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ORIGINAL ARTICLE

Influence of ultrasound application on inlays luting with self-adhesive resin cements

Amerigo Cantoro · Cecilia Goracci · Ivanovic Coniglio · Elisa Magni · Antonella Polimeni · Marco Ferrari

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Abstract The study was aimed at assessing the influence of the cement manipulation and ultrasounds application on the bonding potential of self-adhesive resin cements to dentin by microtensile bond strength testing and microscopic observations of the interface. Fifty-six standardized mesio-occlusal class II cavities were prepared in extracted third molars. Class II inlays were made using the nano-hybrid resin composite Gradia Forte (GC Corp, Tokyo, Japan), following the manufacturer's instruction. The sample was randomly divided into two groups (n=28) according to the luting technique. Half of the specimens were luted under a static seating pressure (P), while the other ones were cemented under vibration (V). The inlays were luted using the following self-adhesive resin cements: G-Cem (G, GC Corp., Tokyo, Japan) Automix (GA) and Capsule (GC); RelyX Unicem (RU, 3 M ESPE, Seefeld, Germany) Clicker (RUC) and Aplicap (RUA). Microtensile sticks and specimens for scanning electron microscope (SEM) observations were obtained from the luted teeth. The interfacial strengths measured for the cements under static pressure or ultrasonic vibration were [median (interquartile range)]: GC/V 4 (2.3–7.9); GC/P 6.8 (4.1–10.1); GA/V 3 (1.9–6.7); GA/P 1.9 (0–5.1); RUC/V 6.6 (4.6–9.8); RUC/P 4.1 (1.8–6.4); RUA/V 6.2 (2.4–10.4); RUA/P 3.4 (0–5.4). The cement formulation influenced dentin bond strength of G. RU bond strength was affected by the luting technique. SEM analysis revealed a homogeneous structure and reduced porosities for both cements as a result of ultrasonic vibration. RU benefited from the application of ultrasounds, while GC achieved higher bond strengths than GA.

Keywords Bonding · Self-adhesive resin cements · Dentin · Ultrasound insertion technique · Inlay

A. Cantoro · C. Goracci (✉) · I. Coniglio · E. Magni · M. Ferrari
Department of Dental Materials, University of Siena,
Policlinico 'Le Scotte', viale Bracci,
Siena 53100, Italy
e-mail: cecilia.goracci@gmail.com

A. Polimeni
Department of Pediatric Dentistry,
University of Rome 'La Sapienza',
Clinica Odontoiatrica, V.le Regina Elena,
Rome 00161, Italy

Introduction

Self-adhesive resin cements are diffusely utilized for luting inlays, onlays, crowns, and endocanalar posts. Their popularity among dentists is obviously based on the simplified application technique, since they do not require any pretreatment of the tooth substrate. Moreover, their use is associated with a reduction in postoperative sensitivity [1], along with moderate pulpal inflammatory response [2] and fluoride release [3].

However, recent in vitro studies reported a relatively reduced bonding effectiveness of self-adhesive resin cements as compared with etch-and-rinse resin cements [4, 5]. A superficial interaction with the dental substrate without the generation of a distinctive hybrid layer or resin tags was described [4, 6–9].

A relatively high viscosity and the presence of voids in the cement layer resulting in insufficient adaptation to the dental substrate were observed for RelyX Unicem [7]. Porosities probably affecting mechanical properties were also described in G-Cem [4].

The application of a static seating force heavier than finger pressure in addition to the use of the cement at room temperature was suggested for improved adhesion of RelyX Unicem. Pre-cure temperature was demonstrated not to have any effect on the bonding ability of G-Cem [4, 10].

It was reported that the ultrasound technique of cementation affects the thixotropic properties of the luting agents, leading to a decrease in viscosity of the resin composites [11]. This may promote an adequate wetting and adaptation of the densely filled resin cements onto the dental substrate [12]. Moreover, ultrasonic vibration increases the temperature imparting an instant set to glass ionomer cements (GICs). In addition, superior mechanical properties, along with a more uniform condensation and reduced porosities are associated with the ultrasonic treatment of GICs [13–16].

