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INFLUENCE OF APPLICATION MODE OF UNIVERSAL ADHESIVES ON BOND STRENGTH PERFORMANCES AND ENZYMATIC ACTIVITY OF CORONAL AND RADICULAR DENTIN

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Influence of application mode of universal adhesives on bond strength performances and enzymatic activity of coronal and radicular dentin

ABSTRACT

Objective:

The primary object of this thesis was to analize the application mode of the universal adhesives and to give clear instructions for reliable clinical procedures. Firstly, we analized the etching mode of universal adhesives on the bond strength to dentin and on the risk of retention, marginal discoloration, marginal adaptation and post-operative sensitivity (POS) by running two systematic reviews. After that we conduct three *in vitro* studies with three different aims: 1) to study the evaporation mode of a universal adhesive on coronal dentin; 2) to examine the cementation approach on radicular dentin (a very different substrate compared with the coronal one); and to evaluate the adhesion of metal brackets to enamel using resin-cements with or without an adhesive.

Materials and methods:

For the first step we have conducted a systematic review. Randomized controlled clinical trials (RCTs) in which resin composites and universal adhesives were used for restoration of non carious cervical lesions (NCCLs) were considered.

Secondary, we proceeded with a different systematic review. Only RCTs in which NCCLs were restored with composites and universal adhesives applied in selective enamel etch (SEE) or self-etch (SE) mode were included.

Further, three different *in vitro* studies were conducted. Regarding the study on evaporation mode, middle/deep dentin of 80 sound extracted human molars were bonded with/without the presence of a simulated pulpal pressure with a universal adhesive applied in total-etch (TE) or SE mode. Two

adhesive evaporation techniques were tested: air-drying or suction with a disposable device. Then, the adhesive was light-cured for 10s, and a 4-mm-thick composite build-up was made. The specimens were stored in artificial saliva at 37°C for 24h (T0) or 6 months (T6), after which they were cut into sticks and submitted to μTBS test. Fractographic analysis was performed using scanning electron microscopy (SEM). Additional teeth of the same groups (n=3) were prepared for *in situ* zymographic analysis. Bonded sticks were ground down and exposed to fluorescein-conjugated gelatin. Enzymatic activity was evaluated on the images obtained using a confocal microscope. Data were statistically analyzed ($p<0.05$)

Secondary, about the *in vitro* study of the cementation of a fiber into radicular dentin, 100 premolars were endodontically treated and assigned to 10 groups with different resin-cements (5 different type used in self-cure mode or in light-cure mode). Half of the teeth from each group were subjected to push-out bond strength (PBS) evaluation after 24h (T0), while other half was tested after 12 months (T12) of artificial saliva aging. Additional 4 teeth per group were prepared for nanoleakage (NL) expression evaluation. PBS values were analyzed using multivariate analysis of variance (ANOVA) and Tukey post hoc test. NL scores were analyzed using Chi-square tests ($\alpha=0.05$).

Finally, for the last study, orthodontic brackets were cemented on 40 freshly extracted human premolars according to 4 different adhesive protocols. Shear bond strength test (SBS) was conducted with an Instron Universal Testing machine. Afterwards, teeth were randomly allocated into subgroups to evaluate two adhesive removal techniques: SL-Sof-Lex on low-speed handpiece; TC-tungsten-carbide multi-laminated high-rotation drill. A 3D scan (3shape) of each model was taken at the beginning (T0), after the SBS (T1) and after cement removal (T2) to assess volumetric differences. To assess the color of buccal enamel surface, spectrophotometry (SpectroShade) was used at room temperature under natural light before the cementation of the bracket, after the bracket removal and after the removal of cement.

Results

After screening of the first systematics review at 12- and 18/24-months the risk for retention loss was higher for SE than for TE groups. No significant differences were observed for marginal discoloration and adaptation. The probability of POS occurrence was less in SE than in TE groups. Using universal adhesives in TE or SEE mode provides more predictable retention, while SE strategy reduces the risk of POS occurrence.

