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Long-term effect of chlorhexidine on the dentin microtensile bond strength of conventional and self-adhesive resin cements: A two-year in vitro study

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ABSTRACT

Since degradation of the adhesive interface plays an important role on dental restoration failure overtime, bonding protocols containing metalloproteinase synthetic inhibitors could be a valuable approach to preserve the bond strength of indirect restorations. A flat dentin surface was created on 40 recently extracted non-carious human third molars ($n=10$). Resin-composite blocks were randomly cemented using two resin cements: a self-adhesive and a conventional. A buffer-free 2% chlorhexidine digluconate solution was used as dentin pretreatment on experimental groups. Microtensile bond strength test was performed immediately and after specimen aging for two-years in artificial saliva. Fracture patterns were determined by SEM. Even after significant reduction in bonding effectiveness with aging, dentin bond strength values of the conventional resin cement remained higher compared to those of the self-adhesive resin cement especially when chlorhexidine pretreatment was performed. No statistical differences were observed between immediate and aged specimens luted with the self-adhesive resin cement. Chlorhexidine was effective to preserve dentin bond strength of indirect restorations when the conventional resin cement was used.

1. Introduction

Resin cements are routinely used in dentistry for luting composite crowns, all ceramic restorations, and posts. A stable union between resin bonding materials and the tooth substrate is highly important to determine the durability of dental restorations [1]. Since the clinical success of indirect restorative procedures depend in part on the technique and materials used for luting [2,3], different bonding mechanisms, methods to preserve the adhesive interface should be further exploited. Regarding the bonding mechanisms of different cements, interest has been increasingly focused on the use of self-adhesive resin cements for: (i) no dental substrate pretreatment is required; (ii) chemical adhesion to dental substrates is promoted [4]; and (iii) relative moisture resistance adhesive interfaces are created [5].

Despite significant improvements, the adhesive interface remains the weakest link of restorations especially when dentin is involved. Two major mechanisms are involved in the loss of dentin bond strength over time: (i) hydrolytic degradation of hydrophilic resin within the hybrid layer [7] and (ii) deterioration of the dentin collagen fibrils [7,8]. Self-adhesive cements differ from conventional or self-etch resin cements for their interaction with dentin is only superficial due to limited decalcification, low diffusion, and partial-exposure of collagen fibrils at the base of the adhesive interface [9]. Meanwhile, the use of conventional resin cements creates a discrepancy between the etching depth and resin penetration into dentin. As a consequence, a zone of exposed collagen non-infiltrated by resin monomers at the base of hybrid layer is formed [10,11]. Deterioration of such unprotected collagen fibrils plays an important role on the degradation of the adhesive interface [12,13] resulting in loss of bond strength [14] and

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consecutively reducing adhesive restorations durability [8]. Host-derived enzymes such as metalloproteinases (MMP) have an important role on degradation of resin–dentin adhesive interfaces [12–14]. MMP are a group of mammalian zinc-and-calcium dependent enzymes capable to hydrolyze collagen fibrils [15,16]. Human dentin contains at least MMP −2, −3, −8, −9, −13, −20 [15–18]. Some of these gelatinolytic/collagenolytic enzymes can be expressed by odontoblasts [19] and are activated from their latent form [20] when pH along the dentin substrate drops [21].

Similarly to adhesive procedures involving self-etching adhesives, increase in MMP expression by the dentin–pulp complex [19] and increased collagenolytic activity [22] to near-maximum levels [20] might be expected when self-adhesive cements are bonded to dentin. In addition, residual unpolymerized acidic monomers might continue to etch the dentin substrate [23] possibly contributing to MMP activation. These events may contribute to resin–dentin bond degradation over time [19,20,22] when low pH self-adhesive resin cements are bonded to dentin. In this context, chlorhexidine, which also presents antibacterial properties [24], has been studied as a promisor synthetic MMP inhibitor at low concentrations [25]. Dentin pretreatment with chlorhexidine has been proven to reduce loss of bond strength over time when total-etch systems are used [14,26,27]. Chlorhexidine potent inhibitory effect against endogenous MMP in dentin is relevant for the current adhesive prosthetic dentistry procedures on account of the degradation of resin–dentin bonds that occurs over time [17,28,29]. Since conflicting information can be found on literature regarding the use of MMP synthetic inhibitors to preserve dentin bond strength of resin cements, the aim of this study was to investigate the long-term bond strength of indirect restorations luted with different resin cements to chlorhexidine-pretreated dentin. The tested hypotheses were that: (i) conventional resin cements produce higher dentin bond strengths when compared to self-adhesive cements irrespective of aging or dentin pretreatment with chlorhexidine; (ii) dentin pretreatment with chlorhexidine reduces loss of bond strength after aging when a self-adhesive resin cement is used; and iii) dentin pretreatment with CHX reduces resin–dentin bond strength loss of conventional resin cements after aging.

