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THE EFFECT OF CROSS-LINKER ON ENDOGENOUS ENZYMATIC ACTIVITY WITHIN RADICULAR DENTIN AND BOND STRENGTH BETWEEN INTRARADICULAR POST AND RADICULAR DENTIN

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Abstract

The primary aim of this study was to evaluate the effect of 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) on endogenous enzymatic activity within radicular dentin and push-out bond strength of adhesively luted fiber posts, at baseline and after artificial aging. Additionally, the effect of different cementation strategies on endogenous enzymatic activity and fiber post retention was evaluated. The experiment was carried out on extracted human premolar teeth, following endodontic treatment and fiber post cementation with different strategies. Briefly, 3 cementation strategies were performed: resin cement in combination with etch-and-rinse (EAR) adhesive system, resin cement in combination with self-etch (SE) system and self-adhesive (SE) cement. Each of the mentioned strategies had a control and experimental (EDC) group in which root canal was irrigated with 0.3M EDC for 1 minute. The push-out bond strength test was performed 24h after cementation and after 40.000 thermocycles. In order to investigate the effect of EDC and different cementation strategies, in situ zymography analyses of the resin-dentin interfaces were conducted. Analysis of variance (ANOVA) was performed to examine the effects of the dependent variables ("EDC pretreatment", "root region", "artificial aging" and "cementation strategy") on push-out bond strength. Kruskal-Wallis one way analysis of variance and pairwise multiple comparison procedures (Dunn's Method) were used to analyze the data obtained from in situ zymography analysis. Statistical analyses were conducted with the software Stata 12.0 (Stata Corp, College Station, Texas, USA) and the significance was set for $p<0.05$. The statistical analysis showed that the variables "EDC", "root region" and "artificial aging" significantly influenced fiber posts' retention to root canal. The highest values were observed in coronal third. The mean values observed after artificial aging were lower when compared to baseline, however EDC was effective in preserving bond strength. The level of enzymatic activity varied between the groups, with highest activity observed in SA groups, and lowest in EAR groups. EDC had a beneficial effect on silencing the enzymatic activity. Within the limitations of the study, it was concluded that the choice of cementation strategy did not influence posts' retention, while EDC contributed to the preservation

of bond strength after artificial aging and reduced enzymatic activity within radicular dentin. In vivo trials are necessary to confirm the results of this in vitro study.

Key words: cross-linker, radicular dentin, adhesion, enzymatic activity

Introduction

Teeth that have undergone root canal therapy are usually characterized by massive loss of coronal and/or radicular dental tissues, which makes them prone to fracture and can eventually result in tooth loss, causing patient's dissatisfaction and difficulties in mastication. The material of choice which is indicated for luting of fiber posts is resin cement. Currently, 3 different cementation strategies can be used during luting procedures: etch-and-rinse (EAR), self-etch (SE) and self-adhesive approach (SA). The first two groups of cements rely on the use of adhesive systems and formation of hybrid layer (HL) - a structure responsible for maintaining the integrity of resin-based restorations. Despite the great progress in the filed of adhesive dentistry and improved characteristics of resin cements, clinical failure in the form of post debonding still occurs. Failure of the post-retained restoration can happen due to the degradation of the HL during which endogenous dentinal enzymes, such as matrix metalloproteinases (MMPs), play an important role. In attempt to overcome this failures, using cross-linking agents and MMPs inhibitors, among which is 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), can be an interesting approach in preserving resin-dentin integrity. So far, promising results have been obtained when using EDC on coronal dentin, however the literature seems to lack information of the EDC effect within radicular dentin when using different adhesive protocols.

Therefore, the aim of this research was to investigate the effect of EDC on push-out bond strength between fiber posts/resin cements and radicular dentin, at baseline and after artificial aging. Furthermore, the influence of EDC and different resin cements on endogenous enzymatic activity within radicular dentin was assessed.

Literature overview

Bonding within the root canal space

Smear layer

The aim of root canal treatment is to eliminate bacteria and infected dentine from within the root canals by means of chemo-mechanical preparation. (1) Similarly to the cutting of coronal dentin by rotary instruments, smear layers are formed on the surfaces of radicular dentin during cleaning and shaping of canals, as well as during preparation for fiber post placement. (2) The creation of smear layer during instrumentation of root canal space is inevitable and its thickness depends the type and sharpness of the endodontic instruments as well as on the degree of dentin moisture. (3) Generally, endodontic smear layers consist of 1-2 μm thick superficial layers of organic and inorganic substances that include fragments of odontoblast processes, microorganisms and necrotic material. These structures are organized into globular aggregates that have 0.05-1 μm diameter. (4) Their small diameter enables them to be packed into the orifices of dentinal tubules, consequently forming smear plugs. The question whether and how to remove smear layer has been widely discussed in the past, and several considerations regarding the properties of this structure should be mentioned.

Firstly, the thickness and volume of the smear layer if difficult to predict. It usually contains bacteria and their products, as well as necrotic tissue which can be a favorable substrate for the development of infection. Furthermore, it may limit the penetration of disinfecting agents and compromise the formation of adequate seal during obturation of root canals. Lastly, it can adversely influence the bond strength between resin-based dental cements and walls of radicular dentin. (5)

In attempt to remove smear layers, several strategies have been suggested and are being used in clinical settings nowadays. The most commonly method of smear layer removal is by applying chemical agents inside the root canal space. (5) Special attention has been given to studying the effect of sodium-hypochlorite (NaOCl) and calcium-chelating agents such as ethylendiaminetetaacetic acid (EDTA) on smear layer removal and their potential effect on

radicular dentin. Most clinicians use the protocol which combines the joint effect of NaOCl and EDTA. (6) NaOCl is a well-known deproteinizing and antibacterial agent that has the ability to dissolve the necrotic tissue (7) and organic components of smear layers (8). During irrigation of canals, it is applied in concentrations varying from 0.5% - 6.15%. (9) On the other hand, 17% EDTA solution leads to the demineralization of the inorganic components of dentin via calcium-chelation. (10) The use of scanning electron microscopy (SEM) has enabled the evaluation of cleanliness of the apical, middle and coronal third of root canal walls following NaOCl/EDTA irrigation. (11, 12) In general, there is a tendency of decrease of canal walls' cleanliness from coronal to apical part (13), and the introduction of active irrigation techniques may enhance the removal of smear layers. (14)

The biggest concern raised when using combination of these two irrigants is the risk of erosion of the underlying radicular dentin which is intact. (15) Studies have been conducted in order to investigate the effect of time exposure and different concentrations of NaOCl and EDTA on the structural integrit of mineralized dentin. (8) A recent study found that 3-5% NaOCl followed by final irrigation of 8-17% EDTA causes minor changes in the relative proportions of Ca and P, leading to minimal erosion of radicular dentin and can be recommended in clinical practice. (16)

Permeability of radicular dentin

The permeability of radicular dentin depends on the thickness of dentin, the density of its tubules and diameter of the tubules. The density of dentinal tubules can vary from 42.000 tubules/mm2 for coronal to as low as 8.000 tubules/mm2 for the apical part. (17) This number can change, depending also on the curvature of the roots. (18) The apical third of the root, close to the apex, is the region showing most variability in morphology and is usually composed of accessory canals, irregular secondary dentin and sometimes cementum-like tissue. (19) Overall, the various morphology found in radicular dentin can lead to different responses to the same etching procedures and, consequently, unequal infiltration of resin cements.

Differently from the coronal dentin, bonding to radicular dentin is less influenced by dentin permeability due to the following reasons: (1) no pulpal pressure is present in the canal space after endodontic therapy; (2) the existence of an intact cementum layer; (3) during root canal shaping and post space preparation, progress is made from deep radicular towards superficial root dentin. (20) However, the creation of the smear layer and smear plugs on the root canal walls' surface can adversely affect the expected increase in dentin permeability which is created by enlarging the intracanal space and by decreasing the thickness of dentin during instrumentation. It is worth mentioning that a promising effect on increasing dentin permeability has been achieved by applying NaOCl and EDTA on radicular dentin surface. (21, 22)

Aspects related to resin polymerization within root canals

After being subjected to root canal shaping, endodotically treated teeth are usually characterized by massive loss of coronal and/or radicular tissue. (23) Fiber reinforced composite (FRC) posts have gained great popularity in restoring structurally compromised teeth since they performed equally well when compared to traditional metal posts in clinical trials. (24, 25) Besides their main indication which is to provide an adequate retention for the coronal restoration, attempts have been made to use FRC posts as intraradicular reinforcing material in order to provide higher fracture resistance with less catastrophic failures. (26-29)

Unlike metal posts which can be cemented using traditional zinc phosphate cement (30), resin cements are considered to be the material of choice for luting of FRC posts. (31) Since the geometry and configuration of root canals is different than coronal cavities, several aspects related to polymerization difficulties of resin cements should be considered.

One of the greatest concerns during polymerization of resin-based dental materials is polymerization shrinkage. (32) The geometry of cavity influences the ease of stress relief during the pre-gelatin phase and is expressed as the ratio of the areas of the bonded to the unbounded cavity surfaces (C-factor). (33) It was reported that for direct composite restorations placed in coronal

cavities, C-factor higher than 3 can cause debonding and leakage. (34) On the other hand, C-factors found within root canal spaces have much higher values and can be over 200. During polymerization procedures, the shrinkage stress relief of resin-based materials is possible due to the presence of large unbonded or freely shrinking surfaces. If the unbonded surface areas are small, there is insufficient stress relief via resin flow and a high probability to cause debonding of the luting material from the intra-radicular dentin. (35) Bearing in mind the specific nature of root canal geometry and that avoiding the development of large contraction stress during the curing procedure is practically impossible, polymerization shrinkage can be controlled by rheological properties of luting materials – which is a direct consequence of its filler content. (36)

Another problem related to the anatomy of root canal is its narrow diameter which, consequently, reduces the penetration of light in deeper intracanal areas, as the distances increases from the light source. (37) The risk of incomplete polymerization is increased when resin cements are placed deeper than 4 or 5 mm due to the restricted transmission of light. (38) For the mentioned reasons, is it advisable not to use light curing resin cements during FRC post cementation, and instead use dual-cure materials. However, even when using dual-cure cements it is generally recommended to light-cure them since it was found that in some cases it can influence bond strength between the cement and dentin. (39)

Different from using self-adhesive cements, multi-step resin cements require application of adhesive system (with or without acid etching, as discussed in other sections) which allow resin penetration into radicular dentin. This is an important consideration, since the use of simplified adhesives for bonding to radicular dentin may further raise the question of incompatibility between the acidic resin monomers that are resident in the oxygen inhibition layers of these adhesives, and the binary peroxide-amine catalysts that are employed in dual-cured resin cements. (40, 41) Nowadays, this problem is usually taken care of by the manufacturers. Dual-cure adhesive versions are available in which tertiary catalysts such as sodium benzene sulphinate are used to offset the

acid-base reaction between the acid monomers and the basic amines along the composite-adhesive interface. (42)

Lastly, remnants found on root canal walls after post space preparation can negatively affect the polymerization of resin cements. An example would be the influence of eugenol-based sealers on bond strength of adhesively luted FRC posts. Eugenol (4-allyl-2-ethoxyphenol) is a phenol component sometimes added to endodontic sealers and can cause delayed polymerization reaction. (43) A recent systematic review with meta-analysis investigated the effect of eugenol-based sealers on push-out strength of FRC posts, and the following conclusions were drawn: (1) eugenol-based sealers reduce the immediate push-out bond strength of FRC posts luted to the root canal with resin cements; (2) the bond strength of all types of adhesive systems was influenced; (3) bond strength of all types of dual-cure resin cements was influenced. (44) Therefore, root canal obturation with this group of sealers is not recommended, if cementation of FRC post with resin cements are further indicated for the reconstruction of the tooth.

Hybrid layer and adhesive systems

Resin-based dental composites are the most commonly used restorative materials in everyday dental practice due to their good mechanical and esthetic characteristics and handling properties. (45-48) In order to achieve long term bonding to enamel and dentin, composite materials require the use of adhesive systems. (49) Based on their interaction with the smear layer and number of steps used during bonding procedures, dental adhesives can be classified into etch-and-rinse (EAR) systems (3- and 2-step) and self-etch (SE) systems (2- and 1-step). (50, 51)

The application of the adhesive system (either EAR or SE) on dentin surface results in the formation of hybrid layer, a structure that is composed of demineralized collagen fibrils reinforced by resin matrix. (52) Etch-and-rinse systems are the oldest adhesives in the evolution of dentin bonding agents. When supplied in the 3-step version, they involve acid-etching with phosphoric acid, priming and application of a separate adhesive. In the 2-step version, after acid-etching, dentin

is simultaneously primed and bonded since the hydrophilic primer and the hydrophobic resin are blended in one solution. (50) On the other hand, simplified self-etch adhesives do not require separate etching step with phosphoric acid. They either come as two- or one-step adhesives, depending whether the self-etching primer and the adhesive resin are provided separately or combined into one single solution. Simplified adhesives are composed from acidic monomers that simultaneously condition and prime dentin, through a partially dissolved smear layer. Since they do not include a separate etching step, the initial substrate for one-step self-etch adhesive systems is mineralized dentin. (51, 53) Generally speaking, thicker hybrid layers are observed when using EAR adhesive systems when compared to SE systems. (54) However, thicker hybrid layers do not necessarily mean higher bond strengths, since both adequate immediate bond strength and good clinical behavior was observed when using SE systems. (55-58) Interestingly, neither EAR or SE adhesive systems are able to prevent the phenomenon of nanoleakage - the diffusion of small ions or molecules within the hybrid layer in the absence of gap formation. (59, 60)

Unlike self-adhesive resin cements which do not form a typical hybrid layer (as discussed in other Chapter), multi-step or conventional resin cements rely on the application of adhesive system, and therefore, a true hybrid layer is formed when using this group of cements for luting procedures. Adhesive cementation of FRC posts can be achieved using also multi-step resin cements, which are modified composite resins with a higher fluidity to improve flow during cementation. (61, 62) Multi-step resin cements require more chair-side time and clinical steps compared to self-adhesive ones, due to the dentin pretreatment which is necessary when using these cements. Also, they are considered to be more technique-sensitive than self-adhesive cements. Although conventional resin cements are more technique sensitive, because they require adhesive cements, these cements are more capable of interpenetrating the demineralized dentin substrate. (63, 64) Since details about classification of resin cements based on their polymerization method is explained in the previous Chapter, a brief overview of comparison between self-adhesive and multi-step cements in bonding performance will be given. A recent systematic review and meta-analysis investigated the data from

laboratory studies that assessed the adhesion performance of indirect restorations to dentin of two different resin cement types: conventional and self-adhesive. The overall results of this article reported that the conventional adhesive approach (resin cement applied in combination with an adhesive system or primer agent) tends to promote higher immediate- and long term bond strength of indirect coronal restorations to dentin. (65) On the other hand, another systematic review revealed that self-adhesive cements seem to improve the retention of FRC posts to radicular dentin when compared to multi-step resin cements. (66)

Degradation of resin-based interfaces

The application of adhesive systems, whether EAR or SE, results in incomplete hybridization of dentin substrate, leaving unprotected collagen fibrils surrounded with water on the bottom of the hybrid layer. (67) Two important aspects, described in the following sections, should be taken into consideration for better understanding of processes that lead to degradation of resin-based restorations.

Resin degradation

Two main mechanisms are considered to be responsible for HL degradation: the disintegration and solubilization of collagen fibers and the hydrolysis and leaching of the adhesive resin material from the interfibrillar spaces. The most important reason for resin degradation between the hybrid layer is hydrolysis. (68) In an attempt to overcome this problem, contemporary adhesive systemts contain a mixture of hydrophilic resin monomers, such as two-hydroxyethyl methacrylate (HEMA),in diluents and organic solvents, usually water, ethanol or acetone. These hydrophilic resin monomers are important for infiltration of the adhesive systems through the wet and demineralized dentin causing the hybridization of the adhesive with the substrate. (69) Still, the mentioned hydrophilic resin monomers in adhesives formulations cause high water sorption by the resin systems and generate a HL that behaves as a pours membrane after polymerization, which permits moving of water throughout the bonded interface. (70)

The penetration of water into the hydrophilic domains of the adhesive enables the leaching of the solubilized resin material. Consequently, resin-infiltrated collagen matrix is solubilized and is slowly leached-out, the underlying insoluble collagen fibrils become exposed and become prone to attack by enzymes, such as matrix metalloproteinases (MMPs). (71) Furthermore, the presence of residual water in the pretreated (etched) dentin can decrease the polymerization of the adhesive monomers which further leads to the increased permeability of the adhesive layer. (72) Even though great advances have been made in the field of adhesive dentistry, all adhesives show variable degrees of incomplete polymerization that correspond to the extent of fluid movement throughout the adhesive layer. (49)

Finally, long-term exposure of resin-based restorations to masticatory forces and repeated changes in temperature and pH which are present in oral cavity may cause deformation of restorative materials (contraction and expansion), affecting resin-dentin interface and allowing penetration of oral fluids. (73) The infiltration of water molecules into the hydrophilic domains of resin-infiltrated matrix collagen matrix can become trapped during the process of photopolymerization. This “trapped” water can further enhances the hydrolysis of collagen and resin polymers, accelerating degradation by abrading the surface, and allowing entrance of both water and salivary enzymes, that can accelerate ester bond hydrolysis, leading to the failure of the adhesive interface. (71, 74)

Degradation of the collagen scaffold/fibers and the role of matrix metalloproteinases

One of the pioneers in explaining collagen degradation over time even in aseptic conditions was Pashley et al., who suggested that this phenomenon occurs due to the endogenous enzymes. (75) The most widely studied group of enzymes which are considered responsible for resin-dentin degradation are MMPs and cysteine cathepsins. In order to understand MMPs mechanism on degradation of HLs as well as hybridization process that occur during adhesive procedures, a short overview of dentin’s structure should be given. Dentin is a collagen-based mineralized tissue

consisting of inorganic apatite crystallites embedded in an extracellular matrix. Type I collagen is the main component of the ECM compartment of dentin, representing up to 90% of the organic material. (76) In addition, several proteins, collectively referred to as noncollagenous proteins, constitute approximately 10% of the matrix. The noncollagenous dentin proteins include proteoglycans, phospholipids, and enzymes. (71, 77) The composition of dentin can vary in different areas of the tooth, depending on its proximity to the pulp tissue, as well as whether the matrix is demineralized or caries affected/infected. These differences can have an effect on the mechanical properties of dentin, as well as the success of bonding to dentin. (78, 79) A collagen molecule is composed of three α-chains, two α-1and one α-2 chain intertwined into a left-handed triple helix. (80) Collagen chains have 3 main domains: a central triple helical region (>95%), a non-helical amino terminal (N-telo peptide) region and a car-boxyterminal (C-telopeptide) region. (81) These peptide chains organize unsoluble collagen fibers by aggregating and stacking in parallel. These collagen fibers contain a 67 nm gap between the adjacent collagen molecules, and are further organized in bundles. (71) During dentin maturation, apatitic mineral crystallites precipitate and inactivate enzymes that are present in the extracellular matrix and were active during the dentinogenesis. (77, 82) Interestingly, dentinal collagen can withstand adhesive procedures that would otherwise destroy the structure of the dermal collagen. (71) However, it is important to underline that dentin over-etching with phosphoric acid (etching longer than 15s) may lead to structural changes in collagen molecules and therefore it is important to limit etching time to not more than 15s. (83)

MMPs are endogenous Zn^{2+}- and Ca^{2+}-dependent enzymes, capable of degrading almost all extracellular matrix components. In human species, the MMPs family consists of 23 members, classified into 6 groups based on substrate specificity and homology. (84) MMPs are typically present as inactive enyzmes in dentin, and the pro-domain requires to be dissociated from the catalytic one in order to be activated. (82) In their non-active form MMPs, the unpaired cysteine in the pro-domain forms a bridge with the catalytic zinc (known as “cysteine switch” mechanism),

preventing enzymatic activity and acting as a ligand for the catalytic zinc atom in the active site, excluding water molecules and rendering the enzyme inactive. (85) Moreover, tissue inhibitors of MMPs have an important role in the local control of MMP activities in tissues, and represent the main inhibitors of MMPs. The MMPs inhibitor family consists of 4 members that all together inhibit MMP activities and prevent breakdown of extracellular matrix. (86)

The most abundant MMP in human dentin is MMP-2, followed by MMP-9. The gelatinases MMP-2 and -9 are not considered to be true collagenases. Yet, they are crucial for the process of collagen degradation. The presence of other enzymes such as collagenase MMP-8, stromelysin-1, MMP-3 and MMP-20 that have been discovered in dentin using different methods. (71)

True collagenases such as MMP-1, -8, -13, -18 are not capable of cleaving intact collagen molecule at the cleavage site, because of the collagen molecule orientation and the position of the C-terminal end, which blocks access to the peptide bonds. (87) Gelatinases, that belong to the large group of telopeptidases, can remove blocking C-terminal telopeptides, allowing access to the true collagenases. Consequently, collagenases can come in contact with the collagen at the cleavage site, turning it into fragments: a 3/4 N-terminal and a 1/4 C-terminal fragment. Removal of the telopeptides also eliminates the C-terminal cross-links, most likely making the collagen more prone to non-specific degradation. (71)

As previously explained, when the dentin is mineralized, its proteases remain structurally stable and inactive. (67) One of the first studies that investigated the influence of application of EAR and SE adhesive systems on MMP-2 and MMP-9 activity by means of gelatin zymography was carried out by Mazzoni et al. (2013). (88) Briefly, the authors mixed dentin powder of sound human teeth with different brands of EAR or SE adhesives, after which the adhesives were rinsed off with acetone. The treated dentin powder was then subjected to zymographic analysis in accordance with the previously established protocol. (89) Interestingly, the activity of MMP-2 and -9 after treatment with either EAR or SE adhesives were adhesive-dependent. Likewise, with SE systems, the exposure of matrix-bound MMPs was followed by increased activity, but sometimes

showed reduced level of activation. To sum up, the authors concluded that there was direct evidence of increased MMP-2 and -9 activities following adhesive application, regardless of the adhesive system used (EAR or SE). (88) Another interesting approach using in situ instead of only using gelatin zymography was suggested by the same groups of authors. This study was one of the firsts to evaluate the activity of endogenous proteases of the HL by means of in situ zymography, showing obvious gelatinolytic activity within HLs created with a two-step EAR adhesive. (90)

Strategies for preservation of the HL

As introduced before, degradation of collagen fibers and hydrophilic resin components lead to degradation of the hybrid layer and can cause the loss of dentin bond strength over time. Currently, the literature suggests two distinct methods of preserving HLs (53, 71, 91):

1. inhibition of enzymatic activity (mostly referring to MMPs activity)
2. increasing the collagen resistance to degradation

Inhibition of the enzymatic activity

The inhibition of endogenous collagenolytic activity can be achieved by chelating mechanisms, considering the fact that the activity of the MMPs is dependent on the metal ions that can be chelated. (71) The most studied MMPs inhibitor is chlorhexidine (CHX) which, apart from having antimicrobial effect, increased the longevity of HL in *in vitro* studies. (89, 92, 93) A recent review by Josic et al. (2021) critically discussed the clinical trials in which CHX was used as a therapeutic primer in order to obtain superior clinical behavior of resin-dentin restorations. (53) The following sections will consider the findings of the review, mechanism of action and potential benefits of using CHX in clinical settings.