About the second systematic review, the SEE approach seems to performe better than SE.

Regarding the first *in vitro* study it reveals that air-drying resulted in significantly higher μTBS values than suction, regardless of the aging and the adhesive application mode. At T6, the SE groups maintained the same bond strength level as at baseline. SEM images showed the presence of sparse water-tree formations at the adhesive interface in the suction groups, irrespective of the application mode. Suction-evaporation, aging and ER mode increased MMPs activity.

About the fiber post adhesion to root dentin, statistical analysis revealed that variables “cement” and “aging” significantly influenced PBS, but not “polymerization” and “root region”. Differences in NL expression were present at T0, and in general the aging process produced an increase in marginal infiltration.

Lastly, brackets cemented with new universal resin-cement with previous etchant application demonstrated bond strength similar to those cemented with the gold standard resin-composite used with the traditional adhesive system. Both adhesive removal techniques successfully cleaned the tooth surface, but also removed a portion of the enamel, particularly SL ($p<0.05$). The changes in the tooth color were not affected significantly neither by the cementation mode with the materials tested nor with different cement removal techniques ($p>0.05$).

Conclusion:

It can be stated that SEE performed better than SE and TE when a universal adhesive is used in terms of uTBS.

Evaporating with air-drying is to be preferred to suction mode for the evaporation of the solvent of universal adhesive in terms of uTBS and enzymatic activity.

Aging and choice of resin cement for cementation of fiber posts influenced the PBS, while root region and polymerization protocol seemed to have no influence on posts' resistance to dislodgment.

Finally brackets cementation with TE with tradition adhesive system and the use of a new resin-cement seems to offer the highest bond strength and leaves more cement remnants after the bracket removal. Cleaning of the remaining cement from the enamel with TC burs seems to be as efficient and less aggressive in terms of healthy tooth tissue preservation compared to the SL.

Keynotes:

universal adhesive; evaporation; dentin; bond strength; bracket; adhesive system.

INTRODUCTION

Developments of bonding systems technologies have completely changed the traditional concepts of adhesive dentistry. Researchers and manufacturers have synergically worked together to simplify clinical procedures, limit operator sensitivity and reduce procedural time. Besides, substantial efforts have been made in the chemistry of adhesive systems to create performable bonding interfaces while maintaining the interfacial integrity stable over time.

Chronologically speaking, universal adhesives represent the latest simplified bonding system launched on the market, constituted by a mixture of hydrophilic and hydrophobic monomers, diluents, and photo-initiator components, all provided in a single bottle solution containing ethanol or acetone as solvents depending on the product. The "universality" of these bonding systems consists of the possibility to be applied in different modes, with/out previous phosphoric acid etching (etch-and-rinse, EAR, and self-etch, SE, mode, respectively) or with only enamel etching (according to the selective enamel etching technique, SEE).

Etching enamel with phosphoric acid has changed the course of restorative dentistry, introducing the modern concept of adhesive dentistry. In general, adhesion mechanism to enamel has remained consistently simple and reliable since the introduction of the acid-etch technique in 1955 by Michael Buonocore [1]. The formation of resin interdigitations into the enamel microporosities created by the dissolution of hydroxyapatite with phosphoric acid is still the crucial mechanism for mechanical bonding of resin-based adhesives to enamel. For dentin, a similar micro-mechanical interdigitating between these two interfaces has been theorized, but it is impeded by the intrinsic humid substrate that usually characterizes this tooth substrate. [2-5] This assumption was based on the abundant resin tags formed by dental adhesives into the dentinal tubules when dentin was previously etched with phosphoric acid (165). Then, the monomer interdiffusion has been demonstrated as the fundamental mechanism in achieving effective dentine bonding (5). Dentin hybridization through monomeric interdiffusion is the key to obtain an effective bond to the dentin tissue. However, the bond between the dental substrate and the restorative material is affected by the presence of residual water or excess solvent.