2. Material and methods

2.1. Tooth preparation

Forty recently extracted non-carious third molars were obtained after patient informed consent under a protocol analyzed and approved by the Ethical Committee of the Piracicaba Dental School, University of Campinas, SP, Brazil. Teeth were collected from eighteen to twenty-two year old patients and stored at 4 °C in 0.02% sodium azide solution for up to one month before use. A flat dentin surface perpendicular to the tooth longitudinal axis was obtained by sectioning off oclusal enamel to expose medium dentine (Isomet 1000 Precision Saw Buehler, Lake Bluff, IL, USA). Dentin roughness was standardized with 600-grit SiC paper (BuehlerMet, Buehler, Lake Bluff, IL, USA) for 1 min under water cooling. Cylindrical composite blocks were prepared using a nanofilled light-activated resin composite (Filtek Supreme Z-350, shade A2, 3M ESPE, St Paul, MN, USA). Three incremental layers measuring no more than 2 mm in thickness each were placed into a Teflon mold (5 mm in thickness and 10 mm in diameter) and individually light-cured using a quartz-tungsten halogen (QTH) unit (3M Curing Light, 3M ESPE) with irradiance of 550 mW/cm^2. Resin blocks were heat treated at 110 °C for 5 min inside an inlay composite chamber (Fotoceram, Goiânia, GO, Brazil) to improve double bond conversion. One side of the composite block was abraded with 600-grit SiC paper (BuehlerMet, Buehler, Lake Bluff, IL, USA) under water cooling to create a flat surface with standardized roughness. The composite blocks were ultrasonically cleaned in distilled water for 5 min, blow-dried, treated with a prehydrolyzed silane agent (Ceramic Primer, 3M-ESPE) for 1 min and blow-dried before bonding.

2.2. Luting procedures

Two resin cements were used to lute the indirect restorations: one self-adhesive luting cement (RelyX U100, 3M-ESPE) and one conventional dual-cured cement (RelyX ARC, 3M-ESPE) (Table 1). Teeth were randomly assigned to four groups ($n=10$) according to resin cement used (ARC or U100) and dentin pretreatment (distilled water or chlorhexidine): (1) ARC, (2) ARC/CHX, (3) U100 and (4) U100/CHX. Adhesive procedures were carried out in a controlled environment with a temperature of 24 °C and a relative humidity of 60%. For all groups, dentin moisture control was performed with sterilized lint-free absorbent papers before and after dentin pretreatments. The absorbent papers were gently placed on top of the flat dentin surface and replaced after 5 s until visible water was no longer absorbed and a surface with a slight glossy appearance was observed. For group ARC/CHX, the dentin surface was etched with 37% phosphoric acid (Scotchbond Etchant, 3M ESPE) for 15 s and rinsed with water for 30 s; excess moisture was removed with absorbent paper. Chlorhexidine pretreatment was performed and consisted of light-pressure application of a 50 μL aliquot of a phosphate buffer-free 2% chlorhexidine digluconate solution (Clorhexidina s, FGM, Brazil) (pH 6.8) for 60 s, using a sterilized disposable microbrush. Excess moisture was removed once again with absorbent papers and one coat of primer (Adper Scotchbond Multi-Purpose, 3M ESPE) was applied actively for 10 s. The primer was gently blow-dried followed by active application of one coat of adhesive (Adper Scotchbond Multi-Purpose, 3M ESPE) for 10 s. Light-activation was performed for 10 s. The indirect restoration was then luted with RelyX ARC. For group U100/CHX, moisture control was performed and chlorhexidine was applied on the smear layer-covered dentin for 60 s with a sterilized microbrush. Excess moisture was removed with absorbent paper and the indirect restoration was luted with RelyX

Table 1
Materials, composition and manufactures.

Brand name	Composition	Manufacturer
Scotchbond etchant	37% Phosphoric acid	3M ESPE Dental Products, St Paul, MN, USA
RelyX ARC	TEGDMA, bis-GMA, zirconia/silica filler (67.5 wt%) initiators	3M ESPE Dental Products, St Paul, MN, USA
RelyX U100	Phosphoric acid methacrylates, dimethacrylates, inorganic fillers (72 wt%), fumed silica, initiators	3M ESPE Dental Products, St Paul, MN, USA
Filtek Z-350	bis-GMA, UDMA, TEGDMA, Ethyl methacrylates, inorganic fillers, photoinitaitors	3M ESPE Dental Products, St Paul, MN, USA
Adper Scothbond	*Primer*: HEMA, polyalkenoic acid methacrylate copolymer. *Adhesive*: bis-GMA, HEMA, photoinitiators	3M ESPE Dental Products, St Paul, MN, USA
Clorhexidina s	2% Chorhexidine	FGM, Joinville, SC, Brazil

Abbreviations: Bis-GMA=bisphenol A-glycidyl methylmethacrylate; HEMA=hydroxyethyl methacrylate; UDMA=urethane dimethacrylate; TEGDMA=triethylene glycol dimethacrylate.