Up to this date, 6 clinical trials investigated the effect of CHX pretreatment on clinical performance of composite restorations. (94-99) Only one out of six trials investigated the effect of

CHX on class II cavities of posterior teeth (97), while the rest of the studies applied CHX within non-carious cervical lesions (NCCLs). The details of these studies are presented in Table 1.

Table 1: Overview of the clinical studies that investigated the effect of CHX on clinical performance of composite restorations

Author, year, location	Study design	Groups and number of restorations placed	CHX application details	Adhesive system / composite resin	Outcome
Neimar Sartori, 2013, Brazil	CT; split-mouth; single blind	2 groups: 1) CHX group; 2) control group. 70 restorations (n=35 CHX group, n=35 control group)	2% CHX solution scrubbed on dentin surface for 30 s	Two-step etch-and rinse adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) + nanofilled composite (Filtek Supreme XT, 3M ESPE)	No difference in the restoration retention and failure rates between the CHX and the control groups, up to 36 months of follow-up
Maristela Dutra-Correa, 2013, Brazil	CT; split-mouth; single blind	4 groups: 1) XP Bond; 2) XP Bond + CHX; 3) Xeno V adhesive; 4) Xeno V adhesive + CHX; 120 restorations (n=30 per each group)	2% CHX solution applied with microbrush for 20 s	Two-step etch-and-rinse adhesive XP Bond (XPB, Dentsply Caulk; Milford, DE, USA) + hybrid composite EsthetX (Dentsply Caulk); One-step self-etch adhesive Xeno V (XEN, Dentsply DeTrey; Konstanz, Germany) + hybrid composite Esthet-X (Dentsply Caulk)	The application of CHX prior to the dentin adhesive did not influence the 6- and 18-months clinical outcome of the two adhesives
M.S.R.G. Araujo, 2015,	RCT, split-mouth	4 groups: 1) Clearfill SE group (CSE); 2) CSE+CHX group; 3) AdhSE group (ADS); 4) ADS + CHX	50 mL of 20% CHX digluconate was incorporated into to 950 mL of the Clearfil SE primer or AdheSE primer to form a mixture with a CHX concentration of	Two-step self-etch adhesives (Clearfil SE Bond, Kuraray, Osaka, Japan) and AdheSE (Ivoclar Vivadent, Schaan, Liechtenstein) +	The incorporation of CHX into the primer of the tested two-step

Brazil	(paired tooth), double blind	group 126 restorations (n=32 in CSE group, n=33 in CSE+CHX group, n=32 in ADS group, n=29 in ADS+ CHX group)	1.0 wt%. Clearfil SE Primer was applied to the enamel and dentine surfaces for 20 s. AdheSE primer was rubbed into the enamel and dentine surfaces for 30 s	nanocomposite resin (Filtek Z-250, 3M ESPE, St. Paul, MN, USA)	self-etch adhesives did not add any clinical advantage up to 2 years of follow-up
Anelise Fernandes Montagner, 2015, Brazil	RCT, split-mouth, triple blind	2 groups: 1) CHX group; 2) control (placebo) group. 169 (n=88 CHX group, n=81 control group)	2% CHX solution applied on dentin surface for 60 s	Two-step etch-and rinse adhesive system (Adper Single Bond 2; 3M ESPE, St. Paul, MN, USA) + nanoparticle composite resin (Filtek Z350; 3M ESPE, Irvine, CA, USA)	No differences in the restoration retention and failure rates between the CHX and the control groups after 6 months of follow-up
Morgana Favetti, 2017, Brazil	RCT, split-mouth, triple blind	2 groups: 1) CHX group; 2) control (placebo) group. 182 restorations (n=91 CHX group, n=91 control group)	2% CHX solution scrubbed on dentin surface for 60 s	Two-step etch-and-rinse adhesive system (Single Bond 2, 3M ESPE, St. Paul, MN, USA) + nanocomposite resin (Filtek Z350, 3M ESPE, Irvine, CA, USA)	No differences in the restoration retention and failure rates between the CHX and the control groups, up to 36 months of follow-up

With the attempt to inhibit endogenous dentinal enzymatic activity and therefore improve bond durability of resin-based restorations, CHX can be used in different modes in clinical settings: (1) as a separate aqueous primer as shown in Figure 1; (2) blended within the primer of two-step SE adhesive systems, or (3) incorporated into adhesives. It has long been known that CHX has a direct inhibitory effect against MMP-2 ,-8 and -9, with MMP-2 being more sensitive than MMP-8 and -9. (100) Although CHX has been widely investigated in *in vitro* and clinical studies, the mechanism responsible for its MMPs inhibiting property has not yet been entirely elucidated. The proposed

mechanism of action involves a chelating mechanism, since CHX is capable of removing zinc and calcium ions which are necessary for the activity of MMPs, but it can also react with catalytic sites within MMPs. (101) Although the mechanism of MMPs inhibition by CHX is thought to be purely electrostatic and therefore reversible, CHX has a high substantivity to dentin, both mineralized and demineralized. (92) In the clinical settings, after orthophosphoric acid etching (3-step or 2-step EAR strategy) or priming (2-step SE strategy), dentin remains partially demineralized, allowing the CHX to exhibit affinity toward the demineralized as well as the underlying mineralized dentinal tissue.

Regardless of the number of steps, when used with etch-and-rinse systems, CHX is usually applied as 2% aqueous solution after the dentin surface had been previously etched with 32-37% phosphoric acid. The separate etching step removes the smear layer and minerals from the dentin surface, and after the phosphoric acid had been rinsed with water, exposed collagen fibrils are left behind. (102) In this case, dentin can be considered as partially demineralized, and applying CHX to this kind of substrate allows it to bind to both collagen matrix, as well as to the underlying mineralized matrix. (92) Once CHX solution is brushed on dentin, no water rinsing is expected to be performed, since the bound CHX could be displaced by the presence of abundant water. Rather, the CHX-impregnated dentin should be immediately covered by the adhesive system which, if followed by an adequate polymerization, should promote the incorporation of CHX within the HL over a prolonged period of time. (103, 104)

On the other hand, simplified SE adhesives do not require separate etching step with phosphoric acid. They either come as two- or one-step adhesives, depending whether the SE primer and the adhesive resin are provided separately or combined into one single solution. Simplified adhesives are composed from acidic monomers that simultaneously condition and prime dentin, through a partially dissolved smear layer. (51) Since they do not include a separate etching step, the initial substrate for one-step self-etch adhesive systems is mineralized dentin. Compared to partially demineralized dentin, mineralized dentin contains inorganic phase in the form of negatively charged

hydroxiapatite which are prone to bind positively charged molecules such those of CHX. (104) However, for the two-step self-etch adhesives, due to the fact that demineralization is achieved by application of primer that contains acidic monomers, the substrate in this specific case can be considered to be partially demineralized dentin, with CHX binding mechanisms similar to the ones previously described for the etch-and-rinse adhesives.

Based on the available literature, it can be concluded that, despite several *in vitro* findings, currently there is still no evidence that supports the use of CHX to improve the prognosis of adhesively-bonded composite restorations.

Increasing the collagen resistance to degradation

Even though CHX inhibits MMPs (89), one of its disadvantages is that it may leach out from hybrid layers over a period of time and loose its protective function. (105, 106) Therefore, other strategies, such as the use of cross linking agents, in preserving the integrity of hybrid layers have been employed and investigated. (107, 108) Both inhibition of MMPs and increase of collages resistance can be achieved by using cross-linking agents. The term "cross-link" refers to the chemical bond between the side chains of amino acids within collagen molecules. (109)

In order to understand the rational behind using chemical cross-linking agents, a detailed explanation of natural cross-linking mechanism that occur within dentinal collagen should be given. Firstly, dentinal collagen does not metabolically turn over and it is not easily degraded. (71, 110) This ability is due to the gradual creation of covalent inter- and intramolecular cross-links, which occur between the C-terminal of one collagen molecule and the N-terminal of the adjacent collagen molecule. (71, 111) Hydrogen bonds are also important for the stabilization of the triple helix by bridging the water-filled gaps between the collagen molecules, and they bring them closer together and facilitate intra- and intermolecular reactions. Because dentinal collagen does not turn over, the natural cross-links accumulate over time and can influence the mechanical properties of collagen fibrils. Dentinal collagen is the most cross-linked collagen in the body. Due to its highly cross-

linked nature, collagen can be acid-etched during bonding procedures without denaturing its structure. (71, 112-114)

So far, the effect of the following cross-linking agents has been investigated and reported in the literature: glutaraldehyde (115-117) and grape-seed extract (118-121). Despite being effective in preserving hybrid layers and bond strength, the biggest remark of glutaraldehyde remains its cytotoxicity (118). Therefore, previous research sought to investigate the effect of 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) on preservation of hybrid layers. Ideally, a cross-linking agent should have inhibiting MMP property and should reinforce denuded collagen fibrils. EDC is known as zero cross-linking agent since it has the ability to cross-link peptides without introducing additional linking groups. (122) Compared to glutaraldehyde, EDC is less cytotoxic and contains functional RN=C=NR group and can react with ionized carboxyl groups in proteins to form an O-acylisourea intermediate that reacts with a non-proteinated amino group and an adjacent protein chain to form a stable covalent amide bond between the two proteins. (71, 123) EDC causes cross-linking to occur in dentinal collagen as well as in dentin matrix-bound MMPs. It has a two-fold activity and is able to cross-link both helical and telopeptide domains in collagen and also to prevent telopeptidase activity that would normally remove telopeptides. (71, 124) When EDC is applied on demineralized dentin, it was shown that its cross-linking effect occurs more rapidly in MMPs as compared to collagen. (124-126) This is usually explained by the fact that carboxyl and amino groups in MMPs are more accessible than those in collagen. (87, 126) Therefore, it may be considered that the inhibitory effect of EDC is much quicker than its cross-linking effect. (126)

Biomodification of dentin by using EDC has shown promising results in previous in vitro research conducted on coronal dentin. Mazzoni et al. (2014) reported that application of 0.3M EDC for 60 seconds can inactivate MMP-2 and -9 after EAR adhesive system has been used during bonding procedures. Furthermore, the images of in situ zymograpy showed that hybrid layers of tested EAR adhesives exhibited intense collagenolytic activity, while almost no fluorescence signal was detected when specimens were pretreated with EDC. (127) EDC was also able to preserve

long-term bond strength of hybrid layers created by 3- and 2-step EAR adhesive systems. Even though immediate bond-strength was not influenced by EDC pretreatment, the beneficial effect of this cross-linker was observed after 1 year of aging in artificial saliva. (128) Interestingly, EDC was able to reduce the matrix-bound collagenolytic enzyme activity over time in hybrid layers created with both EAR and SE adhesives. In case of SE adhesives, EDC is usually applied for 60 seconds after applying SE primer. (129) Another study confirmed the efficacy of EDC on preserving bond-strength, as well as reducing MMP activity after resin composite restorations had been placed with EAR and SE adhesives. (130) EDC was also able to increase the thermal denaturation temperature of dentinal collagen, and this was found to be time and concentration dependent. (131)

Overview of resin-composite cements

The function of dental cements is to retain indirect restorations, orthodontic brackets and post/core restorations in their position in which they have been placed during sitting positions. The mechanism responsible for keeping in place the restorations can be micromechanical (creation of hybrid layer), chemical and mechanical (friction). In the past, non-resin-based cements were used for cementation of restorations made of metal, whereas today esthetic restorations are usually cemented with resin-based cements, which provide adhesion to tooth tissues. (132)

Currently, resin-based dental cements are classified based on their polymerization kinetics (light-cure, auto-cure and dual-cure cements), and based on the number of steps applied during cementation procedure (conventional-multistep and self-adhesive resin cements). (133)

Light-cured resin cements are usually indicated under thin and translucent restorations where there is sufficient light penetration. However, when the restoration thickness is greater than 2 mm or its opacity inhibits light transmission, the light transmission can be compromised. (134) Furthermore, it was reported that the thickness of ceramic restoration has a more important effect on light transmission and polymerization of the cement compared to ceramic shade. (135) Therefore, light-cure cements are used in situations such as cementing veneers (in the anterior region) or thin

inlays in which the thickness and color of the restoration cannot influence in a great manner the ability of the curing light to polymerize the cement. (134)

When dealing with cases of thick indirect restorations or luting of fiber posts where light transmission is relatively limited, dual-cure resin cements are considered to be the material of choice. (133, 136, 137) Like light-cure cements, the polymerization of a dual-cure cement is crucial to provide adequate bond strength in the interface of restoration–resin cement and resin cement–dentin. Dual-cure cements can be photo-polymerized or a redox initiator system can initiate the polymerization. (138, 139) Interestingly, even though they are meant to polymerize well in the absence of light, lower degree of conversions were seen when dual-cure cements were light-cured through thicker ceramics, and resin cement shade and light-exposure time also had an affect on the degree of conversion on this group of cements. (140) Additionally, it has been observed that when light activation was applied, dual-cure resin cements may limit their self-cure mechanism and may compromise their mechanical properties. This property has been reported to be product-dependent, and cannot be generalized to all dual-cure resin cements. (132) Another in vitro study found superior results in terms of post-gel shrinkage when delaying photopolymerization for 5 minutes. (141) However, these results referrer to the cases when indirect restorations were cemented with dual-cure resin cements. A recent study investigated the effect of delayed light-curing when luting FRC posts with dual-cure resin cements. In accordance to the above mentioned studies, the delayed light-activation increased the retention of FRC posts of some dual-cure resin cements to radicular dentin, most likely due to the reduced polymerization stress and higher degree of carbon double bond (C = C) conversion of the cements. (142)

Another classification of resin cements is based on the number of steps used during their application and their interaction with dentin. (132) Although the terminology found in the literature is not always consistent, resin cements that require the application of adhesive systems prior to their application are referred to as (conventional) multi-step resin cements, while those that can be applied directly to the dentin surface without any pretreatment belong to the group of self-adhesive

cements. (143) The detailed explanation of mechanism of action and interaction with dentinal tissue of multistep resin cements will be discussed in the Chapter about formation and preservation of hybrid layer, due to the fact that it depends on the adhesive application. Since it is believed that self-adhesive cements do not form a true hybrid layer (63), a brief description of their main characteristics and their adhesion to dentin will be considered in the following paragraph.

Unlike multi-step resin cements that require adhesive system application, with or without separate acid etching step, self-adhesive cements are considered to be more user friendly and less-technique sensitive. The incorporation of acidic functional methacrylate or related monomers is a critical component in self-adhesive resin cements because effective chemical bonding to tooth tissues requires a polyacid matrix structure, based on a preformed polyalkenoate or one that is created in situ during a curing process involving acidic monomers. (144) The self-adhesive resin cements that can be found on today's market are two-part materials that require either hand mixing, capsule trituration or delivery by an auto-mixing dispenser. (63, 144) According to Ferracane et al. (2011), self-adhesive cements are comprised of conventional mono-, di- and/or multi-methacrylate monomers that are used in a variety of resin-based dental materials: Bis-GMA, urethane oligomers of BisGMA, UDMA, HEMA, TEGDMA, trimethyloylpropane trimethacrylate (TMPTMA). The functional acidic monomers that are utilized to achieve demineralisation and bonding to the tooth surface are still predominantly (meth)acrylate monomers with either carboxylic acid groups, as with 4 methacryloxyethyl trimellitic anhydride (4-META) and pyromellitic glycerol dimethacrylate (PMGDM), or phosphoric acid groups, as with 2-methacryloxyethyl phenyl hydrogen phosphate (Phenyl-P), 10-methacryloxydecyl dihydrogen phosphate (MDP), bis (2-methacryloxyethyl) acid phosphate (BMP) and dipentaerythritol pentaacrylate monophosphate (Penta-P). (144) The presence of the acidic monomers is critically important since it forms a strong, aqueous insoluble salt complex between Ca and the relatively hydrophobic MDP, whereas 4-Met and Phenyl-P produce a Ca-complex with partial stability to dissolution.(144) So far, the proposed mechanism of action of the self-adhesive cements has been studied and, in general, most of the authors are in agreement in

terms of the cements' interaction to dental tissues. Briefly, the setting reaction of RelyX Unicem (the most investigated self-adhesive cement) is based on the the free radical methacrylate polymerisation process as the primary reaction mode. This is then followed by activation by chemical and photochemical routes that initiate the cross-linking polymerisation of monomers with and without phosphoric acid functionality. The acidic groups bind with Ca in the hydroxylapatite to form a stable junction between the methacrylate network and the tooth tissues. Ions released from the acid-soluble filler neutralize the residual acidic groups to form a chelate reinforced three-dimensional methacrylate network. (144) Lastly, there is evidence by X-ray photoelectron spectroscopy of good chemical interaction with Ca from hydroxylapatite, which suggests that micromechanical retention is not the most significant mechanism of adhesion, since infiltration of more than a μm into the dentinal surface is present, and no real resin tag formation can be observed when using self-adhesive resin cements. (145)

FRC posts in adhesive dentistry

As mentioned earlier, the main role of a post system is to retain the coronal restoration of structurally compromised teeth. (146) Although a recent clinical study showed that glass fiber and cast metal posts showed good and similar clinical performance after 5 years, most clinicians today prefer using FRC posts due to their superior esthetic properties and avoiding the laboratory step necessary for metallic posts. (147) Non-metallic posts can traditionally be classified into: epoxy resin posts reinforced with carbon fiber, epoxy or methacrylate resin posts reinforced with quartz or glass fibers, zirconia posts and polyethylene fiber-reinforced posts. (148)

FRC posts are made of carbon, quartz or glass fibers which are embedded in a matrix of epoxy or methacrylate resin. (149) Fibers are oriented parallel to the post's longitudinal axis, and their number per mm^2 varies between 25 and 35, depending on the post type. It is considered that in transverse plane, approximately 30-50% of the posts' surface is filled with fibers. (150) The

adhesion between quartz or glass fibers and resin matrix is achieved by fiber silanization prior to embedding. (149)

FRC posts can be found in different shapes: cylindrical, cylindro-conical, conical or double-tapered. (149) Some authors suggest that parallel-sided posts are more retentive than tapered dowels. (151) Furthermore, it was found that double-tapered posts have the ability to adapt better to shape of the instrumented canal, therefore reducing the amount of dentine tissue to be removed during post space preparation. (152)

The mechanism responsible for the adhesion between resin based dental cements and FRC posts has been widely discussed in the literature, but with inconclusive results. When using a post with epoxy resin matrix, the methacrylate-based resin of the cement or the abutment interacts with a highly cross-linked polymer with limited sites available for copolymerization. (149) Chemical reactions are possible between the resin cement or core material and fibers exposed on the post surface. (149, 153, 154)

In an attempt to secure a more durable adhesion between the posts and cements, several strategies can be found in the literature. One of the frequently proposed strategies is the application of silane coupling agent, which should promote adhesion by increasing the post surface wettability, as well as by chemically bridging methacrylate groups of the resin and hydroxyl groups of quartz and glass fibers. (149) However, a recent systematic review reported that silane application played no role in improving the adhesion between the FRC posts and resin cements. (155) Different results were drawn from in vitro studies investigating the role of pretreatment of FRC post surface with air-borne particle abrasion or with hydrofluoric acid. (155) Interestingly, FRC post pretreatment with 10-20% hydrogen peroxide before silane application resulted in higher push-out bond strength values. (155-157) Generally, phosphoric acid etching can also be recommended for increasing the adhesion of cements to fiber posts, however, it should be considered that all these conclusions were drawn from in vitro studies, and that extrapolation to clinical settings should be done carefully. (155)

Research question

Even though they have been in clinical use for more than 20 years (158), luting of FRC posts is still considered as a technique sensitive procedure and clinical outcome of restorations that are retained by FRC posts is not always predictable. The most common clinical complication which happens in fiber post retained restorations is post debonding. (159) It is influenced by many factors: residual tooth tissues (which is considered to be the most important one), type of occlussion and number of opposing teeth in function, patient's periodontal status, possible signs of parafunction, presence or absence of ferrule, and quality of adhesion. (146, 159) Other complications include loss of retention of single crowns and marginal gaps. (160)

In an attempt to provide better retention and reduce debonding rates, several papers investigated the effect of CHX irrigation on the push-out bond strength between FRC post and radicular dentin. Durski et al. (2018) reported that the use of CHX an additional disinfection treatment with before the application of self-adhesive and multi-step resin cements produced the highest push-out bond strength, regardless of root third. The authors also demonstrated that thermocycling procedure decreased the bond strength for both resin cements long-term when CHX was not applied before cementation. (161) These results are in line with several other studies which reported beneficial effect of CHX on preserving or improving push-out bond strength of FRC posts luted with multi-step resin cements. (162-165)

Since the effect of CHX on long-term bond strength of adhesively luted FRC posts is debatable, mostly due to the fact that CHX tends to leach out of the resin-dentin interface (71), further attempts in preserving bond strength have been made by pretreating radicular dentin with cross-linking agents. So far, positive effect on preserving bond strength after 12 months of artificial aging has been reported when using natural cross-linking agents such as grape seed extract during luting procedures. Furthermore, gelatin zymography analysis revealed that grape seed extract was able to inhibit the MMPs activity when FRC posts were luted with EAR and SE adhesive systems. (166) Similarly to this natural cross-linker, EDC was also reported to preserve bond strength after

12 months of artificial saliva storage, when FRC posts were luted using EAR, SE and self-adhesive strategy. (167) Unlike CHX which was not able to prevent degradation of the adhesive interface after 10 months of storage, EDC was found to be effective in preserving bond strength of FRC posts in nonirradiated teeth, as well as in teeth that were subjected to radiation therapy. (168) Comba et al. (2019) found that the application of 0.3M of EDC solution was able to preserve the bond strength over 1 year of storage, when FRC posts were luted using 3- and 2-step EAR adhesive systems and dual-cure resin cement. Furthermore, the results of their in situ analyses of hybrid layers demonstrated that EDC was successful in inhibiting MMPs activity immediately after the cementation procedure. (169)

Although the effect of EDC on the push-out bond strength of FRC posts has recently been studied, the literature seems to lack information on how this cross-linking agent influences the enzymatic activity within radicular dentin, immediately after cementation and after simulated aging, when three different cementation protocols are employed for luting procedures. Furthermore, the evidence of bonding performance of a recently introduced universal self-adhesive resin compared to commercially available cements is still scarce. Thus the objectives of this thesis were to compare the bonding performance of the new self-adhesive resin cement, as well as to investigate the influence of EDC on push-out bond strength and endogenous enzymatic activity using different cementation strategies.