Several in vitro studies have analyzed the dentin-adhesive bond strength, but not all of them have considered the possibility to replicate the outward fluid flow that clinically occurs through the dentinal tubules due to positive pulpal pressure, estimated to be approximately 15 cm H_2O [9]. Since the accumulation of water at the adhesive interface was demonstrated to decrease bonding performances of adhesive systems, this could count for decreased longevity of the bonded restorations. For this reason, it is mandatory to test bond strength in presence or not of pulpal

pressure, and very few data exist on the bonding performances of the universal adhesives in presence of simulated pulpal pressure.

Within this complicated mechanism, the type of solvent has an important role in adhesive infiltration into the wet dentin substrate. In case of uncomplete solvent evaporation, its residues may interfere with the mechanical properties of resinous materials and consequently have a detrimental impact on the quality and durability of the bonding interface. In addition, uncomplete solvent evaporation may lead to increase in water accumulation within the resin structure and consequently interfere with its polymerization reaction. As a consequence, phenomena of structural plasticization and matrix swelling have been observed [10]. It has been shown that mixing the adhesive with the primer solution decreases the mechanical resistance of adhesive systems because the polymerization process is inhibited by compounds in the primer [8,11]. Among the different methods used for solvent evaporation, no clear indication exists on whether it is the best to be clinically adopted in order to refrain from the deleterious mechanisms previously indicated. Therefore, the evaluation of the effects of different solvent evaporation method is advisable.

Classification of adhesive systems

Traditionally, adhesive bonding systems consist of an acid, primer and adhesive. Acid is used for the removal of mineral crystals and exposure of the collagen fibrils. Primer is a hydrophilic solution of resinous monomers, which allows the infiltration of the resinous monomers, especially in demineralized dentin. The adhesive itself contains mixtures of monomers that penetrate the surfaces treated with the primer, creating a mechanical adhesion to dentin [12]. These components can be presented in separate bottles or together, being carried out in one, two or three clinical application steps.

Ultimately, the adhesive system can be classified according to:

-Generation;

-Solvent;

-Clinical application steps;

-Interaction with the substrate.

The first generation of adhesives, presented by Buonocore in 1956, used NPG-GMA as the main component. This type of adhesive was based on ionic bonds with hydroxyapatite and covalent bonds with collagen, by binding the calcium ions present on the dental surface. The clinical results of these adhesives were very poor with adhesion values hovering around 1-3 MPa [13].

The second generation of adhesives was introduced around the end of the 70s trying to improve the agents used in the first generation. Mainly, polymerizable phosphates such as bis-GMA were added to the resins to promote calcium adhesion of the mineralized tooth structure. This mechanism allowed the formation of ionic bonds which, however, dissolved very quickly in an aqueous environment causing debonding or microleakage (13). The smear layer, as in the first generation, was maintained and the adhesion values obtained were between 4-6 MPa [13].

The third generation introduced in the early 1980s brought with it a major change: acid etching of dentin to modify or partially remove the smear layer. The acid etching was the new milestone of modern adhesion possible thanks to Nakabayashi. By doing so, the dentinal tubules were free and allowed a better application of the primer that was made up mainly of monomers such as 4-META or HEMA [3, 13]. Nakabayashi demonstrated that acid etching of dentin did not cause inflammatory reactions or pulp necrosis [3]. The main disadvantage of this generation was the use of unfilled resins due to their scarcely effective penetration [13].

The fourth generation of adhesive systems has been introduced since the 1990s. It was the first to introduce complete removal of the smear layer and is still considered the gold standard of dentin bonding today. Three separate components are used, sequentially: etchant, primer and bonding. Enamel and dentin are etched simultaneously for 15-20 s, then rinsed and the tooth surface should be left moist to ensure wet bonding and avoid collagen fiber collapse. The formation of the hybrid layer by infiltration of the resins into the dentin surface is the basis of this generation of adhesives and ensures a high bond strength as well as a good dentin seal [13]. Their disadvantage is given by the complexity of using the different components contained individually in separate bottles, due to the multiple steps necessary for their application and by the success of the "wet bonding" which is difficult to reproduce in a standardized way, it is operator dependent.