U100. For group ARC, similar bonding procedures were carried out as for group ARC/CHX except that chlorhexidine pretreatment was replaced by 50 μL of distilled water application for 60 s. For group U100, moisture control was performed, distilled water was applied on the smear layer-covered dentin for 60 s, moisture control was performed again and the indirect restoration was luted with RelyX U100. For all groups, following the application of the respective resin cement on the dentin surface, the composite resin blocks were placed on top of the flat dentin surfaces and received a constant seating pressure of 3 kg for 3 min [30]. Excess cement was removed and then light-activation was performed for 40 s on all tooth surfaces: buccal, lingual, mesial, distal and occlusal.

2.3. Specimen preparation and storage

After storage in 100% relative humidity at 37 °C for 24 h, the bonded teeth were sectioned (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) occluso-gingivally into serial slabs and further into composite-dentin sticks with cross-sectional area of 0.9 mm^2 in accordance with the “non-trimming” technique [31]. A minimum of 16 central sticks were obtained from each tooth and were randomly divided into two subgroups to be tested either immediately or after storage for two years at 37 °C in artificial saliva. The storage solution was prepared in accordance with the protocol previously described [12] and it was changed weekly to avoid drastic pH changes.

2.4. Microtensile bond strength (μTBS) test

Resin–dentin sticks were individually attached to a metallic grip with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and submitted to the microtensile bond test on a mechanical testing machine (DL2000, EMIC, São José dos Pinhais, PR, Brazil) at crosshead speed of 0.5 mm/min until failure using a Geraldeli Device. Immediate and aged bond strengths for each tooth were determined by the μTBS average values of a minimum of eight sticks from each subgroup. μTBS values were expressed in MPa. After exploratory data analysis, mixed models methodology for repeated measures over time (ANOVA using PROC MIXED) was performed in SAS statistical software (SAS 9.4 Software, SAS Institute, NC, USA). Statistical significance was set in advance at $\alpha = 0.05$ and multiple comparison statistical analysis was performed by Tukey Kramer test. Tooth was considered the statistical unit. Sticks with pretest failures were recorded as null bond strengths and such values were included as 0 MPa in the statistical analysis.

2.5. Failure mode analysis

Fractured sticks were chemically dehydrated in ascending concentrations of ethanol (50%, 60%, 70%, 80%, and 90%) for 1 h in each solution and in 100% ethanol for 2 h. The final chemical drying was conducted by immersion in hexamethyldisilazane for 10 min on filter paper inside a covered glass vial and air dried at room temperature. The specimens were mounted on aluminum stubs, gold-sputtered (40 mA for 120 s) (MED 010, Balzers Union, Balzers, Liechtenstein) and analyzed under magnification of 80–1500 × using a scanning electron microscope (LEO 435 VP; LEO Electron Microscopy Ltd, Cambridge, UK) operating on secondary electron mode at 15 Kv. Failure modes were classified into the following categories: Type I—adhesive failure along the cement/dentin interface; Type II—adhesive failure along the cement/resin composite interface; Type III—cohesive failure within resin cement; Type IV—mixed failure: cohesive in resin cement and failure along the cement/dentin interface; Type V—mixed failure: cohesive failure of resin cement and failure along the cement/resin composite interface.

3. Results

Resin cement ($p < 0.001$), aging ($p < 0.001$), and the interaction resin cement/aging/chlorhexidine pretreatment ($p < 0.001$) had significant effects on the μTBSs. RelyX ARC produced significant higher bond strength values compared to RelyX U100 ($p < 0.05$) irrespective of aging and chlorhexidine pretreatment (Table 2). Two-year aging in artificial saliva did not reduce RelyX U100 bond strength ($p > 0.05$) irrespective of chlorhexidine pretreatment. On the contrary, aging significantly reduced RelyX ARC bond strength when chlorhexidine pretreatment ($p < 0.05$) was not performed. When chlorhexidine pretreatment was performed, RelyX ARC bond strength did not significantly change ($p > 0.05$) after aging compared to immediate bond strength values.

