified glass ionomer cement Vitrebond Plus (3M/ESPE) + Composite resin Filtek Supreme Plus (3M/ESPE), *FFS + FS* flowable resin Filtek Supreme Plus Flowable (3M/ESPE) + Composite resin Filtek Supreme Plus (3M/ESPE)

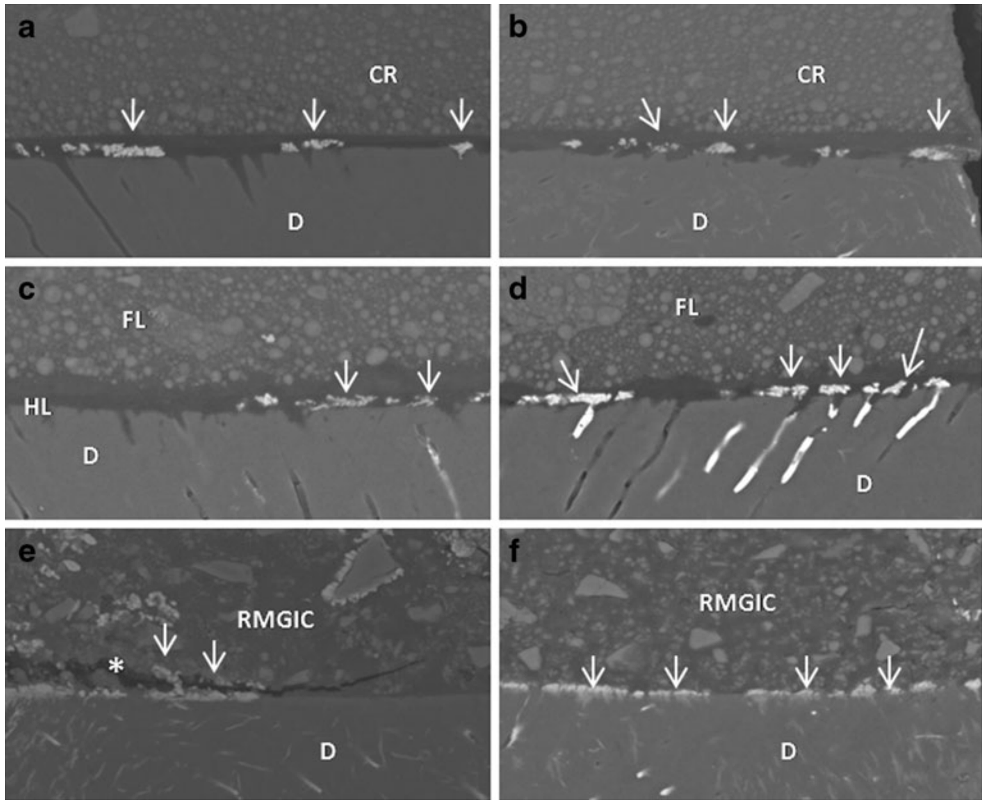

Fig. 2 Representative SEM photomicrograph of all restorative materials used in each test condition (magnification × 1000). The images revealed the silver nitrate deposition (*arrows*) mainly at the bottom and also within the hybrid layer. For FS control (**a**) and aging groups (**b**), a slight difference was observed in the amount of silver deposits in each condition. The leakage pattern is similar for the FFS + FS control (**c**) and aging (**d**) groups, with silver nitrate deposition (*arrows*) within the hybrid layer. Although a higher silver nitrate deposition can be noted in the FFS + FS aging group compared with the control group, this difference was not statistically significant. For RMGIC + FS, in some specimens both in aging and control (**e**) groups, the formation of a gap can be noted in the interface or in the material itself (*asterisk*). Although the intimate contact between dentin and RMGIC can be observed in this representative photomicrograph from RMGIC + FS aging (F), the group presented highest silver deposition at the interface. *CR* composite resin, *D* dentin, *FL* flowable resin, *HL* hybrid layer, *RMGIC* resin-modified glass ionomer cement

on an intermediate layer of flowable resin under hybrid composite resins [17, 26, 27]. In the present study, the microtensile bond strength values for composite resin associated or not with a flowable resin were not statistically different for the control groups. Our findings are in agreement with those reported by Korkmaz et al. [28] that observed higher shear bond strength of a nanofilled resin alone as compared to an intermediate layer of nanofilled flowable resin. Under confined environment (Class II), the polymerization stress is increased due to cavity configuration, and the lower modulus of elasticity of flowable [27] may have partially absorbed the stress resulting in similar bond strength. However, a layer of flowable resin was not strong enough to improve the bond quality and increase the bond strength following simulated aging (Table 2).