Recently, a new paste–paste formulation of RelyX Unicem in a clicker dispenser has been marketed in order to eliminate the need for measuring, as well as to avoid the waste of cement and the use of mixer activator, applier or mixing tips. Also G-Cem has been developed in an automix formula accomplishing mixing and application in a single handling step. Scarce information is available in the literature regarding the bonding effectiveness and adaptation of these newly released self-adhesive resin cements.

Hence, the purpose of the study was to assess the influence of the cement manipulation and the effect of ultrasounds application on the adhesion and interfacial adaptation of self-adhesive resin cements to dentin.

The tested null hypothesis was that neither the luting technique nor the cement packaging and manipulation had a significant influence on the bonding effectiveness achieved on dentin when luting composite inlays.

Materials and methods

Fifty-six caries-free extracted human third molars were collected after the patients' informed consent was obtained under a protocol reviewed and approved by the Institutional Review Board of the University of Siena, Italy. All teeth were stored in a 1% chloramine T solution at 4°C, and were used within 1 month following extraction. Twenty-four hours before the bonding procedure, the teeth were retrieved from the disinfectant solution, abundantly rinsed with distilled water and placed in this same medium at 37°C.

Preparation design

On each tooth, a standardized mesio-occlusal class II cavity was prepared under a copious water spray using 80 µm diamond burs (Inlay Prep-set, Intensiv, Viganello-Lugano, Switzerland), and finished with 25 µm finishing diamond burs. An occlusal reduction of 2 mm was made. The bucco-lingual width of the proximal box was 4 mm, the width of the occlusal cavity was 3 mm and the depth of the pulpal and axial walls measured 2 mm. The proximal boxes were extended 1 mm below the cemento-enamel junction. No bevels were added (Fig. 1).

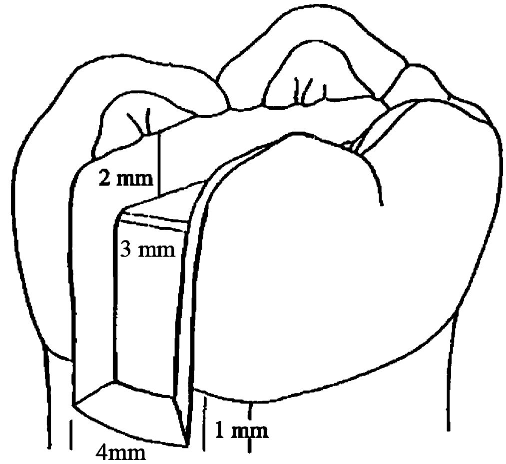

Fig. 1 Picture of standardized class II cavity inlay preparation

Fabrication of inlay restorations

An impression was taken of each prepared tooth using a polyether impression material (Permadyne, 3 M ESPE AG, Seefeld, Germany). Impressions were cast in type 4 stone (Elite Rock, Zhermack, Rovigo, Italy).

The restorations were made in the stone dies, using the nano-hybrid resin composite Gradia Forte (GC Corp, Tokyo, Japan) according to the manufacturer's instruction.

Before the luting procedure, inlays were inspected under an optical microscope at 24×. In case a marginal discrepancy greater than 25 µm was observed, the resin composite inlay was remade.

The inner surface of the inlays was sandblasted with 40 µm aluminum oxide particles, cleaned with ethanol, and air dried.

Luting procedure

The specimens were divided into two equal groups according to the luting technique:

1. Ultrasounds (n=28): the resin composite inlay was luted under vibration. An ultrasonic tip provided with a rubber cap (SONICflex cem, KaVo, Biberach, Germany) was mounted on an ultrasonic handpiece (SONICflex, KaVO, Biberach, Germany), set at medium power according to the manufacturer's recommendation. The tip was oriented perpendicular to the occlusal surface of the inlay. The vibration was maintained until no new resin cement emerged along the inlay margins.
2. Pressure (n=28): the resin composite inlay was placed on the substrate under a seating pressure of 1 kg. The seating force was applied by means of a plunger loaded with a box of lead pellets and maintained for 5 min at 37°C and 100% relative humidity inside a laboratory oven.