Pilot study

Following the cementation strategies for FRC post luting suggested by Radovic et al. (2008) (170) and Mazzoni et al. (2009) (171), the preliminary study aimed to investigate the influence of different adhesive strategies on immediate push-out bond strength between resin cements and radicular dentin. Furthermore, the bonding performance of a recently introduced universal self-adhesive resin cement was compared to bond-strength achieved by commercially available cements.

Lastly, the pilot study was conducted in order to investigate and recommend the most appropriate polymerization protocol (light-cured or self-cured) for all tested dual-cure resin cements.

Bonding performance of a new universal self-adhesive resin cement

Introduction

FRC posts are commonly used for the restoration of endodontically treated teeth that are structurally compromised. (26, 27, 172) The complex root canal geometry, limited visibility within the canal, residual material and smear layer created with chemo-mechanical preparation make the cementation of FRC posts challenging. (173) Resin cements have been considered the material of choice for the cementation of FRC posts. (7) Conventional, multi-step resin cements rely on the use of adhesive systems, used in the EAR or SE mode to obtain hybridization and intimate adhesion through the resin diffusion into the root canal dentin substrate. (71) The tendency to simplify clinical procedures and reduce operator sensitivity, has led to the introduction of resin cements that could adhere both to the dental substrate and restorations without the need of previous surface treatment. (63, 174, 175) Self-adhesive resin cements have enabled shorter working time due to reduction of clinical steps, but the need to mix all the components (hydrophilic and hydrophobic monomers, catalysts, photoinitiators, etc.) in a single material is a concern, and their use, compared to conventional resin cements, is still a matter of interest in dental research. (66)

Further classification of resin cements is made based on their polymerization modality (light-cure, self-cure and dual-cure). (176) In an attempt to overcome the problems related to the decreased light transmission in dark areas, such as the apical region of the root canal, dual-cure resin cements have been introduced. They were developed by combining the most valuable features of the light-cure and self-cure modalities, providing a certain degree of conversion even in the absence of light. (133)

The topic of resin cement polymerization gives rise to a debate that is currently open. Although chemical activation mode is desirable to contrast the clinical adversities related to the so-called shadow areas, many studies suggest that dual-cure resin cements should be light-cured to maximize the polymerization process and optimize the mechanical properties of the materials. (177) The choice of the adequate polymerization protocol that contributes to the attainment of a reliable adhesive bond both at the coronal and apical level of the root canal, is essential when using resin cements for the cementation of FRC posts, whether they are conventional multi-step or self-adhesive luting materials.

Therefore, the purpose of this study was to evaluate the push-out bond strength (PBS) and interfacial nanoleakage expression (NL) of resin cements relying on different adhesive approaches (self-adhesive or conventional multi-steps) for the cementation of FRC fiber posts, and to evaluate the bonding performance of the new universal self-adhesive resin cement. The tested null hypotheses were that PBS and the level of silver grains deposition at the adhesive interface are not influenced by: 1. the type of resin cement; 2. the curing mode (light-cure or self-cure); 3. the root region (coronal or apical)

Materials and methods

Specimen preparation

The study protocol was approved by the Ethics Committee of the Department of Biomedical and Neuromotor Science (DIBINEM), University of Bologna, Italy (protocol N°: 71/2019/OSS/AUSLBO).

Fifty extracted, caries-free, mandibular premolars were stored in 0.5% chloramine solution at 4°C for no longer than 2 months after harvesting. The teeth were sectioned at the cementoenamel junction, perpendicular to the long axis, using a low-speed diamond saw (Microremet, Remet, Bologna, Italy) under water cooling. Root canal treatment was performed using Pathfiles (#1-2-3) and ProTaper (S1-S2-F1-F2-F3) (Dentsply Sirona, York, PA, USA) until the working length.

During instrumentation, the canals were irrigated with 5 mL of 5% sodium hypochlorite (Niclor 5; Ogna, Muggiò, Italy), followed by a final rinse with 1 mL of 10% ethylenediamine tetra-acetic acid (Tubuliclean; Ogna, Muggiò, Italy). In accordance with the continuous wave technique, the canals were filled with endodontic sealer (AH-Plus, Dentsply Sirona), medium-sized gutta-percha points with DownPack (Hu-Friedy, Chicago, IL, USA) and warm gutta-percha (Obtura III, Analytic Technologies, Redmond, WA, USA). The coronal entrance of the filled roots was then temporarily sealed with a glass-ionomer cement (Fuji VII, GC Corp., Tokyo, Japan) and the samples were stored for 24h at 37°C and 100% relative humidity.

Luting of fiber posts

After the removal of the temporary coronal seal, post space preparation was created in a standardized way for each tooth. An 8-mm post space was created by using a low-speed dental hand piece and post drill (RelyX fiber post drill Size 2, 3M, Neuss, Germany). The root canal was then irrigated with 5 ml of distilled water and dried with absorbent paper points (Dentsply-DeTrey, Konstanz, Germany). Before the luting procedures, the fiber post size 2 was inserted into the canal to check if it reached the working length, after which the coronal part outside the canal was cut with a diamond bur. The teeth were then randomly assigned to one of the following groups, according to the luting agent and polymerization protocol employed (N=5):

Group 1a (RXU LC): light-cure RelyX Universal (3M);

Group 1b (RXU SC): self-cure RelyX Universal (3M);

Group 2a (MAX LC): light-cure Maxcem Elite Chroma (Kerr);

Group 2b (MAX SC): self-cure Maxcem Elite Chroma (Kerr);

Group 3a (CAL LC): light-cure Calibra Universal (Dentsply Sirona);

Group 3b (CAL SC): self-cure Calibra Universal (Dentsply Sirona);

Group 4a (MUL LC): light-cure Multilink Automix/Multilink Primer (Ivoclar Vivadent);

Group 4b (MUL SC): self-cure Multilink Automix/Multilink Primer (Ivoclar Vivadent);

Group 5a (LUX LC): light-cure Luxacore Z Dual/LuxaBond TotalEtch System (DMG);

Group 5b (LUX SC): self-cure Luxacore Z Dual/LuxaBond Total Etch System (DMG);

RXU, MAX and CAL are self-adhesive resin cements. MUL is a resin cement that relies on a self-etch approach (SE). LUX is a core build-up and radicular post luting composite used in combination with an EAR bonding system. The details of fiber post surface pretreatments, chemical compositions and application modes of the cements are shown in Table 2.

Table 2. The details of fiber post surface pretreatments, chemical compositions and application modes of the cements.

Resin cement	**Composition**	**Application mode**	**FRC post preparation**
RelyX Universal, 3M (LOT VTGHESP0019)	BPA derivative free dimethacrylate monomers, phosphorylated dimethacrylate adhesion monomers, photoinitiator system, novel amphiphilic redox initiator system, radiopaque fillers and rheological additives, pigments	Dispense in the post space and insert the post.	Clean with alcohol and air-dry for 5 s.
Maxcem Elite Chroma, Kerr	HEMA, GDM, UDMA, 1,1,3,3-tetramethylbutyl hydroperoxide TEGDMA,	Dispense in the post space and insert the post.	Clean with alcohol and air-dry for 5 s. Apply a layer of

(LOT 71887933)	fluoroaluminosilicate glass, GPDM, barium glass filler, fumed silica (69 wt %)		silane coupling agent (Ultradent) for 60 s and gently air-dry.
Calibra Universal, Dentsply Sirona (LOT 170821)	UDMA, trimethylolpropane trimethacrylate TMPTMA, bis-EMA—Bisphenol A ethoxylate dimethacrylate, TEGDMA, HEMA, 3-(acryloyloxy)-2-hydroxypropyl methacrylate, urethane modified bis-GMA, PENTA, silanated barium glass, fumed silica (48 vol %)	Dispense in the post space and insert the post.	Clean with alcohol and air-dry for 5 s. Apply a layer of silane coupling agent (Ultradent) for 60 s and gently air-dry.
Multilink Automix, Ivoclar Vivadent (LOT Y47572)	Dimethacrylate and HEMA, barium glass and silica filler, ytterbiumtrifluoride (68 wt %), catalysts, stabilizers, pigments	Mix Multilink Primer (1:1) and apply with a endobrush to radicular dentin for 30 s. Remove the access with an absorbent paper point. Dispense the cement in the post space and insert the post.	Clean with alcohol and air-dry for 5 sec. Apply a layer of Monobond Plus (Ivoclar Vivadent) for 60 s and gently air-dry.
Luxacore Z Dual, DMG (LOT 211108)	Bis-GMA, UDMA, Barium glass, colloidal silica, nanocomposite, zirconium dioxide 71% weight	Apply DMG etching gel for 15 s on radicular dentin, rinse with water for 15 s. Dry the canal with paper points. Work 1 drop of prebond (Luxacore Total Etch) to dentin for 15 s, remove the access with paper point, gently air-dry. Mix Bond A and Bond B (1:1) and apply to dentin surface for 20 s using a microbrush, gently air-dry. Dispense	Clean with alcohol and air-dry for 5 s. Apply a layer of silane coupling agent (Ultradent) for 60 s and gently air-dry.

		the cement in the post space and insert the post.	

One operator, unaware to the polymerization protocol, performed the fiber post luting procedures. Then, a second operator randomly assigned the specimens either to the LC or SC groups by means of simple randomization (toss of a coin). Light-curing was performed through the fiber post for 60s with a LED curing lamp (1470 mW/cm2, Elipar Deep cure, 3M). The SC groups were put in dark chambers for one hour at 37°C to allow exclusively chemical polymerization of the resin cements.

Afterwards, the specimens were wrapped into humid medical gauze, put into plastic chambers, and stored in an incubator at 37°C for 24 h. After storage, each root was sectioned in at least six 1-mm thick slices using a low-speed diamond saw (Microremet, Remet) under water cooling. The first coronal slices were automatically discarded, the coronal side of each slice was signed with an indelible marker to later ensure the exact positioning during testing. The specimens from each group were immediately processed for PBS test (T_0).

Push-out bond strength test

The thickness of each slice was measured using a digital caliper (Starrett 727, Starrett, Itu, SP, Brazil) with ±0.01 mm accuracy. The slices were then put on 1 mm- square graph paper and photographs were taken with a digital camera (D 7200, Nikon, Japan), after which the coronal and apical diameters of the posts were measured in ImageJ software (National Institute of Health, Bethesda, MD, USA). The push-out test was performed using a universal testing machine (Instron 4465, Instron, Norwood, MA, USA) by applying an axial load force at a crosshead speed of 0.5 mm/min. The apical surface of the slice was placed facing the punch tip to ensure that the load was applied following an apical-coronal direction, so to dislocate the post towards the wider part of the

slice. The load that caused the specimens' failure (manifested by the dislodgment of the post) was recorded in Newtons (N) and it was converted to mega Pascals (MPa) by dividing the load in Newtons by the bonded surface area (SL) in mm^2. (169) The bonded surface area was calculated using the following formula:

$$SL= (\pi(R+r))*((h^2 + (R-r))^2)^{0.5},$$

where R was the coronal diameter of the canal with the post, r the apical diameter and h the thickness of the slice.

The debonded specimens were analyzed by one investigator under a stereomicroscope at 40x magnification (Stemi 2000-C; Carl Zeiss Jena GmbH) and the failure mode was classified as follows: adhesive, between dentin and the cement (AD), adhesive between the cement and the post (AP), cohesive within the cement (CC), cohesive within the post (CP) and mixed (M).

Interfacial nanoleakage expression

Additional mandibular premolars (N=2 per group) were used to quantify the interfacial NL expression. The endodontic treatment, fiber post cementation and cutting procedures were performed as previously described for the PBS test. The specimens were prepared and covered with nail varnish, leaving 1 mm free at the interface, then immersed in a 50 wt% ammoniacal silver nitrate solution for 24 h. Specimens were then photo-developed to reduce the diamine silver ions ($Ag(NH_3)_2^+$) into metallic silver grains. The silver-impregnated specimens were fixed, dehydrated in ascending ethanol solutions, embedded in epoxy resin (Epon 812, Fluka, Switzerland) and processed for light microscopy analysis in accordance with Mazzoni *et al.(171)* Images of the adhesive interfaces were captured (20x magnification) and the extent of interfacial NL was scored by one observer using a four-point scale.

Statistical analysis

After checking the normality (Shapiro-Wilk test) and homoscedastic (modified Levene's test) assumptions of the data sets, an analysis of variance (ANOVA) was performed to examine the effects of the dependent variables "cement", "curing mode" and "root region" and the interaction of these factors on the PBS. Pairwise comparisons were performed using Tukey post-hoc test. In addition, one-way ANOVA test with the post-hoc Bonferroni correction was conducted to evaluate the differences between the groups. NL scores were analyzed using the Chi-square tests. All statistical analyses were conducted with the software Stata 12.0 (Stata Corp, College Station, Texas, USA) and the significance was set for $p<0.05$.

Results

Push-out bond strength test

Mean PBS values (MPa) with standard deviations (SD) of specimens tested at T_0 are presented in Tables 3 for the coronal and apical root regions, respectively. The statistical analysis revealed that the "cement" significantly influenced the PBS ($p<0.05$), but not the variables "polymerization protocol" and "root region" ($p> 0.05$). The results of the one-way ANOVA demonstrated the trend of significantly lower PBS values in the CAL groups compared to other investigated cements ($p<0.05$). RXU cement performed either equally well ($p>0.05$) or better than other self-adhesive and multi-step systems ($p<0.05$).

Table 3: Push-out bond strength values (MPa) with standard deviations in coronal section and apical section after 24h of artificial saliva storage. Different superscript upper case letters indicate differences within the rows, different superscript lower case letters indicate differences within the columns.

Groups	Coronal section		Apical section	
	LC	SC	LC	SC
RXU	16.5±3.7 $^{A a}$	15.0±4.3 $^{A a}$	17.7±6.6 $^{A a}$	19.9±4.8 $^{A a}$
MAX	15.6±4.6 $^{A a}$	19.6±3.1 $^{A a}$	13.1±7.3 $^{A a}$	23.3±3.3 $^{A a}$
CAL	8.6±4.5 $^{A b}$	14.7±6.1 $^{A b}$	5.9±3.9 $^{A b}$	8.1±2.6 $^{A b}$
LUX	17.4±5.4 $^{A a}$	12.6±3.0 $^{A a}$	18.7±6.7 $^{A a}$	20.4±3.3 $^{A a}$
MUL	18.4±6.2 $^{A a}$	20.4±7.3 $^{A a}$	19.0±4.5 $^{A a}$	19.9±6.2 $^{A a}$

The percentage of the types of failure mode within each group is presented in Table 4. A predominance of mixed and adhesive failures at the cement/post interfaces were observed among the groups, independent of the curing mode and aging conditions. Adhesive failures at the dentin side were observed for MAX SC, CAL SC e MUL SC. No cohesive fractures were detected.

Table 4: Failure mode of the dislodged specimens from five experimental groups. Data are expressed as percentages (%) of the total number of specimens tested for each group

Groups	T_0	
	LC	SC
RXU	M: 52 AP: 48 AD: 0 CC: 0 CP: 0	M: 60 AP: 40 AD: 0 CC: 0 CP: 0
MAX	M: 70 AP: 30 AD: 0 CC: 0 CP: 0	M: 41.6 AP: 25 AD: 33.4 CC: 0 CP: 0
CAL	M: 62.5 AP: 37.5 AD: 0 CC: 0 CP: 0	M: 41.6 AP: 25 AD: 33.4 CC: 0 CP: 0
LUX	M: 36.3 AP: 63.7 AD: 0 CC: 0 CP: 0	M: 33.3 AP: 66.7 AD: 0 CC: 0 CP: 0
MUL	M: 55 AP: 45 AD: 0 CC: 0 CP: 0	M: 52.9 AP: 11.7 AD: 35.4 CC: 0 CP: 0

Interfacial nanoleakage expression

Descriptive statistics of interfacial NL scores within the groups in the experimental conditions are presented in Figure 1. The statistical analysis showed differences in the interfacial silver deposition among the tested groups, and this was material-dependent ($p<0.05$). LUX and CAL revealed higher silver nitrate infiltration both in the LC and SC groups ($p<0.05$). RXU, MAX

and MUL showed comparable results, independently from the curing protocol performed. Furthermore, no differences were detected between the apical and the coronal portion of the root, except for CAL SC that exhibited significantly higher NL in the apical portion ($p<0.05$).

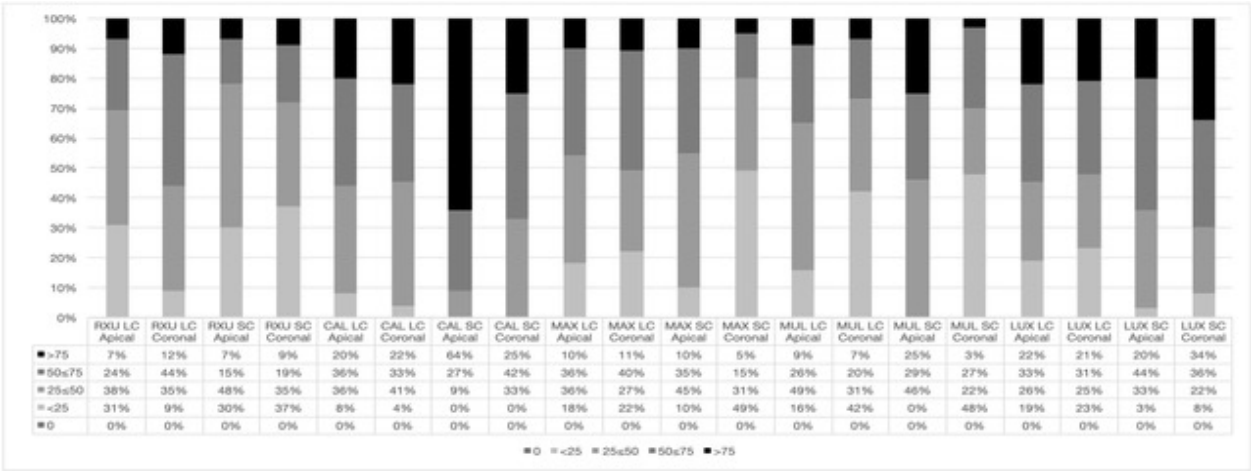

Figure 1. Interfacial NL scores for different types of cement after 24h of artificial saliva storage

Discussion

In vitro studies are usually conducted to test material's performance before their clinical application can be assessed in randomized controlled clinical trials. Although discrepancies between laboratory and clinical conclusions in dental literature exist (178) and it is not always possible to precisely predict clinical behavior of materials based on in vitro results, laboratory studies are still widely performed in dentistry. The most commonly used method for evaluation of the adhesion of FRC posts is the push-out BS test. (179) Even though a recent systematic review found considerable variations in the design of the push-out test among studies (180) and the opinion on the value of methodology of the test is divided (181) it is considered to be more appropriate and reliable for FRC post testing than microtensile BS tests. (182) Therefore, evaluation of the adhesively luted FRC posts by means of push-out BS tests is irreplaceable in the early screening of dental materials' properties.

The bonding performances of a universal self-adhesive resin cement were evaluated and compared to those of other self-adhesive, as well as multi-step resin cements. According to the results obtained, the first null hypothesis must be rejected since PBS values and interfacial NL expression were influenced by the choice of resin cement.

This study used three different bonding strategies for the cementation of FRC posts into root canals. Specifically, LUX and MUL are referred to as multi-step resin cements (E&R and SE respectively), as the luting procedures require more than one clinical step, whereas RXU, MAX and CAL rely on a self-adhesive approach, and no pre-treatment of dentin is necessary. Additionally, the new RXU self-adhesive cement does not require the pretreatment of the post with silane, further simplifying the clinical cementation procedure.

Previous study showed that bonding strategy can influence the hybrid layer appearance, and the integrity of the resin-based restorations. Dentin etching with phosphoric acid performed in the EAR approach removes the smear layer, opens the dentinal tubules and reveals the intertubular dentin collagen network, favoring the penetration of the resin to create longer and thicker resin tags and a more uniform hybrid layer than those achieved with the SE approach. (183) On the other hand, a superficial dentin demineralization was observed with self-adhesive resin cements with very thin and short resin tags. (63, 144) Although it would seem logical to assume that multi-step resin cement systems would exhibit a more durable bond strength (BS) to root canal dentin compared to the simplified self-adhesive resin cements, the results of the present study emphasize that simplified systems can perform equally well or even better, and that the bond strength is correlated to the cement type. This observation is in agreement with a recent systematic review. (66)

The formation of a reliable and stable bond is in part related to the resin cement polymerization process. (74) A proper polymerization reaction of the material translates into better physical and chemical properties (139), increased stability and integrity at the adhesive interface (184), inferior water sorption/solubility phenomena and extended durability of the restoration. (185) In the present study, light-curing did not influence BS of the adhesively luted posts but did impact

the marginal infiltration of some resin cements tested. Consequently, the second null hypothesis had to be only partially accepted. This may be explained by the composition of the resin cements used in this study. As the simplified self-adhesive dual-cure cements are expected to prime and bond the substrate and the restoration at the same time, they contain acidic monomers. (144) Albeit their important role in the interaction with the cementation substrates, these monomers could lead to the inactivation of the conventional organic polymerization initiators, such as benzoyl peroxide/aromatic tertiary amines system, impairing both the chemical and light polymerization process. (132, 139, 184) This particular traditional initiator is present in the CAL cement, possibly underlying the generally poor performance of this material. On the other hand, MAX introduced an amine-free redox initiator system, while the new RXU contains a novel amphiphilic redox initiator system (ARI system). The new self-adhesive resin cement showed comparable or even superior BS both in LC and SC when compared to the other cements tested. According to the claims of RXU manufacturer, the ARI system, alongside with functional monomers, enables the cement to diffuse into the smear layer, achieving a strong bond to dentin. Furthermore, the ARI system and functional monomers in the new self-adhesive cement possibly led to the formation of highly crosslinked 3D polymer network which is considered to be responsible for the long-term stability of the resin-dentin interface.