The fifth generation aims to reduce the steps necessary for the application of fourth generation adhesives, simplifying and speeding up the clinician's work while maintaining the same values in terms of adhesion. In addition, the attempt to remove or at least reduce post-operative sensitivity [13]. Among these, we find the 2-step adhesive systems which consist in the application of etchant and subsequently of primer and bonding in a single solution. This formulation is more susceptible to degradation in water over time than fourth generation adhesives.

The sixth generation of adhesives are also known as "self-etch primers", as the etch step is eliminated. This adhesive system involves the use of an acid primer, followed by the application of the adhesive without rinse the tooth surface. In this way the smear layer is not removed, which is therefore part of the substrate and the "wet bonding" problem is also solved [13]. While the dentin bond values of this generation of adhesives are good, the bond to enamel is less effective. The reason is the use of an

acidic primer that does not have a sufficiently acidic pH. To overcome this problem, a selective pre-etching of the enamel is proposed using traditional orthophosphoric acid, paying attention not to etch the dentin as well, which would lead to "over-etching"and dentinal hypersensitivity problems [13].

With the seventh generation, researchers tried to further reduce the number of steps necessary for the application also to eliminate operator-dependent errors. 1-step formulations are proposed with etchant, primer, and bonding in a single bottle ("all-in-one bottle"). The main problem is represented by the different chemical nature of the various components and their possibility of remaining stable over time when joined together [13]. This generation of adhesives has lower immediate and over time adhesion values than any adhesive on the market today.

The eighth generation of adhesives has been introduced since 2010, with the inclusion of nanometric fillers. These particles with an average size of about 12 nm increase the penetration of the resinous monomers as well as the thickness of the hybrid layer, improving the mechanical properties of the adhesive system. The type of filler and the methodology with which it is incorporated influence the viscosity of the adhesive and the infiltration capacity of the monomer in the spaces between the collagen fibers [13].

Referring to the classification based on the type of solvent, the addition of a solvent within the composition of an adhesive is essential as it significantly improves the wettability of the adhesive itself with respect to the dental substrate [14].

Solvents can dissolve or disperse one or more substances. When this happens, the molecules or ions separate from each other and the space that remains between them is occupied by the solvent molecules (14). The energy required to break the bonds between the solute molecules is given by the formation of bonds between the solute particles and the solvent molecules (14).

The solubility of these molecules is mainly given by their polarity. Solvents can therefore be classified according to their polarity in [14]:

- polar protic: has a hydroxyl group that can form strong hydrogen bonds (for example: water and ethanol);
- dipolar aprotic: forms hydrogen bonds without the need for a hydroxyl group, but by exploiting a dipole moment. It typically has a ketone group (for example: acetone);
- apolar: it has a low dielectric constant and a low dipole moment.

The most used solvents for formulating adhesives are water, ethanol, and acetone. The use of these solvents compared to others is mainly due to their excellent biocompatibility and wide availability [14].

Another important characteristic to take into consideration when talking about solvents is the vapor tension or pressure, the tendency of a particular substance to pass from the condensed to the gaseous

phase. The possibility that the solvent does not evaporate optimally once the adhesive has been applied to the tooth surface could cause voids and therefore increase the permeability of the adhesive layer (14).

The aqueous solvent has a high dielectric constant, this allows the hydrogen bonds between the collagen fibers to be broken, guaranteeing their re-expansion. Furthermore, it allows the dissociation of weak acids to demineralize enamel and dentin and for this reason, it is often used as a solvent in self-etching systems. It keeps the tooth surface moist, but is less volatile than other solvents, it could risk interfering with adhesion by leaving the substrate too wet.

Regarding the acetone solvent, it has a high vapor pression, therefore it evaporates very quickly. This evaporation allows the residual water to be displaced from the tooth surface, and for this characteristic it is often used in etch-and-rinse systems (14). Precisely, because of this characteristic, drying must be delicate.