Each group was further subdivided, according to the self-adhesive resin cement used for inlay cementation:

– G-Cem Capsule (GC Corp, Tokyo, Japan; $n=14$);
– G-Cem Automix (GC Corp, Tokyo, Japan; $n=14$);
– RelyX Unicem Aplicap (3 M ESPE, Seefeld, Germany; $n=14$);
– RelyX Unicem Clicker (3 M ESPE, Seefeld, Germany; $n=14$);

Therefore, the following subgroups were tested:

1. G-Cem/capsule/ultrasounds ($n=7$);
2. G-Cem/capsule/pressure ($n=7$);
3. G-Cem Automix/ultrasounds ($n=7$);
4. G-Cem Automix/pressure ($n=7$);
5. RelyX Unicem/capsule/ultrasounds ($n=7$);
6. RelyX Unicem/capsule/pressure ($n=7$);
7. RelyX Unicem/automix/ultrasounds ($n=7$);
8. RelyX Unicem/automix/pressure ($n=7$);

Chemical composition and batch numbers of the luting agents are reported in Table 1.

All the cements were used in dual-cure mode strictly following the manufacturer's instructions (Table 2).

After luting, excess cement was removed with a scaler and glycerine gel was applied to the margins. The luting agent was then light-cured for 20 s from each side of the restoration (XL3000, 3 M ESPE, St Paul, MN, USA; 550 mW/cm^2). Margins were finished from the restoration toward the tooth structure, using rubber cups and points (Identoflex; Kerr-Hawe SA, Bioggio, Switzerland).

After a 24-h storage period at 37°C and 100% relative humidity, five teeth from each experimental subgroup were vertically sectioned into serial slabs by means of a water-cooled diamond saw (Isomet, Buelher, Lake Bluff, IL, USA), proceeding in the mesio-distal direction at a very low speed and with no additional pressure. Therefore, adhesion to the cavity floor was tested. Each slab was further sectioned into 0.9×0.9 mm composite/dentin sticks, according to the non-trimming version of the microtensile test. The specimens were tested in a universal testing machine (Triax Digital 50, Controls; Milan, Italy) at a crosshead speed of 0.5 mm/min until failure occurred.

Failure modes were evaluated by a single operator under a light microscope (Nikon SMZ645, Nikon, Japan) at 60× magnification, and classified as cohesive within the substrate (dentin or cement), adhesive (between cement and dentin or cement and inlay), or mixed (if adhesive and cohesive fractures occurred simultaneously).

Statistical analysis of microtensile bond strength

Premature failures were included in the statistical analysis as 'zero' values and the frequency of their occurrence in each experimental subgroup were noted (Tables 3, 4).

Bond strength data of RelyX Unicem and G-Cem was analyzed separately, as the pooled population of data from both cements was not normally distributed according to the Kolmogorov–Smirnov test, thus precluding the use of a factorial analysis of variance (ANOVA) to assess the significance of cement formulation, the cementation technique, and of the between-factor interaction.

As the data from either cement were not normally distributed according to the Kolmogorov–Smirnov test, in each data set the Kruskall–Wallis ANOVA on ranks was applied, followed by the Dunn's multiple range test for multiple comparisons. In all the analyses the level of significance was set at $p<0.05$.

Scanning electron microscopy examination

To observe the interface, two teeth from each experimental group were sectioned mesio-distally through the restoration

Table 1 Chemical composition and batch number of the resin cements

RelyX Unicem Clicker (Batch # 350799)	Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, silanated fillers, alkaline fillers	
RelyX Unicem Aplicap (Batch # 345289)	Powder	Liquid
	Glass fillers, silica, calcium hydroxide, self-cure initiators, pigments, light-cure initiators	Methacrylated phosphoric esters, dimethacrylates, acetate, stabilizers, self-cure initiators, light-cure initiators
G-Cem Capsule (Batch # 0611101)	Powder	Liquid
	Fluoro-alumino-silicate glass, initiator, pigment	UDMA, dimethacrylate, 4-META, distilled water, phosphoric acid ester monomer, silicon dioxide, initiator, inhibitor
G-Cem Automix (batch #0807221)	UDMA, fluoro-alumino-silicate glass, dimethacrylate, phosphoric acid ester monomer, silicon dioxide, initiator, inhibitor, pigment,	
Gradia Forte (batch #0807221)	Urethane-based methacrylate, multifunctional methacrylate, silica nanofillers, fine particle glass fillers, prepolymerized fillers, photoinitiator, pigments	

UDMA urethane dimethacrylate, *4-META* 4 methacryloyloxyethyl trimellitate anhydride