The establishment of a fine equilibrium between the different components of the cements, with an efficient polymerization initiation and propagation, would be expected to resolve the issue of differences in the quality of polymerization in different root regions. This is in accordance with the present study, as well as previously published research since the root region did not influence BS and NL expression, requiring the rejection of the third null hypothesis. (186)

Conclusion

Within the limitations of the study, it can be concluded and the choice of material itself, rather than the adhesive approach, can influence the bonding performances of adhesively-luted FRC

posts. Certain simplified systems seem equally suitable for clinical use as the multi-step systems, with the advantage of having lower technique sensitivity and reduced chair-side time. The universal self-adhesive cement showed better or comparable results when compared to marketed cements, even without the pretreatment of the post, and could be recommended in the clinical practice.

Main study

Scientific hypotheses

The null hypotheses tested were:

1. EDC has no effect on post push-out bond strength to radicular dentin achieved by different cementation strategies and resin cements, at baseline or after thermocycling procedure;
2. EDC has no effect on endogenous enzymatic activity within intraradicular dentin, at baseline or after thermocycling procedure;
3. There is no difference in MMPs activity within radicular dentin when different cementation strategies and resin cements are used for FRC post luting;
4. The choice of cementation strategy and resin cement does not influence post push-out bond strength, at baseline or after thermocycling procedure;
5. There is no difference in terms of push-out bond strength values when comparing coronal, middle and apical root region, at baseline of after thermocycling;
6. Thermocyling has no effect of push-out bond strength and endogenous enzymatic activity within radicular dentin.

Materials and methods

One hundred and twenty freshly extracted, intact human mandibular premolar teeth were kept in 0.5 % chloramine solution for not more than 2 months after extraction. The study protocol was approved by the University Ethical Committee.

The teeth were sectioned at the cementoenamel junction, perpendicular to the long axis, using a low-speed diamond saw (Microremet, Remet, Bologna, Italy) under water cooling. Root canal treatment was carried out using Pathfiles (#1-2-3) and ProTaper (S1-S2-F1-F2-F3) (Dentsply Sirona, York, PA, USA) until the instrument reached the working length. During root canal preparation, the canals were irrigated with 5 mL of 5% sodium hypochlorite (Niclor 5; Ogna, Muggiò, Italy), followed by a final rinse with 1 mL of 10% ethylenediamine tetra-acetic acid (Tubuliclean; Ogna, Muggiò, Italy). In accordance with the continuous wave technique, the canals were filled with endodontic sealer (AH-Plus, Dentsply Sirona), medium-sized gutta-percha points with DownPack (Hu-Friedy, Chicago, IL, USA) and warm gutta-percha (Obtura III, Analytic Technologies, Redmond, WA, USA). The coronal entrance of the filled roots was then temporarily sealed with a glass-ionomer cement (Fuji VII, GC Corp., Tokyo, Japan) and the samples were kept for 24h at 37°C and 100% relative humidity.

Luting of fiber posts

After the removal of the temporary coronal seal, post space preparation was created in a standardized way for each tooth. An 8-mm post space was created by using a low-speed dental hand piece and post drill (RelyX fiber post drill Size 2, 3M, Neuss, Germany). The root canal was then irrigated with 5 ml of distilled water and dried with absorbent paper points (Dentsply-DeTrey, Konstanz, Germany). Before the luting procedures, the fiber post size 2 was inserted into the canal to check if it reached the working length, after which the coronal part outside the canal was cut with

a diamond bur. The teeth were then randomly assigned to one of the following groups, according to the luting agent and dentin pretreatment protocol employed (N=20):

Group 1a: cementation with RelyX Universal (3M);

Group 1b: pretreatment with 0.3M EDC-containing aqueous primer for 1 min, drying with absorbent paper points, followed by cementation with RelyX Universal (3M);

Group 2a: dentin treated with Multilink Primer (Ivoclar Vivadent) for 30 s, dried with paper points, followed by cementation with Multilink Automix/Multilink Primer (Ivoclar Vivadent);

Group 2b: dentin treated with Multilink Primer for 30 s, dried with paper points, followed by pretreatment with 0.3M EDC-containing aqueous primer for 1 min. Root canal drying with absorbent paper points, application of a layer of Multilink Primer for 30 s and cementation with Multilink Automix (Ivoclar Vivadent);

Group 3a: dentin etching with 37% phosphoric acid for 15 s (Etching Gel, DMG), abundant water rinsing for 30 s and root canal space drying with paper points. Application of Pre-Bond (DMG) for 15 s, drying with paper points, followed by application of previously prepated Bond A/B (DMG). Gentle air dry and cementation with Luxacore Z Dual (DMG);

Group 3b: the cementation protocol as in Group 3a, with 0.3M EDC-containing aqueous primer pretreatment for 1 min, immediately after the etching step.

The details of FRC surface pretreatment are shown in Table 2.

Light curing was performed immediately after FRC post insertion by placing the light source (1470 mW/cm2, Elipar Deep cure, 3M) in close contact with root canal entrance for 60 s.

After 24h of storage in artificial saliva at 37 ^{o}C, the roots were perpendicularly sectioned using a low-speed diamond saw (Microremet, Remet, Bologna, Italy). The coronal side of each slice was signed with an indelible marker to later ensure the exact positioning during push-out bond

strength testing. Half of the roots from each group was subjected to push-out bond strength test (the randomization was performed by tossing a coin) immediately after sectioning of roots. The other half was put into tea bags and subjected to artificial aging by thermocycling the samples. The thermocycling protocols was as follows: 40.000 cycles, 5-55 ^{o}C (dwell time 30 s) in accordance with Mazzoni et. al. (2009). (171)

Push-out bond strength test

The thickness of each root slice was measured using a digital caliper (Starrett 727, Starrett, Itu, SP, Brazil) with ±0.01 mm accuracy. The slices were then put on 1 mm- square graph paper and photographs were taken with a digital camera (D 7200, Nikon, Japan), after which the coronal and apical diameters of the posts were measured in ImageJ software (National Institute of Health, Bethesda, MD, USA). The push-out test was performed using a universal testing machine (Instron 4465, Instron, Norwood, MA, USA) by applying an axial load force at a crosshead speed of 0.5 mm/min. The apical surface of the slice was placed facing the punch tip to ensure that the load was applied following an apical-coronal direction, so to dislocate the post towards the wider part of the slice. The load that caused the specimens' failure (manifested by the dislodgment of the post) was recorded in Newtons (N) and it was converted to mega Pascals (MPa) by dividing the load in Newtons by the bonded surface area (SL) in mm^2. (169) The bonded surface area was calculated using the following formula:

$$SL= (\pi(R+r))*((h^2 + (R-r))^2)^{0.5},$$

where R was the coronal diameter of the canal with the post, r the apical diameter and h the thickness of the slice.

The debonded specimens were analyzed by one investigator, blinded to the groups, under a stereomicroscope at 40x magnification (Stemi 2000-C; Carl Zeiss Jena GmbH) and the failure mode was classified as follows: adhesive, between dentin and the cement (AD), adhesive between the

cement and the post (AP), cohesive within the cement (CC), cohesive within the post (CP) and mixed (M).

In situ zymography analysis of the resin-dentin interfaces

In order to investigate the effect of EDC on endogenous enzymatic activity following the split-tooth design, the roots of maxillary first premolars (N=4 per group) were endodontically treated as previously described, after which one root (experimental root) received EDC pretreatment for 1 minute while the other root (control root) was left untreated. Cementation of FRC posts was carried out with the same methodology as for the push-out bond strength test.

After 24h hours of storage at 37ºC in humid chabmer, one-millimeter-thick slabs of middle portion of roots were obtained from the prepared specimens using a low-speed diamond saw (Microremet, Remet, Bologna, Italy) under water-cooling. Each specimen was glued to a microscope slide, ground down approximately to the thickness of 50 μm and polished. In situ zymography was performed following the protocol reported by Mazzoni et al. (2014). (127) Self-quenched fluorescein-conjugated gelatin mixture (E-12055; Molecular Probes, Eugene, OR, USA) was placed on the specimen covering the polished resin-dentin surfaces and then protected with a coverslip. The specimens were incubated for 12 h at 37°C in a humid, dark chamber avoiding direct contact with water. Confocal laser scanning microscope was used to examine the specimens after incubation (excitation wavelength, 488 nm; emission wavelength, 530 nm; Model A1-R; Nikon, Tokyo, Japan). For each assembly, a series of images with standardized rectangular selected area were made (one image per each 1 μm into the depth of the sample) to show the hydrolysis of the quenched fluorescein-conjugated gelatin substrate, presented as green fluorescence. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to quantify integrated density of the fluorescence signals, which correspond to the endogenous enzymatic activity. All images were made by one experienced investigator who was blinded to the groups.

Lastly, in order to investigate the effect of 3 different adhesive strategies (cements) on radicular enzymatic activity, freshly extracted first maxillary molars (N=4) were subjected to root

canal treatment, after which in each of the roots FRC post was cemented using one of the investigated three cements. The specimens were then processed for in situ zymography analysis as described earlier.

Tissue processing for Scanning electron microscopy (SEM) analysis

After performing push-out bond strength test, representative root slices from each group (N=2) were selected and processed for scanning electron microscopy (SEM) analysis. The samples were prepared for FEI-SEM analysis following the well established protocol:

- Fixation with 2.5% glutaraldehyde in 0.1 M CaCO pH=7.4 for 3 hours
- Rinsing in 0.1 M CaCO pH=7.4 (3x3 min)
- Dehydration in graded alcohols (50%, 70%, 80%, 90%, 95%, 100%) 2x2 min for each alcohol concentration
- addition of 0.1 ml of HMDS 1x10 min
- 50% alcohol + 50% HMDS 1x10 min
- pure HMDS 1x10 min

The samples were then placed on absorbent paper and air dried for 1 hour. Subsequently, they were mounted on stubs using a conductive tape and coated with a 1.5 nm thick layer of gold-palladium using an Emitech K550X system (Quorum Technologies, UK). Observations were performed using a scanning electron microscope (JSM 5200, JEOL, Tokyo, Japan) under 50x, 100x and 500x magnification.

Statistical analysis

After checking the normality (Shapiro-Wilk test) and homoscedastic (modified Levene's test) assumptions of the data sets, an analysis of variance (ANOVA) was performed to examine the effects of the dependent variables "cement type", "pretreatment", "root region" and "aging" and the

interaction of these factors on the PBS. Pairwise comparisons were performed using Tukey post-hoc test. In addition, one-way ANOVA test with the post-hoc Bonferroni correction was conducted to evaluate the differences between the groups. NL scores were analyzed using the Chi-square tests. Evaluation of the quantified data obtained from in-situ zymography analysis of molar teeth was performed by two-way ANOVA to examine the effect of "cement type" and "pretreatment" on potential gelatinolytic activities. Since the data obtained from in situ zymography analysis of premolar teeth were not normally distributed (failed Shapiro-Wilk test, $p<0.05$) Kruskal-Wallis one way analysis of variance and pairwise multiple comparison procedures (Dunn's Method) were run. Statistical analyses were conducted with the software Stata 12.0 (Stata Corp, College Station, Texas, USA) and the significance was set for $p<0.05$.

Results

Push-out bond strength values

Bond strength data were expressed as means and standard deviations (MPa), and are shown in Tables 5 and 6.

Results of the factorial ANOVA showed that significant differences were observed for the variables: "root region" ($p=0.0000$), "aging" ($p=0.0000$) and "pretreatment" ($p=0.0001$). The variable "cement type" did not influence the PBS ($p>0.05$). The interactions between the variables "root region" and "aging", "pretreatment" and "aging" were significant ($p=0.0215$ and 0.0000, respectively). There was no significant difference ($p>0.05$) for other interactions.

Table 5. Push-out bond strength values (MPa) with standard deviations after 24h of artificial saliva storage. Different superscript letters indicate differences within the rows, different superscript letters indicate differences within the columns.

	Control			**EDC**		
Groups	*Coronal*	*Middle*	*Apical*	*Coronal*	*Middle*	*Apical*
RelyX Universal	19.0 ± 5.6^{Aa}	$13.7 \pm 3.3^{A,B\,a}$	$9.7 \pm 3.2^{B\,a}$	$20.5 \pm 6.9^{A\,a}$	$12.6 \pm 4.0^{A,B\,a}$	$11.2 \pm 4.3^{B\,a}$
Multilink Automix	18.8 ± 7.5^{Aa}	15.8 ± 3.9^{Aa}	$12.9 \pm 4.8^{A\,a}$	$18.0 \pm 5.2^{A\,a}$	$13.6 \pm 4.5^{A\,a}$	$12.0 \pm 4.6^{A\,a}$
Luxacore Z Dual	17.8 ± 3.3^{Aa}	14.5 ± 2.3^{Aa}	$13.9 \pm 3.2^{A\,a}$	$17.5 \pm 4.8^{A\,a}$	$14.7 \pm 4.9^{A\,a}$	$12.2 \pm 4.3^{A\,a}$

Table 6. Push-out bond strength values (MPa) with standard deviations after 40.000 thermocycles. Different superscript upper case letters indicate differences within the rows, different superscript lower case letters indicate differences within the columns.

	Control			**EDC**		
Groups	*Coronal*	*Middle*	*Apical*	*Coronal*	*Middle*	*Apical*
RelyX Universal	$11.6 \pm 3.4^{A,Ba}$	8.8 ± 3.0^{Ba}	9.5 ± 1.6^{Ba}	$18.8 \pm 4.^{Aa}$	$11.6 \pm 4^{A,Ba}$	$11.0 \pm 3.4^{A,Ba}$
Multilink Automix	10.8 ± 4.1^{Aa}	9.9 ± 4.1^{Aa}	9.2 ± 3.5^{Aa}	16.1 ± 8.5^{Aa}	13.8 ± 5.3^{Aa}	13.7 ± 3.9^{Aa}
Luxacore Z Dual	14.0 ± 5.3^{Aa}	10.8 ± 4.0^{Aa}	9.1 ± 3.9^{Aa}	17.4 ± 4.5^{Aa}	14.1 ± 2.3^{Aa}	15.5 ± 3.5^{Aa}

Failure mode

Failure modes distribution of the tested specimens, expressed as percentages of the total number of slices tested, are summarised in Table 7. No cohesive failures within the post nor within cement were observed in any of the groups. In general, artificial aging increased the percentage of adhesive failures between dentin.

Table 7: Failure mode of the dislodged specimens from three experimental groups. Data are expressed as percentages (%) of the total number of specimens tested for each group

Groups	T_0		T_t	
	Control	**EDC**	**Control**	**EDC**
RelyX Universal	M: 47% AP: 18% AD: 35% CC: 0 CP: 0	M: 13% AP: 77% AD: 10% CC: 0 CP: 0	M: 24% AP: 68% AD: 8% CC: 0 CP: 0	M: 7% AP: 79% AD: 14% CC: 0 CP: 0
Luxacore Z Dual	M: 0 AP: 60% AD: 40% CC: 0 CP: 0	M: 28% AP: 66% AD: 6% CC: 0 CP: 0	M: 0 AP: 63% AD: 37% CC: 0 CP: 0	M: 8% AP: 71% AD: 21% CC: 0 CP: 0
Multilink Automix	M: 0 AP: 74% AD: 26% CC: 0 CP: 0	M: 48% AP: 31% AD: 21% CC: 0 CP: 0	M: 12% AP: 27% AD: 61% CC: 0 CP: 0	M: 7% AP: 37% AD: 56% CC: 0 CP: 0

In-situ zymography

The results obtained from in situ zymography analysis of premolar teeth are presented in Figures 2 and 3.

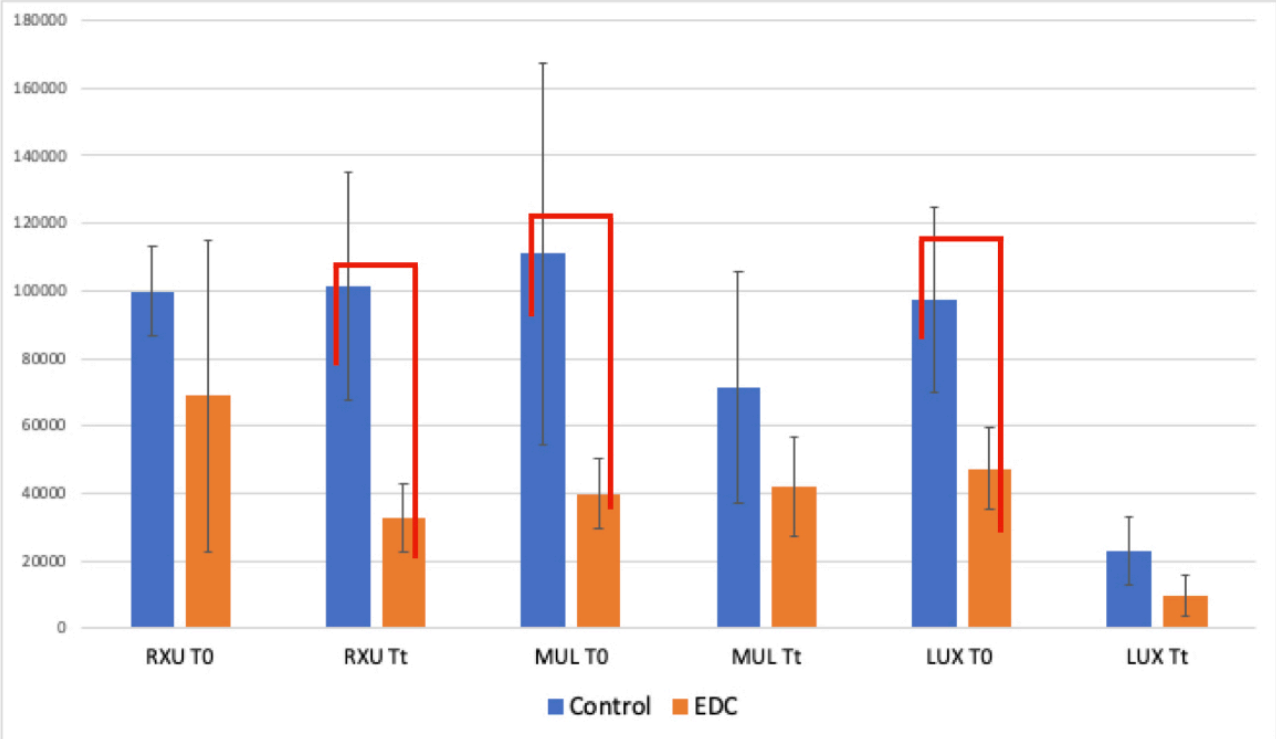

Figure 2: Quantification of the gelatinolytic activity within the resin-dentin interfaces of the tested groups. Values are presented as means ± standard deviations. RXU – RelyX Universal; MUL – Multilink Automix; LUX – Luxacore Z Dual; EDC – EDC pretreatment; T_0 – baseline; Tt – thermocycling. Red lines indicating statistically significant differences between the groups; y-axis: integrated density of the fluorescence signal.

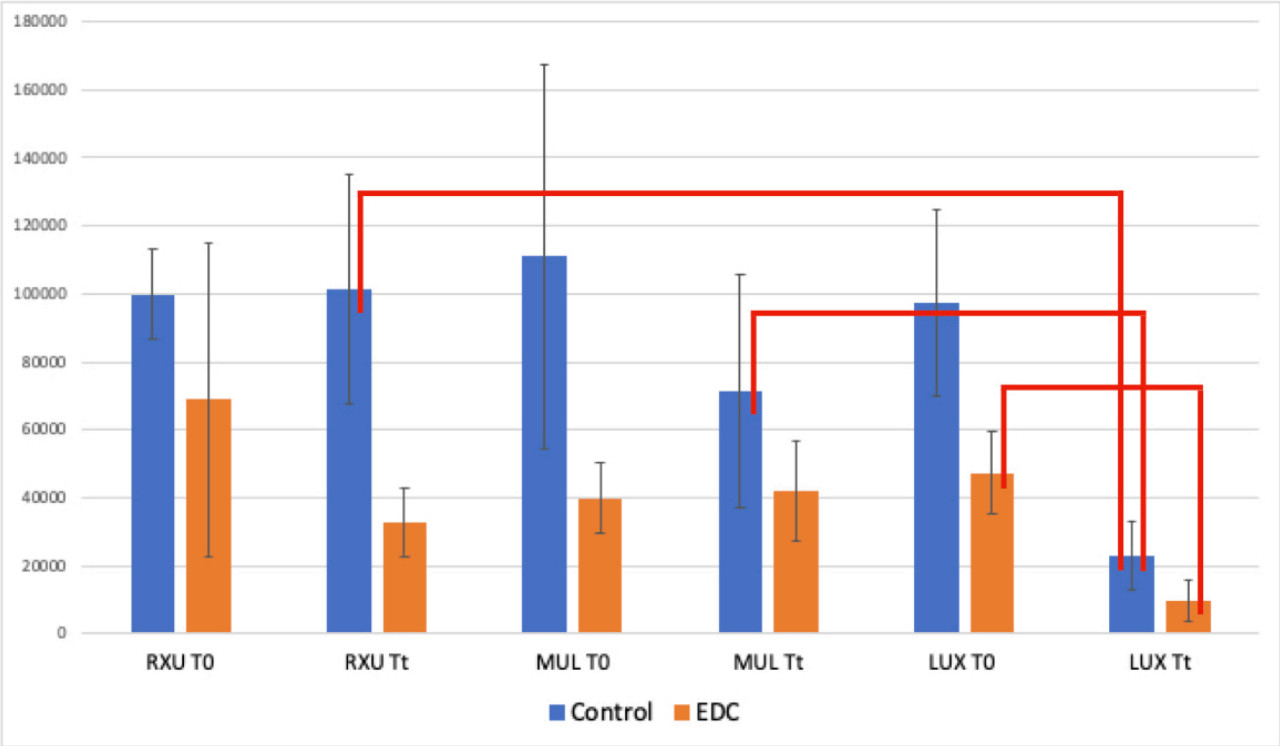

Figure 3: Quantification of the gelatinolytic activity within the resin-dentin interfaces of the tested groups. Values are presented as means ± standard deviations. RXU – RelyX Universal; MUL – Multilink Automix; LUX – Luxacore Z Dual; EDC – EDC pretreatment; T_0 – baseline; Tt – thermocycling. Red lines indicating statistically significant differences between the groups; y-axis: integrated density of the fluorescence signal.

EDC pretreatment significantly reduced enzymatic activity at baseline when fiber posts were cemented using Multilink Automix cement ($p=0.015$) and Luxacore Z Dual ($p=0.01$). Similarly, a significant difference was observed between control and EDC groups after thermocycling when posts were cemented with RelyX Universal cement ($p<0.001$), however there was a lack of significant difference at baseline ($p>0.05$). Interestingly, thermocycling seemed to reduce enzymatic activity in both control and experimental groups when Luxacore Z Dual was used as luting agent ($p<0.05$).