The fracture pattern distribution (%) is demonstrated in Fig. 1. A predominance of Type III failure (cohesive failure along the resin cement) was noted for RelyX U100 when tested immediately and a higher incidence of Type I failures (adhesive failure) was noted after aging irrespective of the use of chlorhexidine. Chlorhexidine did not influence fracture modes for RelyX ARC at the 24 h test period. Type II failure (adhesive failure along the cement/resin composite interface) was predominant. However, an increased number in adhesive failures occurred after aging with a predominance of Type IV failures (mixed involving cohesive failure in resin cement and adhesive failure along the cement/dentin interface) in chlorhexidine untreated specimen and luted with RelyX ARC. When chlorhexidine was used, the predominant failure was Type III (cohesive along resin cement).

4. Discussion

Considering that RelyX ARC produced higher μTBS values compared to RelyX U100 the first hypothesis was accepted. The different bonding mechanism of the tested cements possibly explains the superior bonding efficiency of RelyX ARC compared to RelyX U100. Dentin bonding capacity is influenced by various factors including monomer composition, curing efficiency, tissue hybridization, morphology and extent of dentin demineralization and depth of resin diffusion [32]. Self-adhesive resin cements are composed of acidic monomers that simultaneously demineralize and infiltrate the tooth substrate, producing superficial micromechanical retention and chemical union between the resin monomers and hard dental tissues [4]. The limited ability to demineralize and to infiltrate the dentin substrate [9,33] and its relative high

Table 2
Dentin microtensile bond strength (MPa), standard deviations and pretest failures for all groups.

Resin cement	Pretreatment	Storage	
		24 h	2 years
RelyX ARC	H_2O	*41.54 ($\pm$3.25)^{Aa} [0]	*27.77 ($\pm$3.61)^{Bb} [9]
	Chlorhexidine	*40.24 ($\pm$4.42)^{Aa} [0]	*38.07 ($\pm$3.57)^{Aa} [4]
RelyX U100	H_2O	13.11 ($\pm$3.36)^{Aa} [1]	9.90 ($\pm$2.93)^{Aa} [17]
	Chlorhexidine	11.92 ($\pm$1.37)^{Aa} [2]	7.73 ($\pm$1.24)^{Aa} [15]

Microtensile values followed by lowercase letters indicate significant difference according to Tukey Kramer test ($p < 0.05$) when analyzed per column for each resin cement; different capital letters indicate significant difference according to Tukey Kramer test ($p < 0.05$) when analyzed per row.

* Indicates that RelyX ARC value differs from RelyX U100 respective pretreatment under the same storage period. Numbers inside [ ] represent sticks with pretest failures.

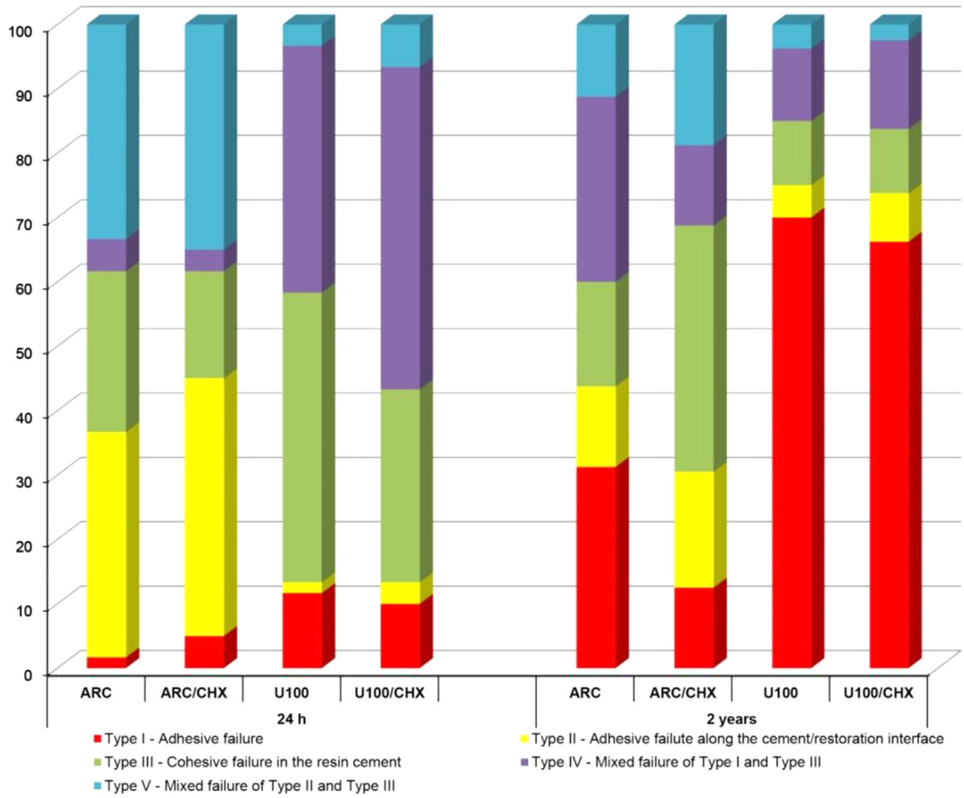

Fig. 1. Incidence of failure modes (%) analyzed by scanning electron micrographs (SEM) of RelyX U100 and RelyX ARC bonded to 2% chlorhexidine pre-treated dentin after 24 h and two years of storage in artificial saliva.