Table 2 Handling of the luting agents

RelyX Unicem Clicker	Extrude the cement from the clicker dispenser
	Mix and apply the cement onto the substrate
RelyX Unicem Aplicap	Activate the capsule for 2 s and mix it for 10 s with Rotomix (3 M ESPE)
	Apply the cement onto the substrate
G-Cem Capsule	Activate the capsule and mix it for 10 s
	Apply the cement onto the substrate
G-Cem Automix	Extrude the cement from the automix syringe
	Apply the cement onto the substrate

with the Isomet saw. The specimens were first polished with increasingly finer grits of SiC papers (Buelher, #600, #1000, #1200) under wet condition. Then, the polished interface was etched for 30 s using 32% silica-free phosphoric acid gel (Uni-Etch, Bisco, Schaumburg, IL, USA). After deproteinization with a 2% sodium hypochlorite solution for 60 s, the coupled interface was rinsed with deionized water. After rinsing, specimens were dehydrated in an ascending series of aqueous ethanol solutions to absolute ethanol, and dried using hexamethyldisilazane (HMDS, Carlo Erba, Rodano, Italy).

Finally, the specimens were mounted on aluminum stubs and inspected without coating with scanning electron microscope (JSM-6060LV, JEOL, Tokyo, Japan) at different magnifications. The microscope operated in low-vacuum condition (11 Pa) at an accelerating voltage of 19 kV.

Results

Microtensile bond strength data

All the specimens failed adhesively at the cement–dentin interface.

Descriptive statistics of microtensile bond strength values, percentages of pre-test failures, and statistically significant differences are reported in Tables 3 and 4 for the G-Cem and the RelyX Unicem data sets, respectively. With regard to G-Cem data, the Kruskall–Wallis Analysis of variance on ranks indicated that subgroups differed significantly ($p<0.001$). Specifically, lower microtensile bond strengths were achieved by the specimens luted with the Automix formulation and, according to the post hoc test, the difference was statistically significant with the capsule/pressure subgroup.

In the RelyX Unicem data set, the Kruskall–Wallis analysis of variance revealed the existence of statistically significant differences ($p<0.001$). Particularly, the microtensile bond strength to dentin was increased following ultrasonic vibration of the inlay and, according to the post hoc test, the difference was statistically significant with the subgroup clicker/pressure ($p<0.05$).

SEM observation

When RelyX Unicem was luted under static seating pressure, the cement appeared porous and air bubbles resulting from mixing could be detected. Although the cement was uniformly adapted on the dental substrate, it shallowly interacted with the underlying dentin (Fig. 2a).

When ultrasounds were applied, a more densely packed and less porous cement layer was observed. The cement was well adapted onto the dentin substrate and the volume of voids within the cement was reduced (Fig. 2b). However, the interaction of the cement with the dental substrate remained superficial. The cement was heavily filled with glass particles of quite relevant size and unable to deeply penetrate into the substrate and to create a distinct hybrid layer with resin tags (Fig. 4b).

G-Cem, applied under oscillating force, exhibited a homogeneous structure. Few porosities could be seen within the cement layer. Nevertheless, no signs of penetration into the dentin tubules could be detected (Figs. 3, 4a).

Discussion

Failure mode analysis showed that all the loaded specimens failed at the cement–dentin interface. This finding pointed

Table 3 Descriptive statistics of microtensile bond strengths measured for G-Cem. Different letters label significantly different groups

G-Cem	Number of microtensile sticks	Premature failures (%)	Median	25–75%	Significance $p<0.05$
Capsule/Ultrasounds	38	8/38 (21.1)	4	2.3–7.9	AB
Capsule/Pressure	27	1/27 (3.7)	6.8	4.1–10.1	A
Automix/Ultrasounds	33	4/33 (12.1)	3	1.9–6.7	B
Automix/Pressure	40	18/40 (45)	1.9	0–5.1	B

Table 4 Descriptive statistics of microtensile bond strengths measured for RelyX Unicem. Different letters label significantly different groups

RelyX Unicem	Number of microtensile sticks	Premature failure (%)	Median	25–75%	Significance $p<0.05$
Aplicap/ultrasounds	39	3/39 (7.7)	6.6	4.6–9.8	A
Aplicap/pressure	38	5/38 (13.2)	4.1	1.8–6.4	AB
Clicker/ultrasounds	40	3/40 (7.5)	6.2	2.4–10.4	A
Clicker/pressure	33	11/33 (33.3)	3.4	0–5.4	BC

out that the adhesion between luting agent and composite inlay was not a critical issue. Therefore, the pretreatment involving sandblasting with aluminum oxide particles and cleaning with alcohol recommended for RelyX Unicem was also effective for G-Cem. For the latter material, no specific indication regarding the interaction with the restorative substrate is indeed provided by the manufacturer.