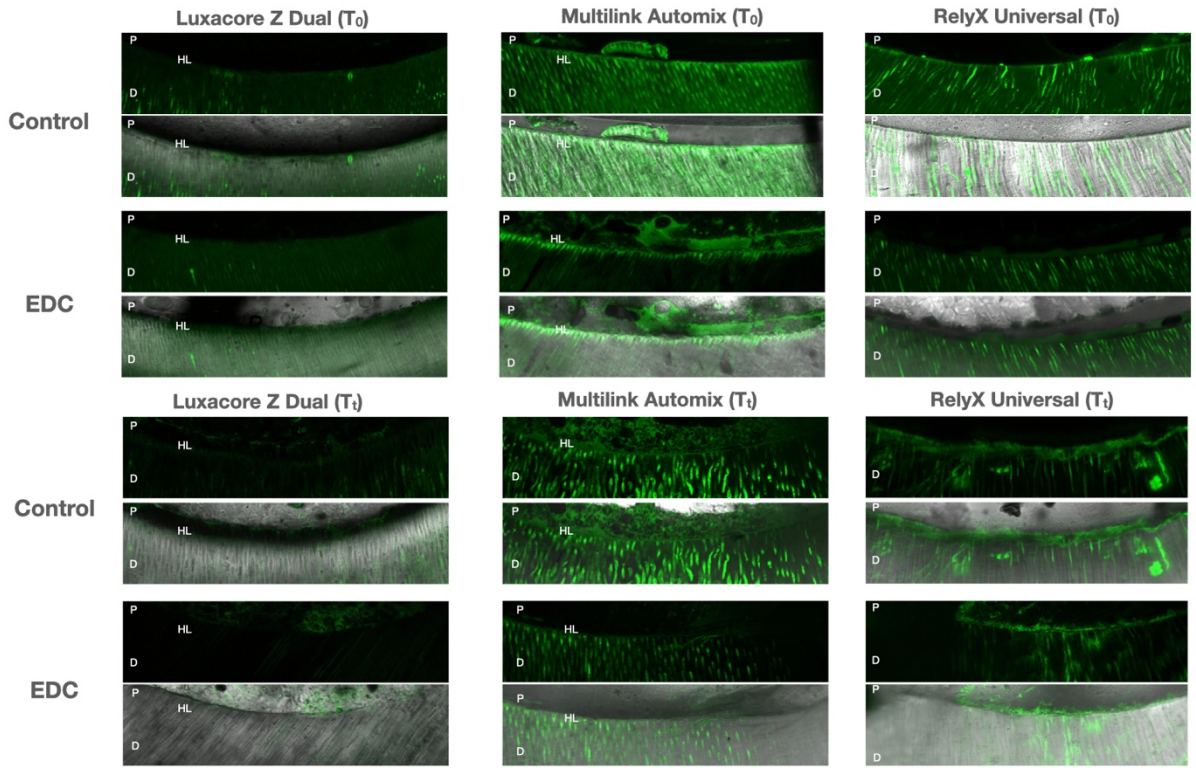

Figure 4: Representative confocal laser scanning microscopy images of resin-bonded radicular dentine interfaces that were incubated with quenched fluorescein-labelled gelatine. Abbreviations: D, dentine; HL, hybrid layer; P, post. T_0, baseline; Tt, thermocycling. For each set of images, the top image was acquired in the green channel. The green fluorescence represented areas with intense endogenous gelationolytic activity within the dentinal tubules and the hybrid layer. The bottom image was produced by merging the differential interference contrast image (showing the optical density of the resin-dentine interface) and the image acquired in green channel.

As for the data obtained from in situ zymography of molars, significant differences ($p<0.05$) were observed among all tested cements (Figure 5). The highest level of enzymatic activity was observed in RelyX Universal group, while Luxacore Z Dual showed lowest enzymatic activity. Considerable level of fluorescence was detected in the dentinal tubules in the RelyX Universal group.

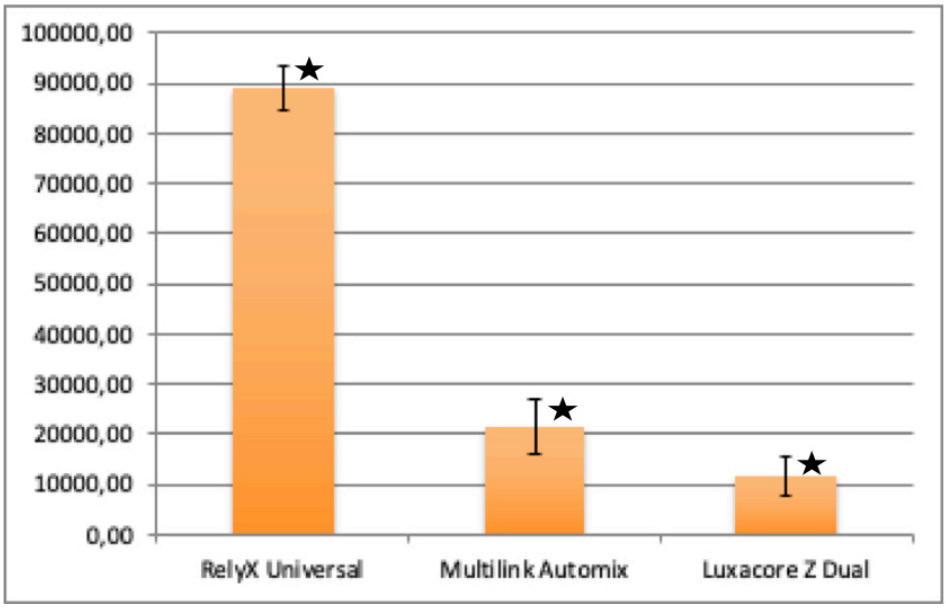

Figure 5: Quantification of the gelatinolytic activity within the resin-dentin interfaces of the tested groups. Values are presented as means ± standard deviations. Stars indicating statistically significant differences between the tested groups.

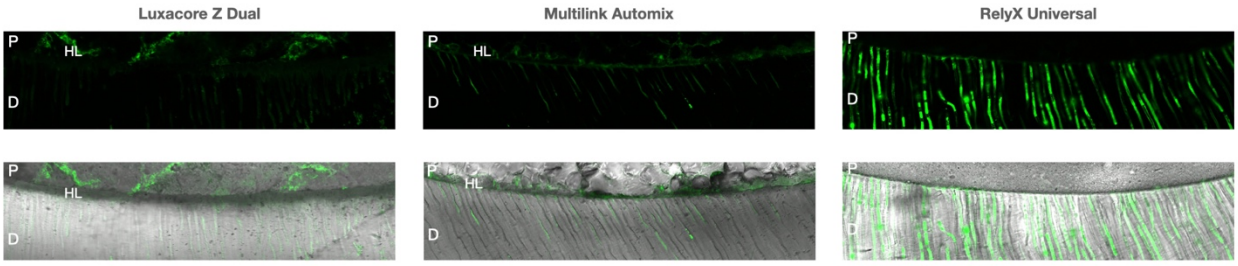

Figure 6: Representative confocal laser scanning microscopy images of resin-bonded radicular dentine interfaces that were incubated with quenched fluorescein-labelled gelatine. Abbreviations: D, dentine; HL, hybrid layer; P, post. For each set of images, the top image was acquired in the green channel. The green fluorescence represented areas with intense endogenous gelationolytic activity within the dentinal tubules and the hybrid layer. The bottom image was produced by merging the differential interference contrast image (showing the optical density of the resin-dentine interface) and the image acquired in green channel.

SEM images of the fractured specimens

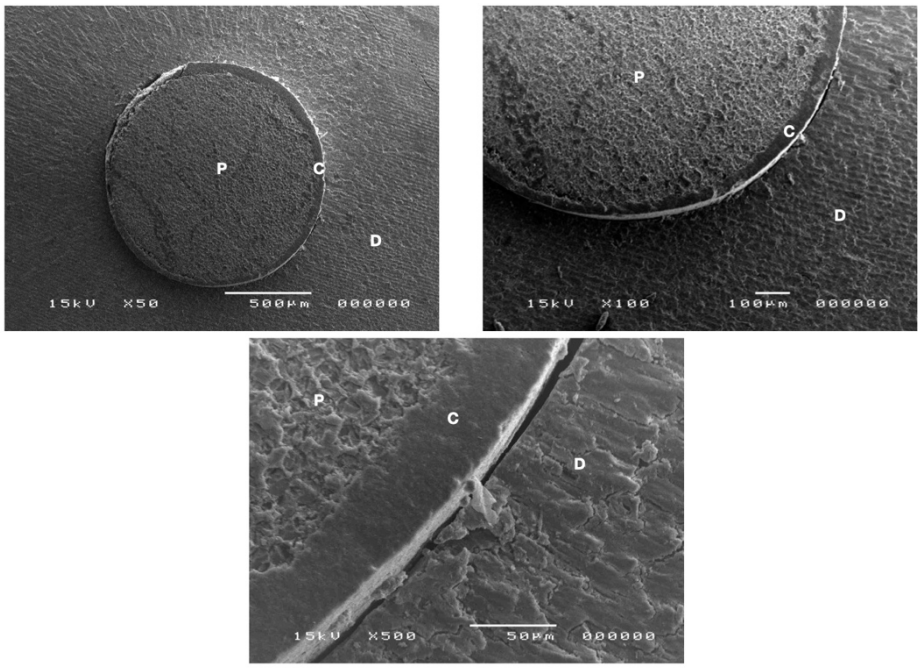

Figure 7: Photomicrographs of the adhesive interface in RelyX Universal control group at baseline. Abbreviations: D, dentine; P, post; C, cement.

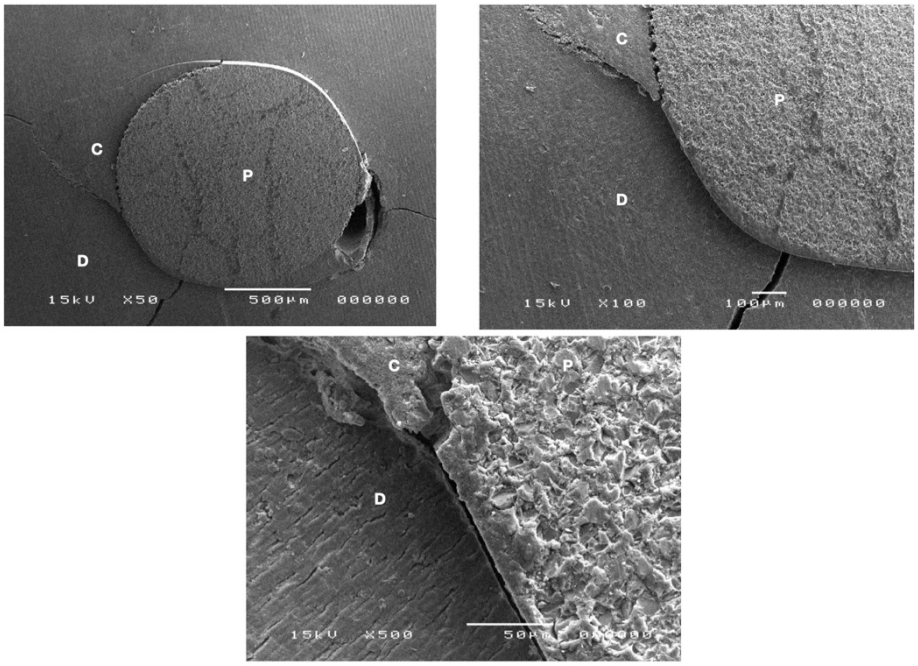

Figure 8: Photomicrographs of the adhesive interface in RelyX Universal EDC group at baseline. Abbreviations: D, dentine; P, post; C, cement.

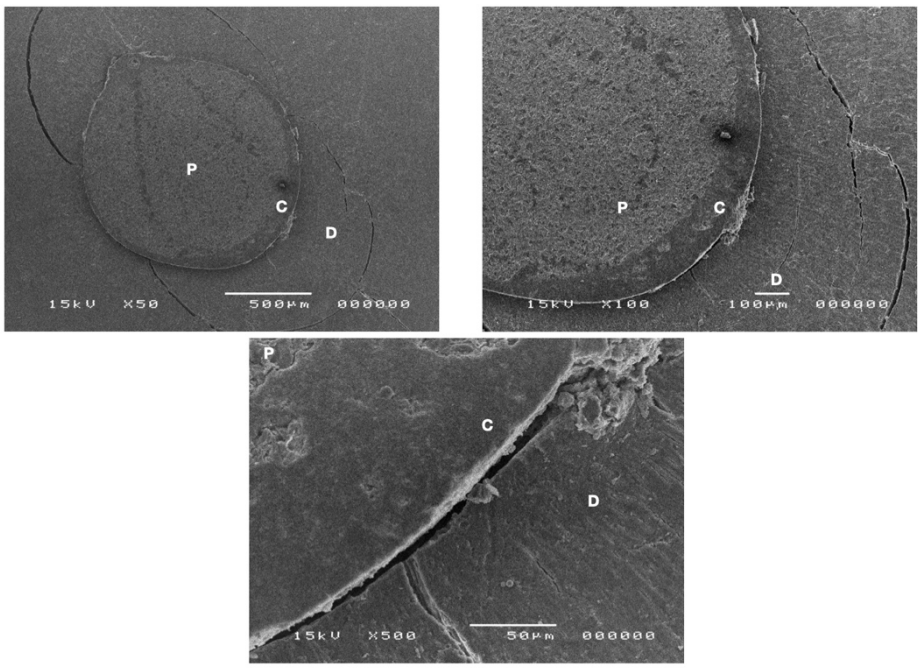

Figure 9: Photomicrographs of the adhesive interface in RelyX Universal control group after thermocycling. Abbreviations: D, dentine; P, post; C, cement.

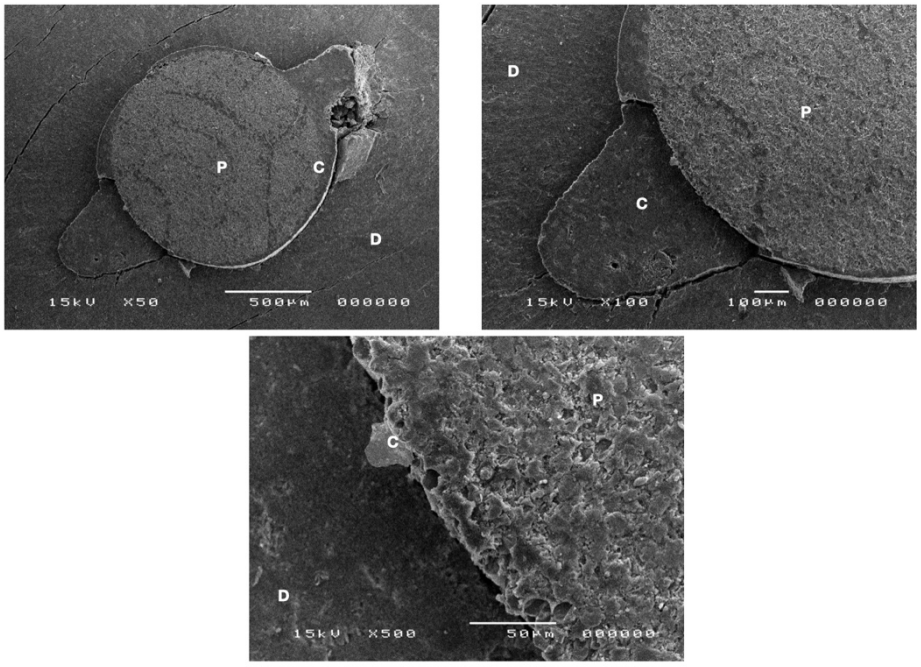

Figure 10: Photomicrographs of the adhesive interface in RelyX Universal EDC group after thermocycling. Abbreviations: D, dentine; P, post; C, cement.

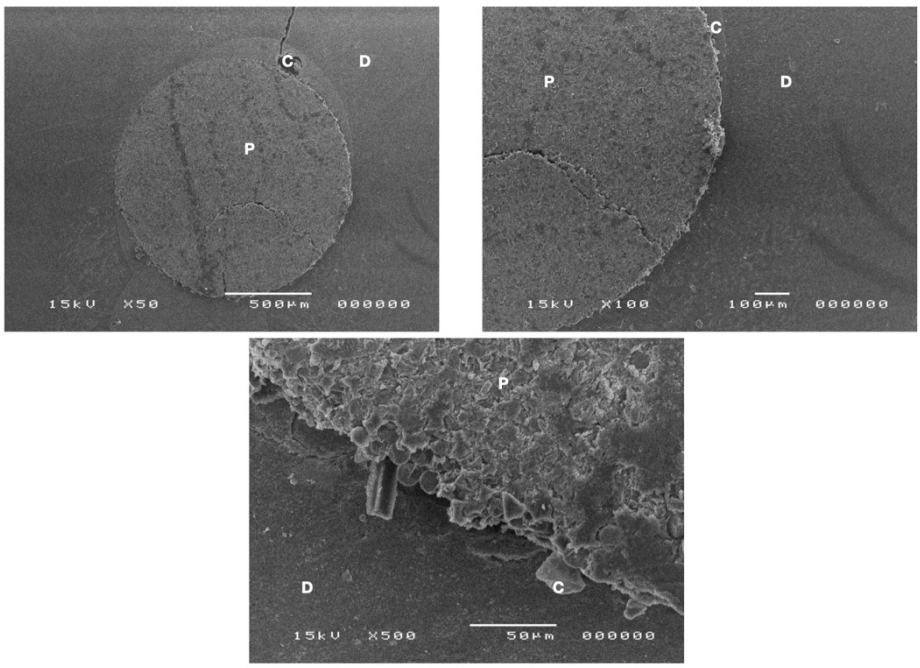

Figure 11: Photomicrographs of the adhesive interface in Multilink Automix control group at baseline. Abbreviations: D, dentine; P, post; C, cement.

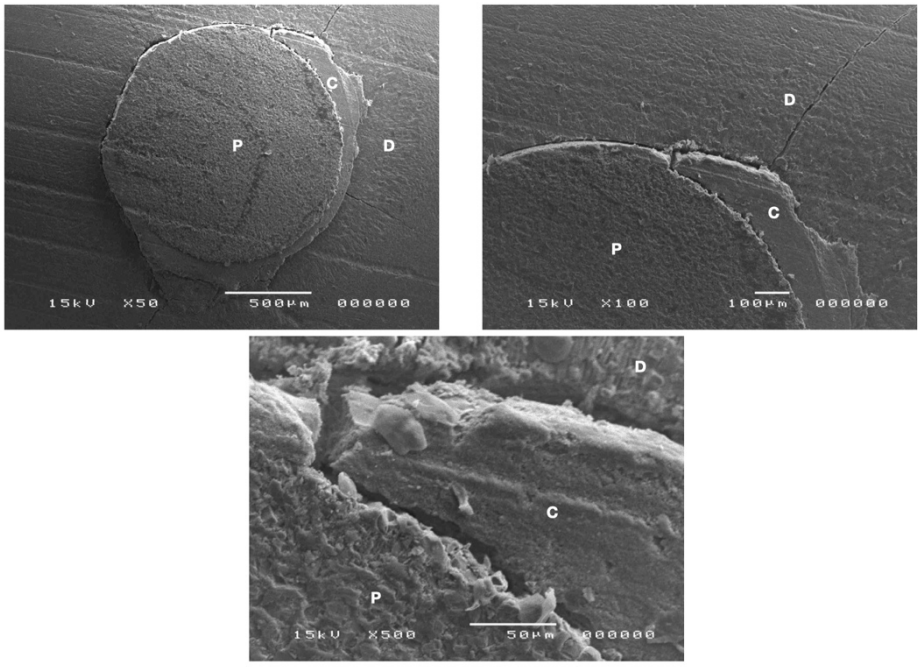

Figure 12: Photomicrographs of the adhesive interface in Multilink Automix EDC group at baseline. Abbreviations: D, dentine; P, post; C, cement.

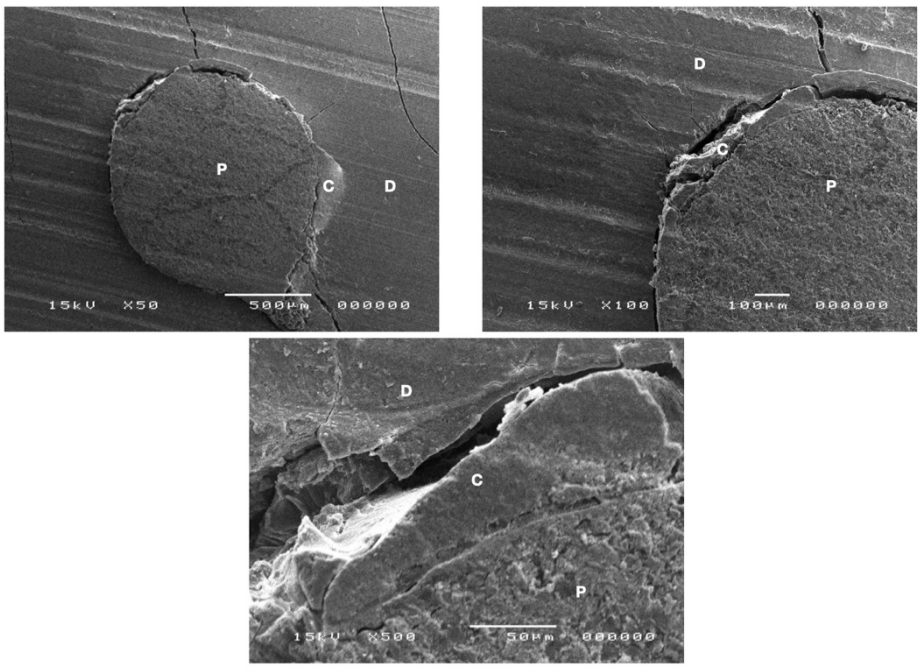

Figure 13: Photomicrographs of the adhesive interface in Multilink Automix control group after thermocycling. Abbreviations: D, dentine; P, post; C, cement.