Finally, the alcoholic solvent, ethanol evaporates better than water, but worse than acetone, consequently the drying process will be intermediate compared to the other two solvents.

According to Van Meerbeek's classification [12], dental adhesives can be classified by mechanism of action into two categories: "etch-and-rinse"(E&R), "self-etch"(SE) or "etch-and-dry" adhesives. The differences lie essentially in the different approaches in removing the smear layer.

The etch and rinse system involves the use of a strong acid (usually 34-37% orthophosphoric acid) for the preparation of the dental substrates and a subsequent water rinsing to eliminate any acid residues. Indeed, water rinsing has the function of blocking acid demineralization and removing the smear layer [6].

Instead no separate acid etching is foresee in the Self-Etch System, as the tooth substrate conditioning takes place simultaneously with the impregnation by the primer and/or bonding and subsequently only one drying is done without rinsing the tooth surface. Consequently, the smear layer is not carried away, but remains as an integral part of the hybrid layer. Drying is a fundamental step here, because it evaporates the solvent and thus carrying away the water that is essential to allow the dissociation of the acid and therefore its action [6].

The simplified application of this methodology reduces the operator errors and allows lower post-operative sensitivity. However, it has the disadvantage of resulting in a lower adhesive effectiveness to the enamel by not using a strong acid.

Finally, dental adhesives can be classified by number of application steps (the most used classification) into: 3-step, 2-step, 1-step.

The 3-Step methodology is applicable only with etch-and-rinse systems, providing for the sequential application of etchant, primer and bonding. Currently, it is regarded as the gold standard for clinically

excellent results. It can be used on different substrates thanks to the possibility of association with coupling agents and/or silanes. The disadvantage is represented by a long time of application and use and by the possibility of post-operative sensitivity.
The 2-Step methodology can be used with both etch-and-rinse and self-etch systems.
In E&R, a first step of etching is performed and then primer and bonding are applied as a single formulation. This saves time, but the adhesion values are considerably lower than with the 3-step (29). The problem of post-operative sensitivity persists.
In the SE, on the other hand, we have the simultaneous application of etchant and primer: an acid primer is used which is subsequently dried without having to rinse it before applying the bonding. Although these are also less effective at the enamel level, they were proven to be very good in terms of bond strength to dentin and do not cause post-operative sensitivity. To overcome the problem concerning poor adhesion to the enamel, added a selective etching of this substrate is usually proposed.
In the 1-Step methodology, etchant, primer and bonding are contained in a single solution, thus allowing only one operating step, optimizing times to the maximum.

Universal adhesives

Universal adhesives are the answer of many manufacturers to the increasingly insistent demand from clinicians for simplified adhesive systems.
They are called "universal", or "multi-mode", due to the possibility of using them with one of the adhesive strategies chosen by the clinician (E&R, SE or SEE). In addition, they are very versatile adhesives capable of bonding dental structures and the different types of materials used for both direct and indirect restorations, such as resins, glass ceramics, zirconia and metals [7].
As self-etch adhesives, however, they have a poor ability to etch enamel, which is why many authors recommend selective etching. As far as dentin is concerned, on the other hand, it has been seen that they are more performant in terms of immediate bond strength used in etch-and-rinse mode for better penetration of the adhesive inside the dentinal tubules.
However, the etch-and-rinse method is less suitable than the self-etch method when the stability of the bond over time is taken into consideration. This could be attributed to the composition of these adhesives due to the presence of the 10-Methacryloyloxydecyl dihydrogen phosphate monomer (10-MDP) capable of establishing a chemical bond with the substrate [7]. The chemical interaction between MDP and hydroxyapatite leads to the surface dissolution of the tooth tissues, which is