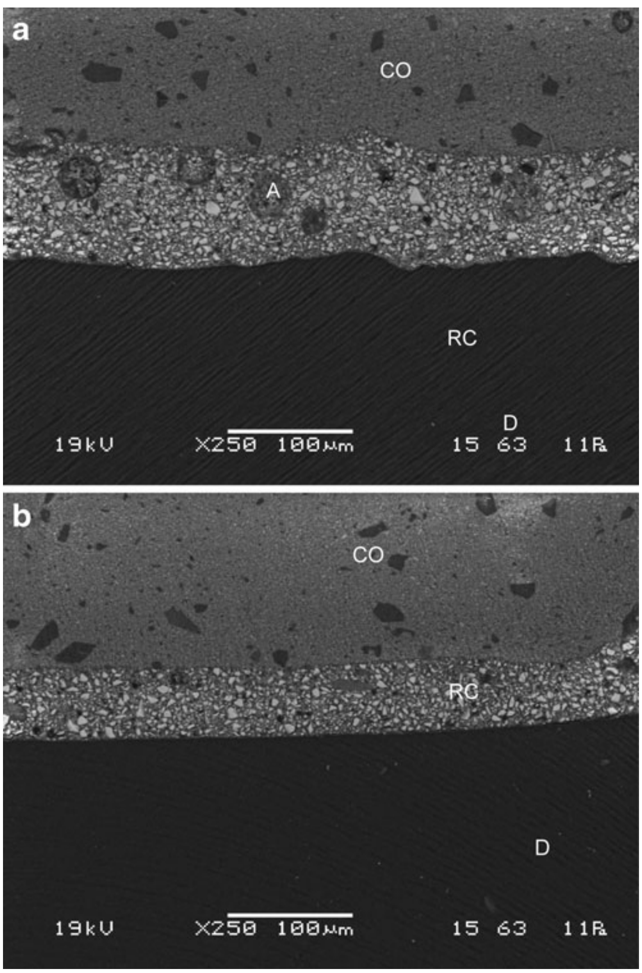

Fig. 2 SEM image of specimens luted with RelyX Unicem Aplicap. (X250, *bar* 100 µm, *CO* composite inlay, *RC* resin cement, *D* dentin). **a** The interface developed when the cement was applied under static seating pressure. Porosities could be detected within the cement layer. *A* Air bubble resulting from mixing. No distinct hybrid layer could be observed at the interface with dentin. **b** When the cement was applied under ultrasonic vibration, the cement layer appeared thinner and less porous

According to the results of this investigation, the null hypothesis should be rejected, since bonding effectiveness achieved on the dentin substrate was affected by both luting technique and cement formulation. In particular, RelyX Unicem benefited from the application of ultrasounds, while G-Cem Capsule achieved higher bond strengths than G-Cem Automix regardless of the technique of cementation.

It was reported that glass-ionomer particles often cluster into larger agglomerates within the cements [13]. The application of ultrasounds is capable of breaking such agglomerates, thus exposing a greater particle surface area to the chemical reaction, thereby accelerating the cement setting [16].

Moreover, ultrasonic vibration was reported to improve mechanical properties of GICs particularly in the first 24 h after setting [14, 15].