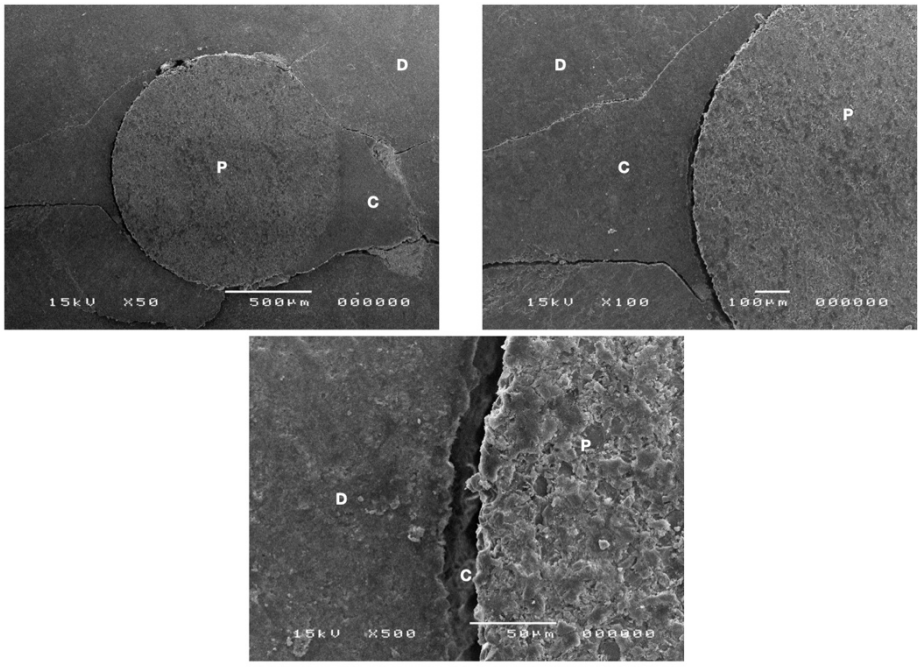

Figure 14: Photomicrographs of the adhesive interface in Multilink Automix EDC group after thermocycling. Abbreviations: D, dentine; P, post; C, cement.

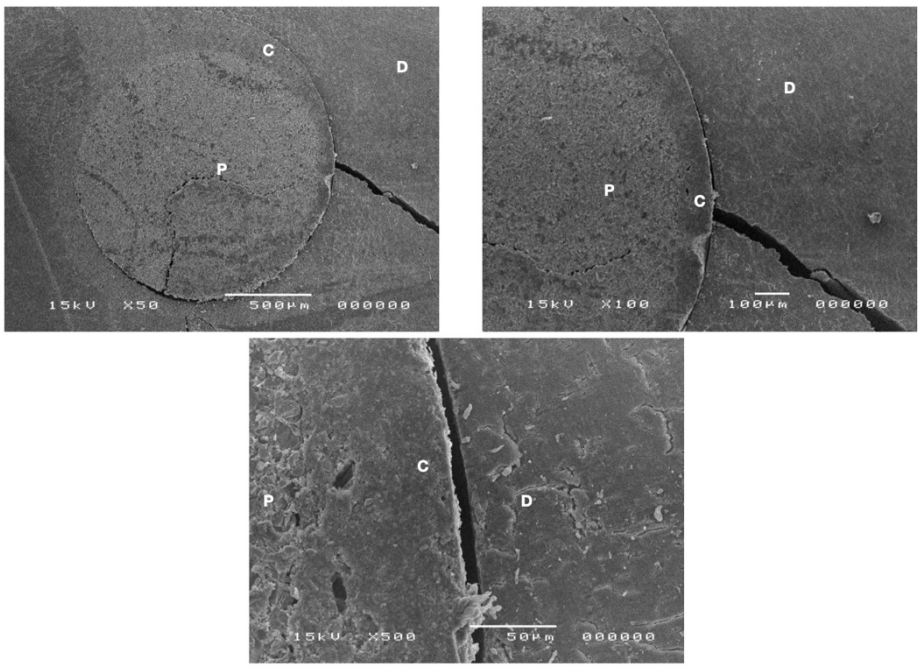

Figure 15: Photomicrographs of the adhesive interface in Luxacore Z Dual control group at baseline. Abbreviations: D, dentine; P, post; C, cement.

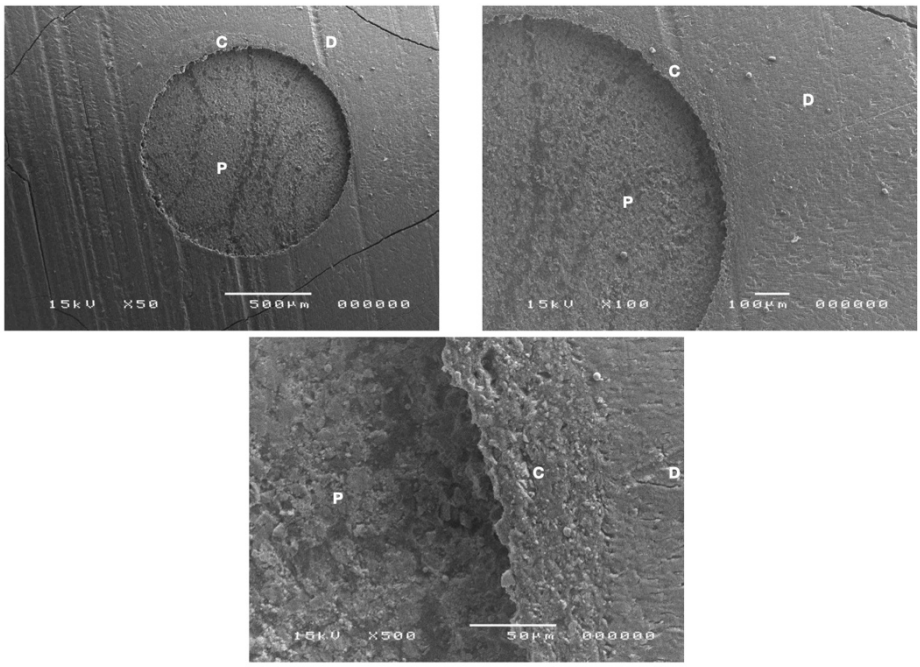

Figure 16: Photomicrographs of the adhesive interface in Luxacore Z Dual EDC group at baseline. Abbreviations: D, dentine; P, post; C, cement.

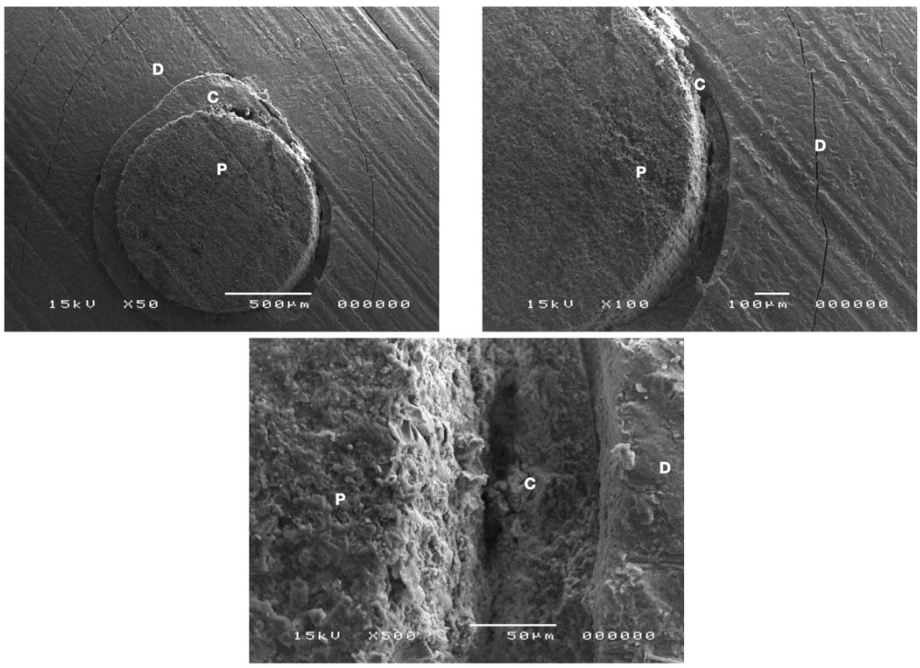

Figure 17: Photomicrographs of the adhesive interface in Luxacore Z Dual control group after thermocycling. Abbreviations: D, dentine; P, post; C, cement.

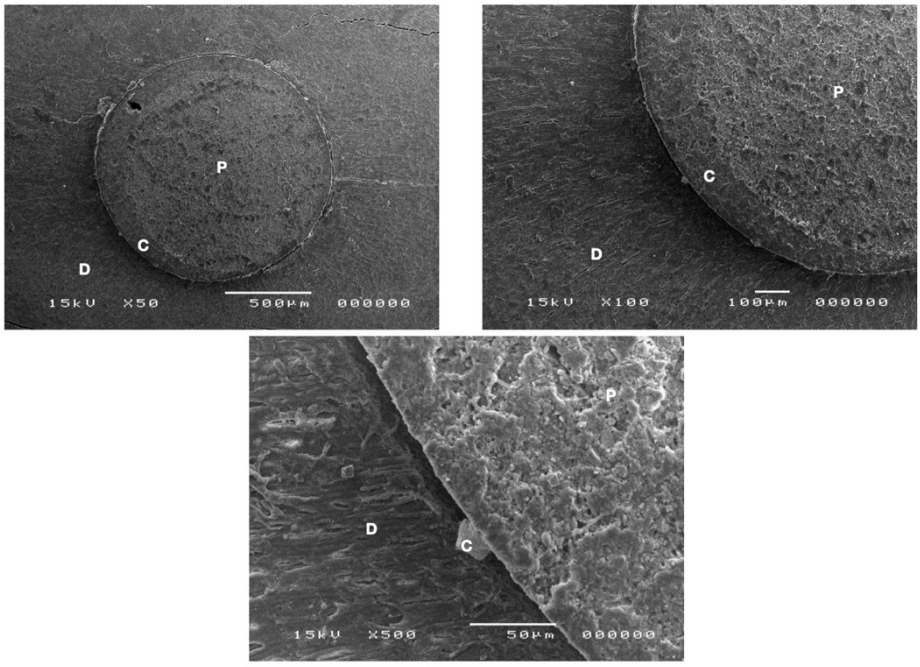

Figure 18: Photomicrographs of the adhesive interface in Luxacore Z Dual EDC group after thermocycling. Abbreviations: D, dentine; P, post; C, cement.

Discussion

The experimental use of collagen cross-linking agents during adhesive procedures has gained great popularity over the past years. The use of cross-linkers can be seen as a biological tissue engineering approach, where dentin tissue repair/regeneration is the development of a biomimetic strategy to enhance the substrate properties by modifying the chemistry of the tissue. (130)

One of the main aims of this study was to evaluate the effect of EDC on push-out bond strength of adhesively luted fiber posts, 24h after cementation and after simulated artificial aging. The first null hypothesis was partially rejected since the application of EDC did not influence the immediate push-out strength, however, it was able to preserve the bond-strength after 40.000 thermocycles. The results are in agreement with previous work which was conducted on coronal dentin in which EDC did not influence the immediate bond-strength values. (128, 130, 187) Our results support the findings from Comba et al. (2019) and Shafiei et al. (2016) in which no beneficial effect of EDC on immediate push-out bond strength of fiber posts was observed when posts were cemented using different adhesive strategies. (167, 169) However, Lopes et al. (2020) did report higher immediate bond-strength values when EDC pretreatment was carried out and fiber posts were luted with SA resin cement. (168) The absence of the positive effect of EDC pretreatment on immediate bond-strength values can be explained by the reduced quantity of exposed collagen network, due to challenges in secondary smear layer removal in root canal spaces, and the degradation of collagen network in endodontically treated teeth. (188-190)

The true beneficial effect of radicular dentin pretreatment with EDC became evident after simulated aging by means of thermocycling. Indeed, previous studies which investigated the effect of EDC on bond-strength on coronal dentin reported that EDC was able to preserve long-term bond strength (127, 128, 130), and promising results were observed even after 5 years of aging in artificial saliva. (187) Similarly, EDC was found to be effective in maintaining the bond-strength of fiber posts and the integrity of resin-dentin interface when posts were luted using different resin

cements (EAR, SE and SA approach) and stored in artificial saliva or water for 12 months. (167, 169) Interestingly, EDC was also demonstrated to be very effective in preserving 10-month bond-strength values in radicular dentin which was submitted to radiation in order to simulate conditions seen in cancer treated patients. No such effect was seen in control groups and in groups where CHX was used for root canal irrigation. (168) This research seems to be the first to report preservation of push-out bond strength values in EDC groups when root slices were submitted to simulated aging by means of 40.000 thermocycles. We further demonstrated reduction in push-out bond-strength values in control groups, regardless of the choice of the adhesive strategy and resin cements used during luting procedures. EDC was most likely able to maintain bond-strength values in EAR and SE groups due to the fact that it can alter the configuration of MMPs catalytic domains which become exposed during etching of radicular dentin. (169) Furthermore, higher bond-strength values in EDC groups can be attributed to the well known fact that EDC can stimulate the formation of cross-links and increase the resistance to degradation of collagen. (191) (124, 168)

The second null hypothesis that EDC has no effect on endogenous enzymatic activity within radicular dentin was rejected since it was able reduce the level of enzymatic activity at baseline when SE (Multilink Autoimix) and EAR (Luxacore Z Dual) resin cements were used for fiber post luting, as well as in SA (RelyX Universal) groups after thermocycling. When cementing fiber posts with a multi-step resin cement that requires application of the phosphoric acid, radicular dentin becomes demineralized with consequent increased endogenous enzymatic activity. Previous study showed that EDC can inactivate matrix-bound dentin proteinases in demineralized coronal dentin matrices with a 1 minute application time. (124) Pretreatment with EDC was also able to inhibit dentin endogenous MMPs as assayed with the zymography when 3- and 2-step EAR adhesive systems were used as bonding agents on coronal dentin. (128) Although conducted on coronal dentin, the findings from the mentioned studies are in line with our results which demonstrated the ability of EDC to silence the enzymatic activity when EAR system was used during luting of fiber posts. Up to this date, two studies evaluated the effect of EDC, by means of in situ zymography, on

enzymatic activity within hybrid layers in radicular dentin created by 3- and 2-step EAR adhesives. Both Comba et al. (2019) and Alonso et al. (2018) reported reduced gelatinolytic activity in EDC groups compared to control groups, thus confirming our findings and the favorable influence EDC has on resin-dentin interface in radicular dentin. (169, 192) Similarly, we also observed reduced enzymatic activity in EDC groups when SE adhesive strategy was used for fiber post luting. These findings are in agreement with Mazzoni et al. (2017) and Maravic et al. (2021) who conducted similar experiments on coronal dentin. (129, 187) We speculate that the full effect of EDC in silencing MMPs seen in Multilink group was also due to experimental design used in this study. Rather then pretreating radicular dentin with EDC prior to adhesive application, Multilink Primer which contains phosphoric acid was applied to radicular dentin according to the manufacturer's instructions, therefore partly demineralizing dentin, after which EDC was applied for 1 minute. These steps enabled application of EDC on demineralized dentin, and silencing of MMP activity even after primer application. (127) To the best of our knowledge, this is the first experiment that showed promising results in reducing enzymatic activity within hybrid layers created with SE approach in radicular dentin. As seen in Figure 2, there was a tendency in reduction of MMPs activity even in RelyX Universal group, however, no statistically significant threshold was reached. This is likely due to the fact that, when using a SA resin cement, EDC is applied on mineralized dentin, thus underestimating the effect this cross-linking agent may have when applied on mineralized dentin. Furthermore, the acidic monomers in SA resin cements, depending on the type and concentration of acid functionality as well as the moisture content, can create a pH value of around 1.5 in freshly mixed cement, thus causing the activation of the MMPs. The true beneficial effect of EDC in silencing enzymatic activity in RelyX Universal group became evident only after artificial aging of the samples, thus confirming the positive effect of EDC on long-term preservation of resin-dentin interface created by SA cements. (168)

The third null hypothesis that there would be no difference in MMPs activity within radicular dentin when different adhesive strategies and resin cements are used for FRC post luting

was rejected, since statistically significant differences were observed among all three tested groups. Contrary to the expectations, the highest level of enzymatic activity was seen when SA resin cement (RelyX Universal) was used for post luting, while the lowest activity was detected when Luxacore Z Dual, a cement used with EAR system, was applied on coronal dentin. Although the results of this study are somehow unexpected, there may be several explanations for this finding. Firstly, when cementing posts with resin cements that require application of phosphoric acid, the etchant needs to be dispensed in the root canal space with the intention of achieving intimate contact with canal walls for 15 s and consequently demineralize radicular dentin. This procedure should lead to the exposure of collagen fibers and activation of latent MMPs. (71) However, applying an etchant into root canal space is more challenging compared to situations when coronal dentin needs to be etched, mostly due to the reduced visibility, narrow root canal space and complex morphology of the canals. (193) Indeed, when etching gel is applied to root canal space passively (by means of needle provided by the manufacturer) SEM images revealed that canal walls are covered with smear layer debris and that dentinal tubules are partially opened due to the presence of smear plugs in all canal thirds. (194) Considering that in this study the etching gel was dispensed into canal space using a needle and that it was left for 15 s, after which it was thoroughly rinsed, it is possible that proper demineralization of radicular dentin was not entirely achieved. Consequently, the well known effect that phosphoric acid has on MMPs activation may have been diminished in this particular case. On the other hand, the increased enzymatic activity observed when SE and SA cements were used for luting can be explained by the technique used for applying the primer in SE groups and chemistry of the SA cements. Multilink Primer which, as previously mentioned, contains phosphoric acid was actively rubbed with microbrush therefore achieving better contact with root canal walls and possibly activating latent MMPs in greater percentage than passive delivery of etching gel. Lastly, very high enzymatic activity detected in RelyX Universal group can be explained by the chemistry of SA resin cements. Self-adhesive resin cements critically rely on strongly acidic monomers that impose formulation stability complications. pH of the freshly mixed

two-component SA cement can be as low as 1.5 which is certainly enough to achieve demineralization of dentin. The neutralization kinetics for RelyX Unicem may take up to 48 h from the moment it has been delivered to root canal. (144) Since it was developed by the same manufacturer, we speculate that RelyX Universal has similar polymerization and neutralization kinetics to RelyX Unicem, and that the time needed for achieving neutral pH value is approximately the same. During neutralization process which may take hours, acidic monomers and low pH level of the cement could have lead to increased level of enzymatic activity within radicular dentin.

In every day dental practice clinicians can choose among one of three different adhesive strategies when luting fiber posts. Even though SA resin cements have been introduced to the market more than a decade ago and the literature reports positive results for this group of cements, concerns still exist within dental community when having to cement fiber posts. Therefore, one of the aims of this study was to evaluate the push-out bond strength achieved with different adhesive approaches and different types of resin cements. Some clinicians claim that multiple steps required when using cements with EAR and SE approach can increase technique sensitivity and, if not performed properly, can reduce the bond strength. On the other hand, some dentists feel more comfortable using multi-step resin cements since, unlike SA cements, they lead to the creation of hybrid layers. (63, 71) The fourth null hypothesis that the choice of adhesive strategy and resin cement does not influence post push-out bond strength was accepted, since no statistically significant difference was observed among the three investigated cements. Sarkis-Onofre et al. (2014) conducted a systematic review of in vitro studies that compared bond-strength values obtained with multi-step (EAR and SE) and SA resin cements. It was reported that the usage of SA cements could improve the retention of fiber posts into root canal. (66) Contrary to the findings of the mentioned systematic review, we did not observe significant differences among the tested resin cements, neither at baseline nor after simulated aging. These results are in line with the pilot study, in which we concluded that retention of fiber posts is more material-dependent than cementation strategy-dependent.

After push-out bond strength test the highest values were observed in the coronal portion of the root, and these values were statistically higher from both middle and apical third. However, no differences were observed between apical and middle part of the root. These results led to the rejection of the fifth null hypothesis. Our findings are in agreement with Machado et al. (2015) and Comba et al. (2019) who reported superior retention of fiber posts in coronal and middle third when FRC posts were luted with multi-step resin cement. (169, 195) Similar findings were reported by Lopes et al. (2020) who investigated bonding properties of SA resin cement. (168) The results are furthermore in line with Rodrigues et al. (2017) who reported the highest bond strength in coronal region of the tooth, regardless of the cementation strategy used. (196) The inferior bond strength values in apical portion can be explained by impaired bonding to radicular dentin in this part of the root, due to reduced access of light which influences polymerization of adhesive systems and resin cements. Lastly, the morphology of dentin is less favorable for achieving adequate bonding and it is usually characterized by presence of smear layer which is difficult to control. (188, 197)

Storage of specimens in artificial saliva or water is the most common way to simulate aging in dental research. It is considered as a low-cost and simple way, with the possibility of aging the resin-dentin interfaces from several months (198), up to 4-5 and even 10 years. (187, 199-201) The decrease in bond strength can be observed even after several months of storage and is supposed to be caused by degradation of interface components by hydrolysis (mainly resin and/or collagen), as explained in the earlier sections. (202) Other proposed mechanisms of degradation of resin-dentin interfaces include: “plasticization” – infiltration and decrease of mechanical properties of the polymer matrix, by swelling and reducing the frictional forces between the polymer chains (203, 204) and breakdown of uncured monomers (202, 205).

Another way of artificial aging is by applying occlusal loading. For this purposes, chewing simulator is usually used to simulate clinical conditions and masticatory forces. (206) (202, 207) Thermocycling is a method commonly used in in vitro research to facilitate the degradation of resin-dentin bonds. The original ISO TR 11450 standard from 1994 suggested a minimum of 500

cycles in water between 5-55 ºC. However, few years later Gale and Darvell (1998) came to a conclusion that 10.000 cycles corresponds to one year of clinical function. (73) Consequently, Academy of dental materials published their guidance for the evaluation and aging methods of resin-based restorations. According to these guidelines, samples should be subjected to a minimum of 10.000 cycles (and by preference more), in aqueous media between 5-55 ºC after 1-7 day storage in the same media. The exposure in each bath should be at least 20 s, and the transfer time should be as short as possible (less than 10 s). (208) The artificial aging occurring within samples exposed to thermocycling occurs in two ways. Hot water (55 C) can accelerate hydrolysis of interface components, and subsequent uptake of water and extraction of breakdown products or poorly polymerized resin oligomers. (209) Next, due to the higher thermal contraction/expansion coefficient of the restorative material (as compared with that of tooth tissue), repetitive contraction/expansion stresses are generated at the material-tooth interface. The generated stresses can cause cracks that propagate along bonded interfaces, and, once a gap is created, changing gap dimensions can cause in- and outflow of oral fluids, which is known as “percolation”. (73, 202)

The rationale for using thermocycling as a way to accelerate degradation of the adhesive interface is supported by the previous unpublished data by Josic et al. Their study concluded that storage of root slices in artificial saliva for 1 year can lead to increased push-out bond strength values when testing 5 different luting agents (stated earlier in the pilot study section). This observation was probably due to the fact that long-term exposure of root slices to aqueous media may have enabled water molecules to enter the resin cement and fiber posts by diffusion. (210, 211) Water diffusion into the material could influence its hygroscopic expansion. The volumetric expansion of resin cement and fiber posts could increase the frictional resistance between the material and canal walls, resulting in its greater resistance towards the axial forces applied during push-out test. (212) It is important to mention that self-adhesive resin cements show different water sorption and solubility characteristics. (185) Also, acidic monomers (such as the ones present in self-adhesive cements) with hydrophilic characteristics can absorb more water than conventional

composites or multi-step resin cements, which would lead to their higher net expansion and more intimate contact to root canal walls. (213) Josic et al. (2020) also reported that cyclic loading in chewing simulator (100.000 cycles) caused no visible reduction in fracture resistance of endodoctically treated teeth restored with FRC posts. (27) Finally, it was reported the elastic modulus and shear strength of an endodontic FRC post material is more influenced by thermocycling fatigue than by water storage alone. (214) Therefore, thermocycling seems like an adequate method to challenge the adhesive interfaces between resin cements – dentin and resin cement – FRC post. Even though a standardized thermocycling protocol does not seem to exist and high heterogeneity is observed among in vitro research (215), root slices were exposed to 40.000 thermal cycles (with dwell time of 30 s) in order to stress the adhesive interface and to gain more clinical relevance. (171) Lastly, the literature reports no information on how thermocycling influences the endogenous enzymatic activity.