viscosity [6] contribute to low monomer diffusion, reducing micromechanical retention. During cement setting, calcium atoms present in the dentin substrate/smear layer act as electron acceptors promoting chemical union between the acidic resin monomers and the hard dental tissues [4]. Calcium phosphates are mostly formed [33] and do not exhibit a high bonding energy. The ability of this bonding mechanism to overcome the resin cement limited diffusion into dentin and reduced micromechanical retention is questionable since such chemical bonds involve calcium atoms present in low-adhered smear layer. Similar to glass-ionomer cements [34], the concept of using the smear layer as a bonding substrate might be the weak link in obtaining high bond strengths. This bonding mechanism may explain the reduced effectiveness of the tested self-adhesive resin cement in bonding to dentin compared to the conventional resin cement in the present study.

On the contrary, the conventional resin cement tested relies on the micromechanical retention of the adhesive system and the dentin substrate. Our findings are not in agreement with other studies where self-adhesive cements bonded equally effectively to dentin when compared to conventional resin cements after 24 h [2,35]. The microtensile test methodology used in the present study is one of the most accepted methods to evaluate bond strength durability [8,36]. The use of microshear bond strength test [2] and the non-inclusion of pretest failures on the statistical analyses [35] in previous studies might explain the conflicting results. In the present study, pretest failures were included into the results as 0 MPa reducing the overall bond strength of groups with increased number of pretest failures. The self-adhesive resin cement tested presented pretest failures which were taken into consideration, differently from previous studies [2,35]. This fact might explain the higher immediate dentin bond strength for the conventional resin cement in the present study compared to the self-adhesive cement. When pretest failures are not included in the results, overestimation of bond strengths values might occur, leading to possible incorrect data evaluation. Higher immediate bonding effectiveness of conventional resin cements compared to self-adhesive resin cements was also reported in a previous study [3] when different dentin depths were evaluated using the microtensile test.

Indirect restorations must reside for long periods in the oral cavity. As a consequence, water uptake plays an important hole in the long-term in vivo resin–dentin bond degradation. Storage in aqueous media is a valid method to simulate aging of resin–dentin bonded restorations [37]. After aging in artificial saliva at 37 °C, RelyX ARC presented a significant reduction in dentin bond strength compared to the 24 h period. Aging for two-years did not result in significant lower dentin microtensile bond strength for RelyX U100. It is still debatable whether the enzymatic degradation of adhesive–dentin interfaces by MMPs [14] or the hydrolysis of the resin components is the principal mechanism of resin–dentin bond degradation. Most likely, both processes occur simultaneously for subsequent resin elution from hydrolytically unstable polymers within the hybrid layer leaves the collagen fibrils unprotected and vulnerable to degradation [12,38]. It is possible that low water sorption [5] contributed to the maintenance of RelyX U100 dentin bond strength by reducing hydrolysis of the resin cement during the storage period. Even with a low initial pH of 2.1, nearly no demineralization or infiltration of the dentin surface below the smear layer is noticed [9] with only mild/partial exposure of collagen fibrils [39]. When dentin is luted with conventional resin cements, the use of hydrophilic HEMA/BisGMA adhesive systems creates bonding interfaces more prone to degradation. Exposed collagen at the base of the hybrid becomes more susceptible to endogenous degradation by host-derived enzymes such as MMP [12,14,27]. The aging protocol using artificial saliva at 37 °C, neutral pH, presence of water and calcium ions produce ideal conditions for endogenous MMPs present in dentin to degrade collagen [40]. While the use of hydrophilic resins allows more efficient resin diffusion into the wet dentin-etched substrate, the occurrence of hydrolysis increases over time [41] due to higher resin affinity for water molecules. As a result, more permeable interfaces [42] are produced. Even though aging in artificial saliva did not affect RelyX U100 bond strength, RelyX ARC still presented significantly higher dentin bond strengths compared to RelyX U100 after aging. Therefore, one may speculate which criteria is clinically more relevant: whether the relative stability of lower bond strengths produced by self-adhesive resin cements; or higher bond strengths produced by conventional resin cements that reduce over time.