A similar behavior might have been manifested in this study by RelyX Unicem. The application of ultrasounds could have promoted the initial acid-base reaction between calcium hydroxide and acidic resin monomers by which the water needed for functional monomers ionization is generated. Moreover, also the acid-base reaction between the acidic monomers and the basic inorganic fillers of the material might have been enhanced. In addition, the local

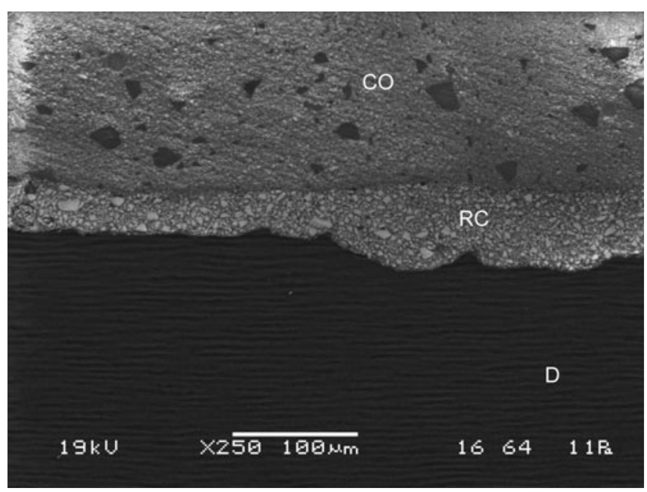

Fig. 3 SEM image of specimens luted with G-Cem Capsule under ultrasonic vibration. (X250, *bar* 100 µm, *CO* composite inlay, *RC* resin cement, *D* dentin). The cement layer appeared homogeneous and no gaps were seen along the interface with dentin

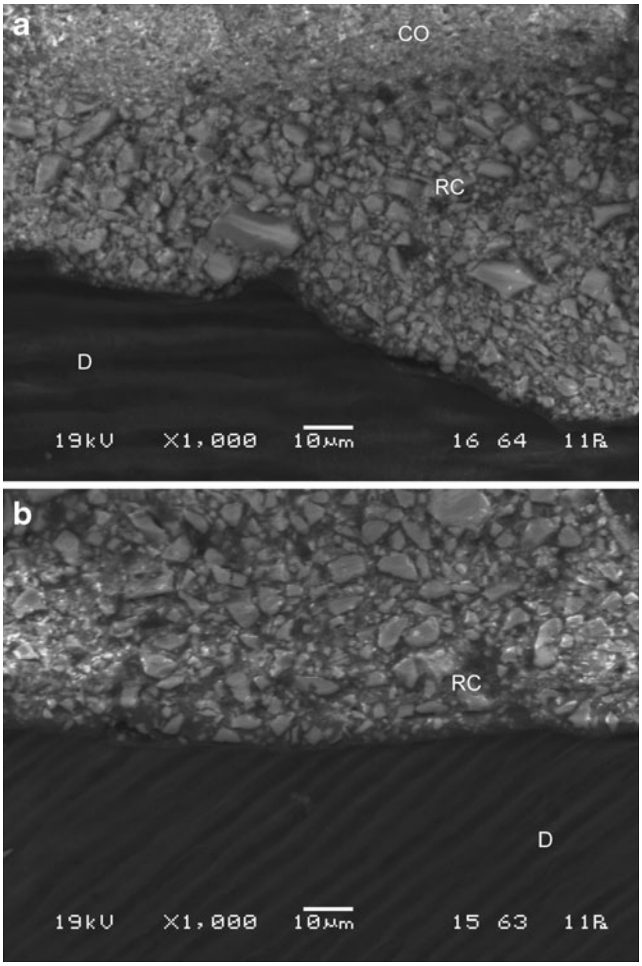

Fig. 4 Higher magnification of specimens luted under ultrasonic vibration (X1000, *bar* 10 μm, *CO* composite inlay, *RC* resin cement, *D* dentin). **a** G-Cem capsule; **b** RelyX Unicem Aplicap. Both cements appeared densely filled with particles of relevant size and unable to penetrate into the dentin tubules

heating produced during ultrasounds application could have catalyzed free radical polymerization [16].

Furthermore, the application of ultrasounds was reported to reduce porosity and improve glass particle packing [13]. In the present investigation, SEM analysis revealed a reduction in density and size of porosities within the luting agent when the ultrasonic insertion technique was used (Fig. 2b). Air bubbles were more diffuse within the cements when static pressure was applied (Fig. 2a). The presence of such discontinuities could influence the clinical longevity of the restoration. The rate at which fatigue failure occurs was indeed found to be related to discontinuities in the cement layer [17].