The final null hypothesis, that thermocyling has no effect on push-out bond strength and endogenous enzymatic activity within radicular dentin had to be rejected, since this method of artificial aging statistically influenced both retention of fiber posts as well as endogenous enzymatic activity. Previous research published by Yaman et al. (2014) and Mazzoni et al. (2008) who reported that thermocycling was able to reduce push-out bond strengths of adhesively luted fiber posts. (171, 216) On the other hand, Dimitrouli et al. (2012) did not observe decrease in bond strength when posts luted with SA and multi-step resin cements were subjected to thermocycling. (217) The absence of difference can be due to the study design and the low number of cycles (5.000) used in this research, since 5.000 thermocycles is insufficient to produce a significant effect to materials' properties.

This study investigated, for the first time, the influence of thermocycling on endogenous enzymatic activity within radicular dentin. When observing Figure 2, a general trend in reduction of enzymatic activity can be observed in all groups. However, the only significant difference was observed in Luxacore Z Dual group, where differences were detected in both control and EDC

groups. These results may seem unexpected, since the level of enzymatic activity usually increases with aging and is considered to be responsible for the loss of bond strength in resin-dentin restorations. (187) Although surprising, the explanation to this phenomenon may lie in the fact that instead of aging samples in artificial saliva as reported by Maravic et al. (2021) (187) and Breschi et al. (2020) (200), the root slices in this study were exposed to thermocycling procedure in dwells that were filled with distilled water. Unlike artificial saliva which contains Zn and Ca ions necessary for MMPs activity, distilled water clearly represents a different storage medium for aging of samples due to the lack of ions. Tezvergil-Mutlua et al. (2010) investigated the requirement of Zn and Ca ions for functional MMP activity in demineralized dentin matrices. (218) The authors reported that "the common use of water as an aging medium may underestimate the hydrolytic activity of endogenous dentin MMPs and should be discouraged because it would promote the loss of calcium and zinc ions from dentin matrices, rather than restore them". Since Luxacore groups were the only groups in which dentin demineralization was performed with phosphoric acid, we speculate that the loss of Ca and Zn ions in this group was higher than in partially demineralized (Multilink Automix) or mineralized radicular dentin (RelyX Universal), thus leading to considerable decrease in MMP activity.

Finally, for the first time we demonstrated that the level of MMPs activity seemed to have no direct correlation with retention of fiber posts in root canals after simulated aging. This further highlights the complex mechanism of posts' retention, and confirms the previous findings that the retention of fiber posts in the root canal cannot be attributed to the mode of interaction of the luting cements with dentine nor to their ability to diffuse into dentine. (219) However, the cross-linking effect of EDC within radicular dentin should be investigated in future studies, since this zero cross-linking agent was able to preserve bond strength values.

Conclusions and Future Directions

Within the limitations of this in vitro research, the following conclusions can be made:

- The investigated cross-linking agent EDC does not influence immediate push-out bond strength, however, it contributes to the preservation of resin-dentin interfaces within radicular dentin created by different cementation protocols after simulated aging
- EDC was effective in reducing endogenous enzymatic activity within hybrid layers in radicular dentin
- The choice of resin cement and cementation strategy did not influence posts' retention in the canal space
- Coronal third yielded the highest bond strength values, demonstrating the most predictable retention of fiber posts in the mentioned root region
- Thermocycling, as means of simulated aging, significantly reduced retention of fiber posts in self-etch and self-adhesive control groups, as well as endogenous enzymatic activity when posts were cemented with EAR adhesive and multi-step resin cement.

Considering that pretreating radicular dentin with EDC during 1 minute represents clinically acceptable timeframe, randomized controlled clinical trials investigating the potential benefits of this cross-linking agent are necessary to confirm the findings of this in vitro study.

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References

1. Chugal N, Mallya SM, Kahler B, Lin LM. Endodontic Treatment Outcomes. Dent Clin North Am. 2017;61(1):59-80.

2. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(6):658-66.

3. Cameron JA. The use of ultrasound for the removal of the smear layer. The effect of sodium hypochlorite concentration; SEM study. Aust Dent J. 1988;33(3):193-200.

4. Pashley DH, Tao L, Boyd L, King GE, Horner JA. Scanning electron microscopy of the substructure of smear layers in human dentine. Arch Oral Biol. 1988;33(4):265-70.

5. Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J. 2010;43(1):2-15.

6. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98.

7. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod. 2004;30(11):785-7.

8. Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod. 2010;36(1):105-9.

9. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216(6):299-303.

10. Saito K, Webb TD, Imamura GM, Goodell GG. Effect of shortened irrigation times with 17% ethylene diamine tetra-acetic acid on smear layer removal after rotary canal instrumentation. J Endod. 2008;34(8):1011-4.

11. Teixeira CS, Felippe MC, Felippe WT. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis. Int Endod J. 2005;38(5):285-90.

12. Prati C, Foschi F, Nucci C, Montebugnoli L, Marchionni S. Appearance of the root canal walls after preparation with NiTi rotary instruments: a comparative SEM investigation. Clin Oral Investig. 2004;8(2):102-10.

13. Urban K, Donnermeyer D, Schafer E, Burklein S. Canal cleanliness using different irrigation activation systems: a SEM evaluation. Clin Oral Investig. 2017;21(9):2681-7.

14. Virdee SS, Seymour DW, Farnell D, Bhamra G, Bhakta S. Efficacy of irrigant activation techniques in removing intracanal smear layer and debris from mature permanent teeth: a systematic review and meta-analysis. Int Endod J. 2018;51(6):605-21.

15. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int Endod J. 2002;35(11):934-9.

16. Wang Z, Maezono H, Shen Y, Haapasalo M. Evaluation of Root Canal Dentin Erosion after Different Irrigation Methods Using Energy-dispersive X-ray Spectroscopy. J Endod. 2016;42(12):1834-9.

17. Carrigan PJ, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. J Endod. 1984;10(8):359-63.

18. Schellenberg U, Krey G, Bosshardt D, Nair PN. Numerical density of dentinal tubules at the pulpal wall of human permanent premolars and third molars. J Endod. 1992;18(3):104-9.

19. Mjor IA, Smith MR, Ferrari M, Mannocci F. The structure of dentine in the apical region of human teeth. Int Endod J. 2001;34(5):346-53.

20. Al-Karadaghi TS, Franzen R, Jawad HA, Gutknecht N. Investigations of radicular dentin permeability and ultrastructural changes after irradiation with Er,Cr:YSGG laser and dual wavelength (2780 and 940 nm) laser. Lasers Med Sci. 2015;30(8):2115-21.

21. Surapipongpuntr P, Duangcharee W, Kwangsamai S, Ekka A. Effect of root canal irrigants on cervical dentine permeability to hydrogen peroxide. Int Endod J. 2008;41(10):821-7.

22. Guignes P, Faure J, Maurette A. Relationship between endodontic preparations and human dentin permeability measured in situ. J Endod. 1996;22(2):60-7.

23. Faria AC, Rodrigues RC, de Almeida Antunes RP, de Mattos Mda G, Ribeiro RF. Endodontically treated teeth: characteristics and considerations to restore them. J Prosthodont Res. 2011;55(2):69-74.

24. Sarkis-Onofre R, Jacinto RC, Boscato N, Cenci MS, Pereira-Cenci T. Cast metal vs. glass fibre posts: a randomized controlled trial with up to 3 years of follow up. J Dent. 2014;42(5):582-7.

25. Figueiredo FE, Martins-Filho PR, Faria ESAL. Do metal post-retained restorations result in more root fractures than fiber post-retained restorations? A systematic review and meta-analysis. J Endod. 2015;41(3):309-16.

26. Torres-Sanchez C, Montoya-Salazar V, Cordoba P, Velez C, Guzman-Duran A, Gutierrez-Perez JL, et al. Fracture resistance of endodontically treated teeth restored with glass fiber reinforced posts and cast gold post and cores cemented with three cements. J Prosthet Dent. 2013;110(2):127-33.

27. Josic U, Radovic I, Juloski J, Beloica M, Popovic M, Alil A, et al. Can Fiber-post Placement Reinforce Structurally Compromised Roots? J Adhes Dent. 2020;22(4):409-14.

28. Qing H, Zhu Z, Chao Y, Zhang W. In vitro evaluation of the fracture resistance of anterior endodontically treated teeth restored with glass fiber and zircon posts. J Prosthet Dent. 2007;97(2):93-8.

29. Giovani AR, Vansan LP, de Sousa Neto MD, Paulino SM. In vitro fracture resistance of glass-fiber and cast metal posts with different lengths. J Prosthet Dent. 2009;101(3):183-8.

30. Behr M, Rosentritt M, Wimmer J, Lang R, Kolbeck C, Burgers R, et al. Self-adhesive resin cement versus zinc phosphate luting material: a prospective clinical trial begun 2003. Dent Mater. 2009;25(5):601-4.

31. Naumann M, Sterzenbach G, Rosentritt M, Beuer F, Frankenberger R. Is adhesive cementation of endodontic posts necessary? J Endod. 2008;34(8):1006-10.

32. Ferracane JL. Developing a more complete understanding of stresses produced in dental composites during polymerization. Dent Mater. 2005;21(1):36-42.

33. Feilzer AJ, De Gee AJ, Davidson CL. Setting stress in composite resin in relation to configuration of the restoration. J Dent Res. 1987;66(11):1636-9.

34. Choi KK, Ryu GJ, Choi SM, Lee MJ, Park SJ, Ferracane JL. Effects of cavity configuration on composite restoration. Oper Dent. 2004;29(4):462-9.

35. Tay FR, Loushine RJ, Lambrechts P, Weller RN, Pashley DH. Geometric factors affecting dentin bonding in root canals: a theoretical modeling approach. J Endod. 2005;31(8):584-9.

36. Ferrari M, Carvalho CA, Goracci C, Antoniolli F, Mazzoni A, Mazzotti G, et al. Influence of luting material filler content on post cementation. J Dent Res. 2009;88(10):951-6.

37. Aravamudhan K, Rakowski D, Fan PL. Variation of depth of cure and intensity with distance using LED curing lights. Dent Mater. 2006;22(11):988-94.

38. Lui JL. Depth of composite polymerization within simulated root canals using light-transmitting posts. Oper Dent. 1994;19(5):165-8.

39. Tagami A, Takahashi R, Nikaido T, Tagami J. The effect of curing conditions on the dentin bond strength of two dual-cure resin cements. J Prosthodont Res. 2017;61(4):412-8.

40. Pfeifer C, Shih D, Braga RR. Compatibility of dental adhesives and dual-cure cements. Am J Dent. 2003;16(4):235-8.

41. Cheong C, King NM, Pashley DH, Ferrari M, Toledano M, Tay FR. Incompatibility of self-etch adhesives with chemical/dual-cured composites: two-step vs one-step systems. Oper Dent. 2003;28(6):747-55.

42. Suh BI, Feng L, Pashley DH, Tay FR. Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part III. Effect of acidic resin monomers. J Adhes Dent. 2003;5(4):267-82.

43. Fujisawa S, Kadoma Y. Effect of phenolic compounds on the polymerization of methyl methacrylate. Dent Mater. 1992;8(5):324-6.

44. Altmann AS, Leitune VC, Collares FM. Influence of Eugenol-based Sealers on Push-out Bond Strength of Fiber Post Luted with Resin Cement: Systematic Review and Meta-analysis. J Endod. 2015;41(9):1418-23.

45. Ferracane JL. Resin composite--state of the art. Dent Mater. 2011;27(1):29-38.

46. Ferracane JL. Resin-based composite performance: are there some things we can't predict? Dent Mater. 2013;29(1):51-8.

47. Ferracane JL, Mitchem JC. Properties of posterior composite: results of round robin testing for a specification. Dent Mater. 1994;10(2):92-9.

48. Hilton TJ, Ferracane JL. Cavity preparation factors and microleakage of Class II composite restorations filled at intraoral temperatures. Am J Dent. 1999;12(3):123-30.

49. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24(1):90-101.

50. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27(1):1-16.

51. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011;27(1):17-28.

52. Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. J Esthet Dent. 1991;3(4):133-8.

53. Josic U, Maravic T, Mazzitelli C, Del Bianco F, Mazzoni A, Breschi L. The effect of chlorhexidine primer application on the clinical performance of composite restorations: a literature review. J Esthet Restor Dent. 2021;33(1):69-77.

54. Albaladejo A, Osorio R, Toledano M, Ferrari M. Hybrid layers of etch-and-rinse versus self-etching adhesive systems. Med Oral Patol Oral Cir Bucal. 2010;15(1):e112-8.

55. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater. 2001;17(4):296-308.

56. Digole VR, Warhadpande MM, Dua P, Dakshindas D. Comparative evaluation of clinical performance of two self-etch adhesive systems with total-etch adhesive system in noncarious cervical lesions: An in vivo study. J Conserv Dent. 2020;23(2):190-5.

57. Bekes K, Boeckler L, Gernhardt CR, Schaller HG. Clinical performance of a self-etching and a total-etch adhesive system - 2-year results. J Oral Rehabil. 2007;34(11):855-61.

58. Kurokawa H, Takamizawa T, Rikuta A, Tsubota K, Miyazaki M. Three-year clinical evaluation of posterior composite restorations placed with a single-step self-etch adhesive. J Oral Sci. 2015;57(2):101-8.

59. Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, et al. Comparative SEM and TEM observations of nanoleakage within the hybrid layer. Oper Dent. 1995;20(4):160-7.

60. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent. 1995;20(1):18-25.

61. Sousa SJL, Poubel D, Rezende L, Almeida FT, de Toledo IP, Garcia FCP. Early clinical performance of resin cements in glass-ceramic posterior restorations in adult vital teeth: A systematic review and meta-analysis. J Prosthet Dent. 2020;123(1):61-70.

62. Cantoro A, Goracci C, Papacchini F, Mazzitelli C, Fadda GM, Ferrari M. Effect of pre-cure temperature on the bonding potential of self-etch and self-adhesive resin cements. Dent Mater. 2008;24(5):577-83.

63. Radovic I, Monticelli F, Goracci C, Vulicevic ZR, Ferrari M. Self-adhesive resin cements: a literature review. J Adhes Dent. 2008;10(4):251-8.

64. Viotti RG, Kasaz A, Pena CE, Alexandre RS, Arrais CA, Reis AF. Microtensile bond strength of new self-adhesive luting agents and conventional multistep systems. The Journal of prosthetic dentistry. 2009;102(5):306-12.

65. Miotti LL, Follak AC, Montagner AF, Pozzobon RT, da Silveira BL, Susin AH. Is Conventional Resin Cement Adhesive Performance to Dentin Better Than Self-adhesive? A Systematic Review and Meta-Analysis of Laboratory Studies. Oper Dent. 2020;45(5):484-95.

66. Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Oper Dent. 2014;39(1):E31-44.

67. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjaderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials. 2006;27(25):4470-6.

68. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability--A literature review. Dent Mater. 2016;32(2):e41-53.

69. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. Dent Mater J. 2005;24(1):1-13.

70. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent. 2002;30(7-8):371-82.

71. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjaderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater. 2018;34(1):78-96.

72. Jacobsen T, Soderholm KJ. Some effects of water on dentin bonding. Dent Mater. 1995;11(2):132-6.

73. Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent. 1999;27(2):89-99.

74. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, et al. Degree of conversion and permeability of dental adhesives. Eur J Oral Sci. 2005;113(6):525-30.

75. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res. 2004;83(3):216-21.

76. Linde A, Goldberg M. Dentinogenesis. Crit Rev Oral Biol Med. 1993;4(5):679-728.

77. Josic U, Maravic T, Bossù M, Cadenaro M, Comba A, Ierardo G, et al. Morphological Characterization of Deciduous Enamel and Dentin in Patients Affected by Osteogenesis Imperfecta. Applied Sciences. 2020;10(21):7835.

78. Goldberg M, Kulkarni AB, Young M, Boskey A. Dentin: Structure, Composition and Mineralization: The role of dentin ECM in dentin formation and mineralization. Frontiers in bioscience (Elite edition). 2011;3:711.

79. Perdigão J. Dentin bonding as a function of dentin structure. Dental Clinics. 2002;46(2):277-301.

80. Rainey JK, Goh MC. A statistically derived parameterization for the collagen triple-helix. Protein Sci. 2002;11(11):2748-54.

81. Yamauchi M, Terajima M, Shiiba M. Lysine Hydroxylation and Cross-Linking of Collagen. Methods Mol Biol. 2019;1934:309-24.

82. Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand. 2007;65(1):1-13.

83. Breschi L, Perdigao J, Gobbi P, Mazzotti G, Falconi M, Lopes M. Immunocytochemical identification of type I collagen in acid-etched dentin. J Biomed Mater Res A. 2003;66(4):764-9.

84. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92(8):827-39.

85. Tjaderhane L, Salo T. Expression of matrix metalloproteinase-13 in odontoblast-like cells. Int Endod J. 2013;46(10):1006-7.

86. Mazzoni A, Tjaderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res. 2015;94(2):241-51.

87. Perumal S, Antipova O, Orgel JP. Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis. Proc Natl Acad Sci U S A. 2008;105(8):2824-9.

88. Mazzoni A, Scaffa P, Carrilho M, Tjaderhane L, Di Lenarda R, Polimeni A, et al. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res. 2013;92(1):82-6.

89. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjaderhane L, et al. Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. Dent Mater. 2010;26(4):320-5.

90. Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjaderhane L, et al. MMP activity in the hybrid layer detected with in situ zymography. J Dent Res. 2012;91(5):467-72.

91. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res. 2011;90(8):953-68.

92. Carrilho MR, Carvalho RM, Sousa EN, Nicolau J, Breschi L, Mazzoni A, et al. Substantivity of chlorhexidine to human dentin. Dent Mater. 2010;26(8):779-85.

93. Zhang X, Wang L, Liu S, Bai H, Niu W. Evaluation of the bond strength of chlorhexidine incorporated into the adhesive system composition: A PRISMA guided meta-analysis. J Dent Sci. 2020;15(3):315-28.

94. Araujo MS, Souza LC, Apolonio FM, Barros LO, Reis A, Loguercio AD, et al. Two-year clinical evaluation of chlorhexidine incorporation in two-step self-etch adhesive. J Dent. 2015;43(1):140-8.

95. Dutra-Correa M, Saraceni CH, Ciaramicoli MT, Kiyan VH, Queiroz CS. Effect of chlorhexidine on the 18-month clinical performance of two adhesives. J Adhes Dent. 2013;15(3):287-92.

96. Favetti M, Schroeder T, Montagner AF, Correa MB, Pereira-Cenci T, Cenci MS. Effectiveness of pre-treatment with chlorhexidine in restoration retention: A 36-month follow-up randomized clinical trial. J Dent. 2017;60:44-9.

97. Hajizadeh H, Ghavamnasiri M, Majidinia S. Randomized clinical evaluation of the effect of chlorhexidine on postoperative sensitivity of posterior composite resin restorations. Quintessence Int. 2013;44(10):793-8.

98. Montagner AF, Perroni AP, Correa MB, Masotti AS, Pereira-Cenci T, Cenci MS. Effect of pre-treatment with chlorhexidine on the retention of restorations: a randomized controlled trial. Braz Dent J. 2015;26(3):234-41.

99. Sartori N, Stolf SC, Silva SB, Lopes GC, Carrilho M. Influence of chlorhexidine digluconate on the clinical performance of adhesive restorations: a 3-year follow-up. J Dent. 2013;41(12):1188-95.

100. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol. 1999;6(3):437-9.

101. Uitto VJ, Turto H, Huttunen A, Lindy S, Uitto J. Activation of human leukocyte collagenase by compounds reacting with sulfhydryl groups. Biochim Biophys Acta. 1980;613(1):168-77.

102. Carvalho RM, Yoshiyama M, Pashley EL, Pashley DH. In vitro study on the dimensional changes of human dentine after demineralization. Arch Oral Biol. 1996;41(4):369-77.

103. Cadenaro M, Maravic T, Comba A, Mazzoni A, Fanfoni L, Hilton T, et al. The role of polymerization in adhesive dentistry. Dent Mater. 2019;35(1):e1-e22.

104. Kim J, Uchiyama T, Carrilho M, Agee KA, Mazzoni A, Breschi L, et al. Chlorhexidine binding to mineralized versus demineralized dentin powder. Dent Mater. 2010;26(8):771-8.

105. Ricci HA, Sanabe ME, de Souza Costa CA, Pashley DH, Hebling J. Chlorhexidine increases the longevity of in vivo resin-dentin bonds. Eur J Oral Sci. 2010;118(4):411-6.

106. Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR. Ethanol wet-bonding challenges current anti-degradation strategy. J Dent Res. 2010;89(12):1499-504.

107. Hass V, de Paula AM, Parreiras S, Gutierrez MF, Luque-Martinez I, de Paris Matos T, et al. Degradation of dentin-bonded interfaces treated with collagen cross-linking agents in a cariogenic oral environment: An in situ study. J Dent. 2016;49:60-7.

108. Hass V, Luque-Martinez IV, Gutierrez MF, Moreira CG, Gotti VB, Feitosa VP, et al. Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition. Dent Mater. 2016;32(6):732-41.

109. Parise Gre C, Pedrollo Lise D, Ayres AP, De Munck J, Tezvergil-Mutluay A, Seseogullari-Dirihan R, et al. Do collagen cross-linkers improve dentin's bonding receptiveness? Dent Mater. 2018;34(11):1679-89.