Chlorhexidine did not affect bond strength of RelyX ARC and RelyX U100 after 24 h. Previous studies have reported that the use of chlorhexidine along with self-adhesive cements [21,43,44] and conventional resin cements [45] does not interfere in the immediate dentin bond strength. Regarding conventional resin cements, the use of a three-step etch-and-rinse adhesive system contributed to the fact that chlorhexidine pretreatment did not affect the 24 h dentin bond strength of RelyX ARC. The priming step was performed with a HEMA based primer that does not debond chlorhexidine molecules from the dentin substrate [46] after chlorhexidine pretreatment. Chlorhexidine molecules become trapped along the collagen interfibrillar spaces beneath the bonding resin and after primer/adhesive application chlorhexidine molecules remain bonded to collagen fibrils.

In this study, chlorhexidine did not impair RelyX U100 immediate dentin bond strength. It has been reported that improper moisture control might have contributed to dentin bond reduction and not chlorhexidine by itself [47]. Moreover, the formation of crystal-shaped micrometric-sized precipitates on top of the smear may also reduce self-adhesive resin cement bond strength when chlorhexidine is applied to dentin [48]. Unlike the 2% chlorhexidine digluconate solution (pH 7.4) used in a previous experiment [48], the 2% chlorhexidine digluconate water/ethanol based solution (pH 6.8) used in the current experiment did not contain phosphate buffers to regulate pH. Since dentin pretreatment with chlorhexidine did not impair dentin bond strength in the present study, a possible chemical interaction between chlorhexidine and the phosphate buffer present in the previous study might have formed precipitates which might explain the reported immediate bond strength reduction [48]. Increase in pH (from 6.0 to 8.2) occurs when 2% chlorhexidine solution is mixed with dentin powder [49]. Changes in pH produced by chlorhexidine application might trigger the phosphate buffer to maintain the solution pH constant: the positively charged chlorhexidine molecules might chemically interact with the more readily available phosphate ions present in the solution to form the crystal-shaped precipitates [48]. In the present study, chlorhexidine bonding to the trivalent phosphate groups in hydroxyapatite certainly also occurred [46], but probably to a lesser degree, so the formation of micrometric-sized chlorhexidine crystals most likely was reduced. As a consequence, the immediate bonding effectiveness of self-adhesive tested was not impaired. Similar results were reported in previous studies where a phosphate-free solution of water/ethanol chlorhexidine was used [43–45].

Even though overall bond strength values for chlorhexidine-pretreated specimen were lower than untreated specimen when RelyX U100 was evaluated after aging, no statistical significant differences for dentin microtensile values were observed. The second hypothesis was rejected for the chlorhexidine pretreatment did not reduce loss of dentin bond strength of the tested self-adhesive resin cement after aging. Our findings are in agreement with previous studies where chlorhexidine did not interfere in the shear bond strength of coronal [43] and root dentin [21] after one year when self-adhesive resin cements were tested. In the present study, the use of chlorhexidine was irrelevant to prevent bond degradation of the self-adhesive resin cement tested. Chlorhexidine molecules do not reach the partially exposed collagen fibrils in chlorhexidine-pretreated dentin; they mostly bind to the superficial mineralized portion of the smear layer. When the resin cement is applied, smear layer and superficial chlorhexidine molecules are partially dissolved. Chlorhexidine diffusion into the dentin substrate is different when total-etch systems are used due to the presence of exposed collagen fibrils. When self-adhesive cements are used, chlorhexidine does not directly bond to the unprotected collagen which probably impairs chlorhexidine capacity to inhibit MMPs activity beneath the resin-infiltrated collagen interfibrillar spaces. So the use of chlorhexidine with the sole purpose to prevent bond degradation of self-adhesive resin cements might not be indicated.

Differently from bond strength results, fracture modes considerably changed in RelyX U100 after aging (Fig. 2): increased number in adhesive failures occurred in both chlorhexidine-pretreated and untreated specimen. This is possibly related to: (i) the self-adhesive resin cement superficial interaction with dentin [9,33], and (ii) principally due to the low-energy chemical union formed between resin monomers and calcium present in dentin. Water present in the artificial saliva certainly degraded the calcium phosphate salts at the bonding interface to a higher extent compared to the hydrophobic resin cement matrix. As a result, the resin cement/dentin interface after aging became more prone to rupture during the microtensile test producing predominantly adhesive fractures. It is important to observe that the number of adhesive fractures for RelyX ARC after aging did not increase as much as for RelyX U100, which might be related to the fact that RelyX ARC does not rely on chemical union for retention.