Moreover, it was shown that oscillation loading of the cement improved the flow of dental cements [11]. In particular, the flow of more heavily filled cements was increased to a significant extent [12, 18]. RelyX Unicem has a filler load of 70%wt. The improved flow of RelyX Unicem under ultrasonic application may explain the gain in inlay retention in comparison with the application under static pressure, as chemical and physical interactions with dentin are expectedly promoted by a more intimate cement–substrate adaptation.

Smear layer and underlining dentin have been regarded as a solid buffer, limiting the etching ability of acidic monomers [8]. The pH of RelyX Unicem from an initial value below 2 was found to rise to 2.8 within 90 s and to be neutral at 48 h [19]. A different behavior was reported for G-Cem. Its pH was 1.8 after 90 s and 3.6 after 48 h [19]. Hence, it can be speculated that the maintenance of a low pH during setting sustained the cement demineralising action on dentin, resulting in a bond strength that could not be further helped by ultrasounds application.

For G-Cem, capsule reached higher bond strengths than the Automix formulation. The capsule formulation features the presence of 4-methacryloyloxyethyl trimellitate anhydride (4-META) and water. 4-META needs water to be hydrolysed and demineralise dentin. Moreover, it is capable of a chemical reaction with hydroxyapatite [20]. Conversely, in the Automix composition the inclusion of 4-META is not mentioned. This may result in a more limited chemical interaction with dentin of G-Cem Automix. Additionally, in the absence of water, also the ionization of the phosphoric acid monomer responsible for demineralization and interaction with dentin may be less effective.

Regardless of the luting technique applied, the self-adhesive resin cements proved unable to develop a well distinct hybrid layer, interacted superficially with the surface of the smear layer covered dentin and did not penetrate into dentin tubules (Figs. 2, 3, and 4). The cements appeared densely filled with glass particles measuring 10 μm and over in diameter.

The specimens of group B were luted under a sustained seating pressure for 5 min. The choice was based on a previous study which showed enhanced interfacial strength and improved adaptation of resin cements using a seating force greater than finger pressure [9]. However, some limitations are associated with the use of a heavy static seating pressure. Critical dimensions in restoration thickness or width may result in microfractures during or even prior to definitive seating of the restoration. Also, high loads may distort the inlay and a prolonged period is needed for the inlay seating [12, 21]. In addition, in the mentioned study, the pressure was applied uniformly on a flat surface, therefore in the presence of a favorable C-factor. In this study, the class II inlay cavity could have influenced stress dissipation and consequently the development of the interfacial bond.

In this study, bond strength at the cavity floor was assessed. However, different conditions for bonding may have occurred at the other cavity walls, not only in relation to local differences in the dentin substrate [8], but also, owing to the cavity geometry, the luting agent was under compressive stress in some areas, under shear stress in some others.

When ultrasonic vibration is used, loads significantly decrease. Walmsley et al. [21] demonstrated that loads applied by the tip on the substrate corresponded to 250 g. Moreover, ultrasound insertion results in faster seating [12, 21]. However, the occurrence of damage to the inlay surface was reported when using ultrasounds. Damages were associated with the orientation of the tip perpendicular or parallel to the inlay surface and to the presence of a shielded tip. In the present study, the tip was applied perpendicularly to the substrate and a rubber shields was used according to the manufacturer's instruction. The use of a rubber shielded tip seems to reduce indentation on the surface and to create a smoother surface than when utilizing an unguarded tip. However, a better energy transfer for a thin layer of composite luting agent was related to the use of an unguarded tip orientated perpendicular to the surface [22].

Several studies demonstrated that oscillation of seating forces positively influences the seating of indirect restorations during cementation [12, 18]. However, Peutzfeldt [18] showed an improvement in seating when oscillating forces was applied to high-viscosity cements. In the presence of low-viscosity luting agents the seating was similar to that reached with finger pressure. It could be interesting to evaluate the seating of inlays when luted using self-adhesive resin cements under oscillating forces or moderate static loads.

Conclusions

The luting technique influenced the bond strength of RelyX Unicem. Specifically, ultrasounds were effective on microtensile bond strength. Formulation was relevant for G-Cem. G-Cem capsule reached higher bond strengths than G-Cem automix. Faster and more controlled procedure is a clinically appreciated aspect of using the ultrasound technique.

Conflict of interest The authors declare that they have no conflict of interest.

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