110. Tjäderhane L, Carrilho MR, Breschi L, Tay FR, Pashley DH. Dentin basic structure and composition—an overview. Endodontic Topics. 2009;20(1):3-29.

111. Yamauchi M, Shiiba M. Lysine hydroxylation and cross-linking of collagen. Methods Mol Biol. 2008;446:95-108.

112. Miura J, Nishikawa K, Kubo M, Fukushima S, Hashimoto M, Takeshige F, et al. Accumulation of advanced glycation end-products in human dentine. Arch Oral Biol. 2014;59(2):119-24.

113. Miguez PA, Pereira PN, Atsawasuwan P, Yamauchi M. Collagen cross-linking and ultimate tensile strength in dentin. J Dent Res. 2004;83(10):807-10.

114. Shinno Y, Ishimoto T, Saito M, Uemura R, Arino M, Marumo K, et al. Comprehensive analyses of how tubule occlusion and advanced glycation end-products diminish strength of aged dentin. Scientific reports. 2016;6(1):1-10.

115. Lee J, Sabatini C. Glutaraldehyde collagen cross-linking stabilizes resin–dentin interfaces and reduces bond degradation. European journal of oral sciences. 2017;125(1):63-71.

116. Chen C, Mao C, Sun J, Chen Y, Wang W, Pan H, et al. Glutaraldehyde-induced remineralization improves the mechanical properties and biostability of dentin collagen. Materials Science and Engineering: C. 2016;67:657-65.

117. Al-Ammar A, Drummond JL, Bedran-Russo AK. The use of collagen cross-linking agents to enhance dentin bond strength. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2009;91(1):419-24.

118. Fang M, Liu R, Xiao Y, Li F, Wang D, Hou R, et al. Biomodification to dentin by a natural crosslinker improved the resin–dentin bonds. Journal of dentistry. 2012;40(6):458-66.

119. Seseogullari-Dirihan R, Apollonio F, Mazzoni A, Tjaderhane L, Pashley D, Breschi L, et al. Use of crosslinkers to inactivate dentin MMPs. Dental Materials. 2016;32(3):423-32.

120. Liu Y, Bai X, Li S, Liu Y, Keightley A, Wang Y. Molecular weight and galloylation affect grape seed extract constituents' ability to cross-link dentin collagen in clinically relevant time. Dental Materials. 2015;31(7):814-21.

121. Moreira M, Souza N, Sousa R, Freitas D, Lemos M, De Paula D, et al. Efficacy of new natural biomodification agents from Anacardiaceae extracts on dentin collagen cross-linking. Dental Materials. 2017;33(10):1103-9.

122. Olde Damink LH, Dijkstra PJ, van Luyn MJ, van Wachem PB, Nieuwenhuis P, Feijen J. Cross-linking of dermal sheep collagen using a water-soluble carbodiimide. Biomaterials. 1996;17(8):765-73.

123. Zeeman R, Dijkstra PJ, van Wachem PB, van Luyn MJ, Hendriks M, Cahalan PT, et al. Successive epoxy and carbodiimide cross-linking of dermal sheep collagen. Biomaterials. 1999;20(10):921-31.

124. Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogullari-Dirihan R, Hoshika T, Cadenaro M, et al. Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs, in vitro. J Dent Res. 2012;91(2):192-6.

125. Scheffel DL, Hebling J, Scheffel RH, Agee K, Turco G, de Souza Costa CA, et al. Inactivation of matrix-bound matrix metalloproteinases by cross-linking agents in acid-etched dentin. Oper Dent. 2014;39(2):152-8.

126. Singh P, Nagpal R, Singh UP, Manuja N. Effect of carbodiimide on the structural stability of resin/dentin interface. J Conserv Dent. 2016;19(6):501-9.

127. Mazzoni A, Apolonio FM, Saboia VP, Santi S, Angeloni V, Checchi V, et al. Carbodiimide inactivation of MMPs and effect on dentin bonding. J Dent Res. 2014;93(3):263-8.

128. Mazzoni A, Angeloni V, Apolonio FM, Scotti N, Tjaderhane L, Tezvergil-Mutluay A, et al. Effect of carbodiimide (EDC) on the bond stability of etch-and-rinse adhesive systems. Dent Mater. 2013;29(10):1040-7.

129. Mazzoni A, Angeloni V, Sartori N, Duarte S, Jr., Maravic T, Tjaderhane L, et al. Substantivity of Carbodiimide Inhibition on Dentinal Enzyme Activity over Time. J Dent Res. 2017;96(8):902-8.

130. Mazzoni A, Angeloni V, Comba A, Maravic T, Cadenaro M, Tezvergil-Mutluay A, et al. Cross-linking effect on dentin bond strength and MMPs activity. Dent Mater. 2018;34(2):288-95.

131. Cadenaro M, Fontanive L, Navarra CO, Gobbi P, Mazzoni A, Di Lenarda R, et al. Effect of carboidiimide on thermal denaturation temperature of dentin collagen. Dent Mater. 2016;32(4):492-8.

132. Pegoraro TA, da Silva NR, Carvalho RM. Cements for use in esthetic dentistry. Dent Clin North Am. 2007;51(2):453-71, x.

133. De Souza G, Braga RR, Cesar PF, Lopes GC. Correlation between clinical performance and degree of conversion of resin cements: a literature review. J Appl Oral Sci. 2015;23(4):358-68.

134. Barghi N, McAlister EH. LED and halogen lights: effect of ceramic thickness and shade on curing luting resin. Compend Contin Educ Dent. 2003;24(7):497-500, 2, 4 passim; quiz 8.

135. Kilinc E, Antonson SA, Hardigan PC, Kesercioglu A. The effect of ceramic restoration shade and thickness on the polymerization of light- and dual-cure resin cements. Oper Dent. 2011;36(6):661-9.

136. Savatier L, Curnier F, Krejci I. Micro-CT evaluation of cavities prepared with with different Er:YAG handpieces. Eur J Paediatr Dent. 2014;15(2):95-100.

137. Jang Y, Ferracane JL, Pfeifer CS, Park JW, Shin Y, Roh BD. Effect of Insufficient Light Exposure on Polymerization Kinetics of Conventional and Self-adhesive Dual-cure Resin Cements. Oper Dent. 2017;42(1):E1-E9.

138. Alpoz AR, Ertugrul F, Cogulu D, Ak AT, Tanoglu M, Kaya E. Effects of light curing method and exposure time on mechanical properties of resin based dental materials. Eur J Dent. 2008;2(1):37-42.

139. Tanoue N, Koishi Y, Atsuta M, Matsumura H. Properties of dual-curable luting composites polymerized with single and dual curing modes. J Oral Rehabil. 2003;30(10):1015-21.

140. Passos SP, Kimpara ET, Bottino MA, Santos GC, Jr., Rizkalla AS. Effect of ceramic shade on the degree of conversion of a dual-cure resin cement analyzed by FTIR. Dent Mater. 2013;29(3):317-23.

141. Soares CJ, Bicalho AA, Verissimo C, Soares P, Tantbirojn D, Versluis A. Delayed Photo-activation Effects on Mechanical Properties of Dual Cured Resin Cements and Finite Element Analysis of Shrinkage Stresses in Teeth Restored With Ceramic Inlays. Oper Dent. 2016;41(5):491-500.

142. Faria-e-Silva AL, Peixoto AC, Borges MG, Menezes MdS, Moraes RRd. Immediate and delayed photoactivation of self-adhesive resin cements and retention of glass-fiber posts. Brazilian oral research. 2014;28(1):1-6.

143. Amaral M, Rippe MP, Bergoli CD, Monaco C, Valandro LF. Multi-step adhesive cementation versus one-step adhesive cementation: push-out bond strength between fiber post and root dentin before and after mechanical cycling. Gen Dent. 2011;59(5):e185-91.

144. Ferracane JL, Stansbury JW, Burke FJ. Self-adhesive resin cements - chemistry, properties and clinical considerations. J Oral Rehabil. 2011;38(4):295-314.

145. Monticelli F, Osorio R, Mazzitelli C, Ferrari M, Toledano M. Limited decalcification/diffusion of self-adhesive cements into dentin. J Dent Res. 2008;87(10):974-9.

146. Juloski J, Radovic I, Goracci C, Vulicevic ZR, Ferrari M. Ferrule effect: a literature review. J Endod. 2012;38(1):11-9.

147. Sarkis-Onofre R, Amaral Pinheiro H, Poletto-Neto V, Bergoli CD, Cenci MS, Pereira-Cenci T. Randomized controlled trial comparing glass fiber posts and cast metal posts. J Dent. 2020;96:103334.

148. Dietschi D, Duc O, Krejci I, Sadan A. Biomechanical considerations for the restoration of endodontically treated teeth: a systematic review of the literature, Part II (Evaluation of fatigue behavior, interfaces, and in vivo studies). Quintessence Int. 2008;39(2):117-29.

149. Goracci C, Ferrari M. Current perspectives on post systems: a literature review. Aust Dent J. 2011;56 Suppl 1:77-83.

150. Vichi A, Ferrari M, Davidson CL. Influence of ceramic and cement thickness on the masking of various types of opaque posts. J Prosthet Dent. 2000;83(4):412-7.

151. Schwartz RS, Robbins JW. Post placement and restoration of endodontically treated teeth: a literature review. J Endod. 2004;30(5):289-301.

152. Cagidiaco MC, Goracci C, Garcia-Godoy F, Ferrari M. Clinical studies of fiber posts: a literature review. Int J Prosthodont. 2008;21(4):328-36.

153. Monticelli F, Osorio R, Sadek FT, Radovic I, Toledano M, Ferrari M. Surface treatments for improving bond strength to prefabricated fiber posts: a literature review. Oper Dent. 2008;33(3):346-55.

154. Goracci C. Laboratory data and their clinical implications. Fiber posts and endodontically treated teeth: a compendium of scientific and clinical perspectives. 2008:95.

155. Mishra L, Khan AS, Velo M, Panda S, Zavattini A, Rizzante FAP, et al. Effects of Surface Treatments of Glass Fiber-Reinforced Post on Bond Strength to Root Dentine: A Systematic Review. Materials (Basel). 2020;13(8).

156. Majeti C, Veeramachaneni C, Morisetty PK, Rao SA, Tummala M. A simplified etching technique to improve the adhesion of fiber post. J Adv Prosthodont. 2014;6(4):295-301.

157. Mosharraf R, Ranjbarian P. Effects of post surface conditioning before silanization on bond strength between fiber post and resin cement. J Adv Prosthodont. 2013;5(2):126-32.

158. Stewardson DA. Non-metal post systems. Dent Update. 2001;28(7):326-32, 34, 36.

159. Sorrentino R, Di Mauro MI, Ferrari M, Leone R, Zarone F. Complications of endodontically treated teeth restored with fiber posts and single crowns or fixed dental prostheses-a systematic review. Clin Oral Investig. 2016;20(7):1449-57.

160. Reis N, Bergamini M, Silvestre T, Veitz-Keenan A. Are fibre posts associated with the occurrence of prosthetic complications? Evid Based Dent. 2018;19(2):62.

161. Durski M, Metz M, Crim G, Hass S, Mazur R, Vieira S. Effect of Chlorhexidine Treatment Prior to Fiber Post Cementation on Long-Term Resin Cement Bond Strength. Oper Dent. 2018;43(2):E72-E80.

162. Gomes Franca FM, Vaneli RC, Conti Cde M, Basting RT, do Amaral FL, Turssi CP. Effect of Chlorhexidine and Ethanol Application on Long-term Push-out Bond Strength of Fiber Posts to Dentin. J Contemp Dent Pract. 2015;16(7):547-53.

163. Cecchin D, Giacomin M, Farina AP, Bhering CL, Mesquita MF, Ferraz CC. Effect of chlorhexidine and ethanol on push-out bond strength of fiber posts under cyclic loading. J Adhes Dent. 2014;16(1):87-92.

164. Cecchin D, Farina AP, Giacomin M, Vidal Cde M, Carlini-Junior B, Ferraz CC. Influence of chlorhexidine application time on the bond strength between fiber posts and dentin. J Endod. 2014;40(12):2045-8.

165. Cecchin D, de Almeida JF, Gomes BP, Zaia AA, Ferraz CC. Influence of chlorhexidine and ethanol on the bond strength and durability of the adhesion of the fiber posts to root dentin using a total etching adhesive system. J Endod. 2011;37(9):1310-5.

166. Cecchin D, Pin LC, Farina AP, Souza M, Vidal Cde M, Bello YD, et al. Bond Strength between Fiber Posts and Root Dentin Treated with Natural Cross-linkers. J Endod. 2015;41(10):1667-71.

167. Shafiei F, Yousefipour B, Mohammadi-Bassir M. Effect of Carbodiimide on Bonding Durability of Adhesive-cemented Fiber Posts in Root Canals. Oper Dent. 2016;41(4):432-40.

168. Lopes FC, Roperto R, Akkus A, de Queiroz AM, Francisco de Oliveira H, Sousa-Neto MD. Effect of carbodiimide and chlorhexidine on the bond strength longevity of resin cement to root dentine after radiation therapy. Int Endod J. 2020;53(4):539-52.

169. Comba A, Scotti N, Mazzoni A, Maravic T, Ribeiro Cunha S, Michelotto Tempesta R, et al. Carbodiimide inactivation of matrix metalloproteinases in radicular dentine. J Dent. 2019;82:56-62.

170. Radovic I, Mazzitelli C, Chieffi N, Ferrari M. Evaluation of the adhesion of fiber posts cemented using different adhesive approaches. Eur J Oral Sci. 2008;116(6):557-63.

171. Mazzoni A, Marchesi G, Cadenaro M, Mazzotti G, Di Lenarda R, Ferrari M, et al. Push-out stress for fibre posts luted using different adhesive strategies. Eur J Oral Sci. 2009;117(4):447-53.

172. Zicari F, Van Meerbeek B, Scotti R, Naert I. Effect of ferrule and post placement on fracture resistance of endodontically treated teeth after fatigue loading. J Dent. 2013;41(3):207-15.

173. Maroulakos G, He J, Nagy WW. The Post-endodontic Adhesive Interface: Theoretical Perspectives and Potential Flaws. J Endod. 2018;44(3):363-71.

174. Manso AP, Carvalho RM. Dental Cements for Luting and Bonding Restorations: Self-Adhesive Resin Cements. Dent Clin North Am. 2017;61(4):821-34.

175. Mazzitelli C, Monticelli F, Toledano M, Ferrari M, Osorio R. Effect of thermal cycling on the bond strength of self-adhesive cements to fiber posts. Clin Oral Investig. 2012;16(3):909-15.

176. Umetsubo LS, Yui KCK, Borges AB, Barcellos DC, Gonçalves SEdP. Additional chemical polymerization of dual resin cements: reality or a goal to be achieved? Revista de Odontologia da UNESP. 2016;45(3):159-64.

177. Braga RR, Cesar PF, Gonzaga CC. Mechanical properties of resin cements with different activation modes. J Oral Rehabil. 2002;29(3):257-62.

178. Josic U, Maravic T, Mazzitelli C, Del Bianco F, Mazzoni A, Breschi L. The effect of chlorhexidine primer application on the clinical performance of composite restorations: a literature review. J Esthet Restor Dent. 2020.

179. Goracci C, Grandini S, Bossu M, Bertelli E, Ferrari M. Laboratory assessment of the retentive potential of adhesive posts: a review. J Dent. 2007;35(11):827-35.

180. Brichko J, Burrow MF, Parashos P. Design Variability of the Push-out Bond Test in Endodontic Research: A Systematic Review. J Endod. 2018;44(8):1237-45.

181. De-Deus G, Souza E, Versiani M. Methodological considerations on push-out tests in Endodontics. Int Endod J. 2015;48(5):501-3.

182. Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, et al. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci. 2004;112(4):353-61.

183. Bitter K, Paris S, Martus P, Schartner R, Kielbassa AM. A Confocal Laser Scanning Microscope investigation of different dental adhesives bonded to root canal dentine. Int Endod J. 2004;37(12):840-8.

184. Moraes RR, Boscato N, Jardim PS, Schneider LF. Dual and self-curing potential of self-adhesive resin cements as thin films. Oper Dent. 2011;36(6):635-42.

185. Mazzitelli C, Monticelli F, Osorio R, Casucci A, Toledano M, Ferrari M. Water sorption and solubility of different self-adhesive cements. Dental Materials. 2009;5(25):e37.

186. Pala K, Demirbuga S, Gümüs HÖ, Arslan S, Zorba YO. Effect of different bonding techniques on the bond strength of two different fiber posts. Journal of Restorative Dentistry. 2014;2(1):32.

187. Maravic T, Mancuso E, Comba A, Checchi V, Generali L, Mazzitelli C, et al. Dentin Cross-linking Effect of Carbodiimide After 5 Years. J Dent Res. 2021:220345211014799.

188. Serafino C, Gallina G, Cumbo E, Ferrari M. Surface debris of canal walls after post space preparation in endodontically treated teeth: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97(3):381-7.

189. Gu XH, Mao CY, Liang C, Wang HM, Kern M. Does endodontic post space irrigation affect smear layer removal and bonding effectiveness? Eur J Oral Sci. 2009;117(5):597-603.

190. Ferrari M, Mason PN, Goracci C, Pashley DH, Tay FR. Collagen degradation in endodontically treated teeth after clinical function. J Dent Res. 2004;83(5):414-9.

191. Scheffel DL, Delgado CC, Soares DG, Basso FG, de Souza Costa CA, Pashley DH, et al. Increased Durability of Resin-Dentin Bonds Following Cross-Linking Treatment. Oper Dent. 2015;40(5):533-9.

192. Alonso JRL, Basso FG, Scheffel DLS, de-Souza-Costa CA, Hebling J. Effect of crosslinkers on bond strength stability of fiber posts to root canal dentin and in situ proteolytic activity. J Prosthet Dent. 2018;119(3):494 e1- e9.

193. Vertucci FJ. Root canal morphology and its relationship to endodontic procedures. Endodontic topics. 2005;10(1):3-29.

194. Costa Scholz MF, Aboud Matos de Almeida R, Scholz N, Gomes GM, Masson PM, Loguercio AD, et al. The Effect of Viscosity and Application Mode of Phosphoric Acid on Bond Strength of GlassFiber Post. Clin Cosmet Investig Dent. 2020;12:61-70.

195. Machado FW, Bossardi M, Ramos Tdos S, Valente LL, Munchow EA, Piva E. Application of resin adhesive on the surface of a silanized glass fiber-reinforced post and its effect on the retention to root dentin. J Endod. 2015;41(1):106-10.

196. Rodrigues RV, Sampaio CS, Pacheco RR, Pascon FM, Puppin-Rontani RM, Giannini M. Influence of adhesive cementation systems on the bond strength of relined fiber posts to root dentin. J Prosthet Dent. 2017;118(4):493-9.

197. Mohsen CA. Evaluation of push-out bond strength of surface treatments of two esthetic posts. Indian J Dent Res. 2012;23(5):596-602.

198. Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho RM, Russell CM, et al. Durability of resin-dentin bonds. J Adhes Dent. 1999;1(3):211-8.

199. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res. 2003;82(2):136-40.

200. Breschi L, Maravic T, Comba A, Cunha SR, Loguercio AD, Reis A, et al. Chlorhexidine preserves the hybrid layer in vitro after 10-years aging. Dent Mater. 2020;36(5):672-80.

201. Fukushima T, Inoue Y, Miyazaki K, Itoh T. Effect of primers containing N-methylolacrylamide or N-methylolmethacrylamide on dentin bond durability of a resin composite after 5 years. J Dent. 2001;29(3):227-34.

202. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005;84(2):118-32.

203. Ferracane JL, Berge HX, Condon JR. In vitro aging of dental composites in water--effect of degree of conversion, filler volume, and filler/matrix coupling. J Biomed Mater Res. 1998;42(3):465-72.

204. Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med. 2001;12(2):136-51.

205. Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, et al. Micromorphological changes in resin-dentin bonds after 1 year of water storage. J Biomed Mater Res. 2002;63(3):306-11.

206. Frankenberger R, Roth S, Kramer N, Pelka M, Petschelt A. Effect of preparation mode on Class II resin composite repair. J Oral Rehabil. 2003;30(6):559-64.

207. Nikaido T, Kunzelmann KH, Chen H, Ogata M, Harada N, Yamaguchi S, et al. Evaluation of thermal cycling and mechanical loading on bond strength of a self-etching primer system to dentin. Dent Mater. 2002;18(3):269-75.

208. Armstrong S, Breschi L, Ozcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (muTBS) approach. Dent Mater. 2017;33(2):133-43.

209. Miyazaki M, Sato M, Onose H, Moore BK. Influence of thermal cycling on dentin bond strength of two-step bonding systems. Am J Dent. 1998;11(3):118-22.

210. Chai J, Takahashi Y, Hisama K, Shimizu H. Water sorption and dimensional stability of three glass fiber-reinforced composites. Int J Prosthodont. 2004;17(2):195-9.

211. Vichi A, Vano M, Ferrari M. The effect of different storage conditions and duration on the fracture strength of three types of translucent fiber posts. Dent Mater. 2008;24(6):832-8.

212. Goracci C, Fabianelli A, Sadek FT, Papacchini F, Tay FR, Ferrari M. The contribution of friction to the dislocation resistance of bonded fiber posts. J Endod. 2005;31(8):608-12.

213. Park JW, Ferracane JL. Water aging reverses residual stresses in hydrophilic dental composites. J Dent Res. 2014;93(2):195-200.

214. Barbizam JV, White SN. Fatigue susceptibility of an endodontic fibre post material. Int Endod J. 2014;47(2):202-9.

215. Morresi AL, D'Amario M, Capogreco M, Gatto R, Marzo G, D'Arcangelo C, et al. Thermal cycling for restorative materials: does a standardized protocol exist in laboratory testing? A literature review. Journal of the mechanical behavior of biomedical materials. 2014;29:295-308.

216. Yaman BC, Ozer F, Takeichi T, Karabucak B, Koray F, Blatz MB. Effect of thermomechanical aging on bond strength and interface morphology of glass fiber and zirconia posts bonded with a self-etch adhesive and a self-adhesive resin cement to natural teeth. J Prosthet Dent. 2014;112(3):455-64.

217. Dimitrouli M, Geurtsen W, Luhrs AK. Comparison of the push-out strength of two fiber post systems dependent on different types of resin cements. Clin Oral Investig. 2012;16(3):899-908.

218. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjaderhane L, et al. The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dent Mater. 2010;26(11):1059-67.

219. Ubaldini ALM, Benetti AR, Sato F, Pascotto RC, Medina Neto A, Baesso ML, et al. Challenges in luting fibre posts: Adhesion to the post and to the dentine. Dent Mater. 2018;34(7):1054-62.