Since chlorhexidine-pretreated aged specimens presented higher dentin μTBS than untreated aged specimens when RelyX ARC was used, the third hypothesis was accepted: chlorhexidine was effective to decrease resin–dentin bond strength loss of the tested conventional resin cement after aging. This finding is in agreement with a previous study [43] where dentin shear bond strength was evaluated after one year of water storage. Moreover, in the present study the total number of adhesive failures after aging of chlorhexidine-pretreated specimen was considerably lower than untreated specimen when RelyX ARC was used (Fig. 3). The positive effect of chlorhexidine to preserve collagen fibrils against MMP hydrolytically degradation has been confirmed both in vivo [14,26] and in vitro [12]. It is speculated that: (i) chlorhexidine molecules compete with MMP specific calcium and zinc sites inactivating MMP proteolytic activity; or (ii) chlorhexidine may simply inhibit collagen degradation via MMP denaturation [25]. Therefore, dentin pretreatment with 2% chlorhexidine seems promising to preserve dentin bond strength of indirect restorations luted with conventional resin cements. Further studies are needed to investigate the effect of chlorhexidine on the dentin bonding degradation of resin cements over time. Different aging protocols including temperature and pH variation, mechanical loading, and also *in vivo* studies should be performed, particularly for self-adhesive resin cements.

5. Conclusions

Based on the obtained results and considering the limitations of this study the following conclusions were drawn: (i) the conventional resin cement tested produced higher immediate microtensile dentin bond strengths when compared to the self-adhesive resin cement tested; (ii) higher bond strengths were sustained even after long-term storage when the conventional resin cement was used; (iii) 2% chlorhexidine was effective to

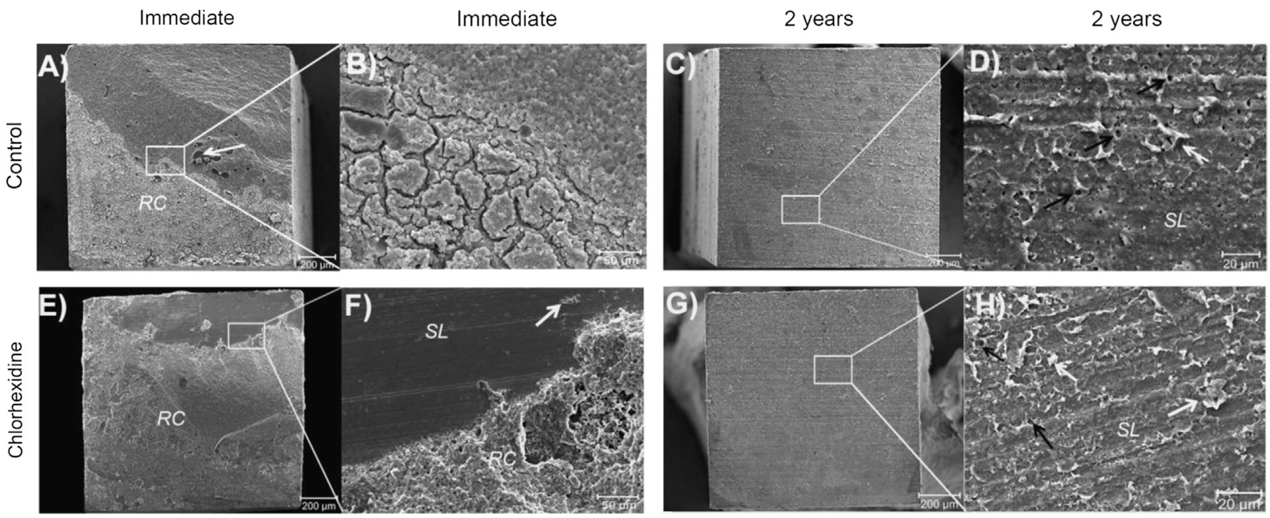

Fig. 2. Representative SEM of the dentin side of fractured specimens in groups luted with RelyX U100 showing the predominant failure modes for each group. (A) Immediate testing, specimen presenting cohesive failure along resin cement (*RC*) (80× magnification), dentin areas not involved by resin cement (white arrow) during the luting procedure were sparsely observe when RelyX U100 failed cohesively; (B) higher magnification (550×) of the area limited by the rectangle in (A) showing cohesive failure of resin cement at different levels; (C) specimen stored for two-year in artificial saliva showing adhesive failure along the cement dentin interface (80× magnification); (D) higher magnification (1000×) of the area limited by the rectangle in (C), resin cement remnants (white arrows), exposed dentinal tubules (black arrows) were observed possible due to smear layer (*SL*) partial dissolution over time; (E) specimen pre-treated with chlorhexidine showing a mixed failure mode involving cohesive failure of resin cement (*RC*) and adhesive failure along resin cement and dentin; (F) higher magnification (550×) of the area limited by the rectangle in (E) showing the presence smear layer (*SL*) no open dentinal tubules were observed; (G) adhesive failure between resin cement and dentin was the predominant failure mode observed on when was pre-treated with chlorhexidine after ageing; (H) higher magnification (1000×) of the area limited by the rectangle in (G), similarly to (C) and (D), resin cement remnants were observed (white arrows) along with sparse open dentinal tubules (black arrows) partially covered by smear layer (*SL*).