While in vitro studies reported no influence of simulated aging procedures in nanoleakage [11, 18], others showed increased leakage values after aging procedures [22]. Awliya and El-Sahn [29] reported a typical nanoleakage pattern only for one of three flowable materials placed in Class V cavities. In the same study, specimens were thermal cycled and the percentage of leakage using flowable resin was significantly lower when compared to resin composite. It is important to note that the cavity configuration may have played important role on the differing results. The low viscosity of flowable resins results in inferior creep strain due to reduced filler content [30] that leads to high shrinkage and less stiffness than conventional hybrid composite resins [31]. The contraction strain can exceed the low elastic modulus, resulting in stress level similar to those obtained with non-flowable materials [17]. It can be suggested that, in the present study the flowable resin was not able to perform as a stress absorbing layer most likely due to its poor mechanical properties and the stress was hastily transmitted to the bonded interface.

The use of RMGIC in open sandwich restorations has been investigated in vivo [32, 33] and in vitro [34]. Lindberg et al. [32] reported a similar failure rate and secondary caries frequency between RMGIC in open sandwich and composite resin of Class II restorations after 9 years. Our findings are in agreement with other studies that observed low dentin bond strength of resin-modified GICs [28, 35], even with the application of a surface primer prior to material placement [28]. On the other hand, some studies have reported better marginal adaptation for the sandwich technique when compared to composite resin [19, 36], where dentin surface was conditioned with polyacrylic acid [37]. The lack of surface conditioning may have influenced the results by reducing surface interaction due to presence of smear layer.

The poor performance of the RMGIC might also be associated with its mechanism of bonding, which is believed to be mainly chemical for Vitrebond [38] and Vitrebond Plus [39]. The RMGIC Vitrebond (3M/ESPE) has been reported to present an intimate contact with the dentin substrate but an absence of partial demineralization of dentin surface and absence of gel phase, which suggests a primary chemical interaction [38]. Even though a stable chemical bond may be achieved, it does not correlate to an efficient seal of the interface. Increased microtensile bond strength was reported when the dentin was treated with polyacrylic acid before placing the glass ionomer material [36]; however, another study showed that the influence of cavity conditioner in marginal adaptation is material-dependent [37].

Glass ionomer materials have a matrix composed by water, which can be gained or lost according to the environment, causing contraction of the material at elevated temperature [40]. Yan et al. [40] demonstrated that the resin-modified GICs showed greater expansion when heated in wet condition, probably due to expansion of the resin phase and affinity for water of the HEMA content. Such expansion might have also influenced the results of the present study during thermal cycling, and may explain the high values for silver nitrate deposition, although statistically significant differences were not observed between control and simulated aging. Hence, the presence of hydrophilic functional groups in the newer glass ionomer based materials can absorb water more easily over time, acting as a plasticizer [41]. It can be speculated that the water sorption by the RMGIC may occur in the same manner as by hydrophilic resin monomers in dentin–restoration interfaces, contributing to its degradation and increasing the nanoleakage phenomenon. A high area of silver particles deposition was observed on the material itself (Fig. 2), which may reinforce previous findings regarding increased porosity of the material [42, 43]. In addition, due to more hydrophilic monomers, the volumetric shrinkage of the RMGIC can vary between 2 % and 3 % [43, 44] which is very similar to the composite resin.

According to our in vitro results, the use of a restorative layering technique with the RMGIC or flowable resin was not beneficial to reduce nanoleakage or improve bond strength to dentin of the materials tested. Hence, the flowable resin may increase the damage to the dentin bonded interfaces subjected to simulated oral challenges. Even though the RMGIC was not affected by simulated aging, caution must be taken when using such technique since a more vulnerable interface may be present due to low bond strength values and high silver nitrate deposition. However, fluoride release and the fact that RMGICs might be less sensitive to contamination by saliva and blood than composites [45] must be considered for the clinical indication of this material. Furthermore, in vivo long-term studies should be conducted to better characterize the clinical performance of different RMGIC and flowable resins.

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Conflict of interest The authors declare that they have no financial arrangement or affiliation with any corporate organization offering financial support for the present research.

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