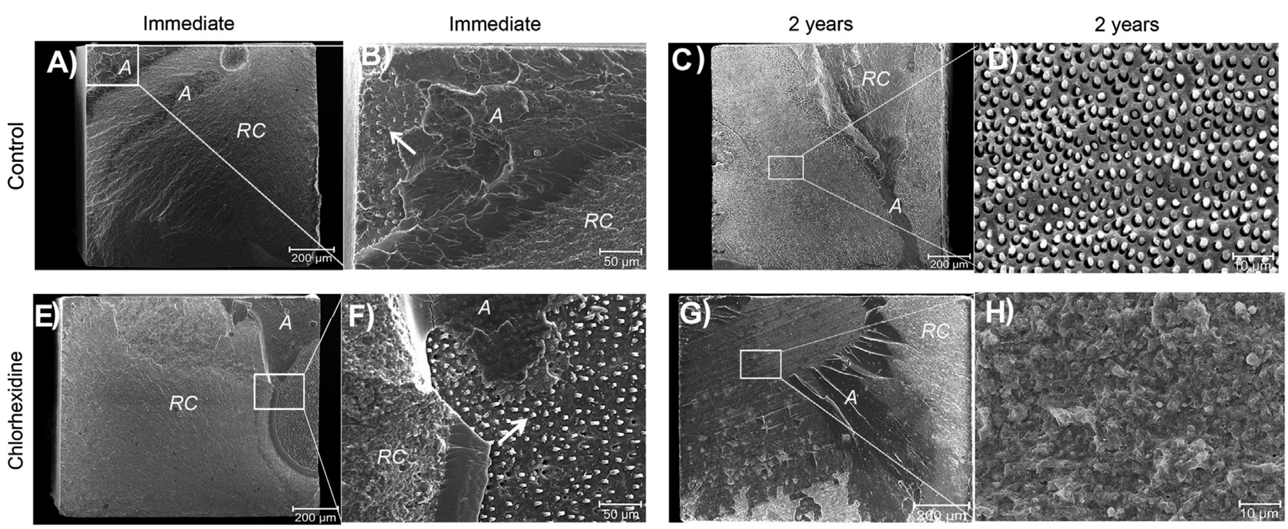

Fig. 3. Representative SEM of the dentin side of fractured specimens in groups luted with RelyX ARC showing the predominant failure modes for each group. (A) Immediate testing showing a mixed failure pattern involving predominantly cohesive failure of resin cement (*RC*) and adhesive failure between resin cement and restoration (80× magnification) (*A*=adhesive system); (B) higher magnification (550×) of the area limited by the rectangle in (A) showing a small area of cohesive failure along the adhesive system (*A*) and fracture on top of the hybrid layer (white arrow); (C) specimen after two-years of storage in artificial saliva with predominant adhesive failure along cement and dentin (80× magnification), a small portion of cohesive failure of resin cement (*RC*) could be observed; (D): higher magnification (550×) of the area limited by the rectangle in (C), note that the fracture occurred at the bottom of the hybrid layer exposing resin tags; (E) chlorhexidine treated dentin immediately tested presenting a Type 4 failure similarly to A) (80× magnification); (F): higher magnification (1500×) of the area limited by the rectangle in (E), a portion of cohesive failure involving the adhesive system (*A*) and exposed resin tags were observed; (G) chlorhexidine treated dentin after two-years of storage, showing a mixed failure pattern involving cohesive failure of resin cement (*RC*) and adhesive failure along dentin/cement interface, note a large area presenting cohesive failure of the adhesive system (*A*); (H) higher magnification (1500×) of the area limited by the rectangle in (G) showing that fracture occurs mostly on top of the hybrid layer, open dentinal tubules or resin tags were sparsely observed in small areas.

preserve dentin bond strength of the conventional resin cement tested when an adhesive system containing HEMA/BisGMA monomers was used; and (iv) dentin bond strength of the self-adhesive resin cement tested was neither affected by aging nor by the use of 2% chlorhexidine, therefore the use of chlorhexidine to preserve resin–dentin bond strength of self-adhesive resin cements should not be encouraged.

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