followed by the adsorption of MDP on the dentin/enamel surface and the deposition of MDP-Ca salts with lower solubility than that of the salts produced from other functional monomers.
Another aspect to take into consideration is the fact that dentin is a moist substrate and to allow good compatibility with hydrophobic resins, in recent years, attempts have been made to use monomers that are as hydrophilic as possible. Precisely for this reason, universal adhesives (especially the more recent ones), also contain water in association with organic solvents such as ethanol and acetone, so that there is better penetration of the monomer into dentin [8].
Although these components are essential for the composition of such adhesives, they should be adequately removed and/or evaporated during clinical procedures to avoid an inhibition of monomer polymerization.
This evaporation can occur by rubbing the adhesive on the tooth surface, for example with the help of a brush, followed by air-drying [8]. Currently there is no clear indication regarding the correct evaporation time. However, it can be said that a prolonged evaporation time of the solvent leads to an increase in the adhesive bond [8], but finally it depends on the formulation of the specific universal adhesive.

Application of dental adhesives

As previously mentioned, on the basis of the application mode dental adhesive can be classified in “etch and rinse” and “self-etch”.
E&R adhesives are applied after complete phosphoric acid etching of dental substrate, the etching should be applied 15 seconds for dentin and 30 seconds for enamel and then rinse well for the same time of acid application. Afterwards it follows the application of the primer with a microbrush that prevent the collapse of demineralized collagen fibrils, promote the formation of the hybrid layer and that should be evaporated in order to allow the bonding to penetrate into the dentinal tubules. The passage of the evaporation is controversial because it doesn’t be too long, but at the same time not too short even. [12, 15, 16] Finally the application with a microbrush of the adhesive, that should be not too dense to allow a good adhesion in terms of longevity and marginal adaptation.
Self-etch adhesives instead foresee a selected enamel etching of 15 seconds, rinse well for the same time and then application of etching-primer with a microbrush for 20 seconds, then evaporation. In this adhesive in the first bottle there is a mild acid with the primer. Finally with different microbrush, application of the bonding, gently air-drying and then polymerization for 20 seconds.

In this different type of dental adhesive there is 10-MDP that chemically interacts with dentin during demineralization; this adhesives also contain hydrophilic components and water, for that reason they require extended drying. [17]
Finally, Universal adhesive foresee one single bottle in which primer and bonding are together. The application of the adhesive is preceded to a selective enamel etching with orthophosphoric acid for 15 seconds, then with a microbrush the application for 20 seconds and at last evaporation and polymerization for 20 seconds. It contains 10-MDP and may contains Silane, it reduces risk of over-dried or moist dentin, it interacts with different materials, it is a mixture of hydrophobic and hydrophilic components and for this reason it requires extended drying. [18]

Dentin hybridization

The application of the adhesive system (EAR, SE, SEE) on dentin surface results in the formation of "hybrid layer" (HL), a tissue that is composed of demineralized collagen fibrils reinforced by resin matrix. This resin-impregnation creates a transitional "hybrid" layer, that is neither resin nor tooth, but a hybrid of the two. The thin layer of resin-reinforced dentin locks the two dissimilar substances together on a molecular level, sealing the surface against leakage and imparting a high degree of acid resistance [19].
Generally, thicker hybrid layers are observed when using EAR adhesive systems when compared to SE systems, because of the action of a more acid etchant used in the first mode, that completely dissolves the smear layer. [20] But 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. [21-24] Neither EAR or SE adhesive systems can prevent the phenomenon of nanoleakage - the diffusion of small ions or molecules within the hybrid layer in the absence of gap formation. [25,26]
About adhesion to radicular dentin we must consider the structural features of the root canal. Ferrari et al. (2000) evaluated dentine morphology in root canals in terms of tubule orientation and density. Root dentin is a very different structure compared to coronal dentin, it contains accessory root canals, areas of resorption, embedded and free pulp stones, and varying amounts of irregular secondary dentine 155].
Adhesion to radicular dentin can be obtained by 3 different cementation strategies: 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. Hybrid layer thickness in root canal dentine has not been reported in the dental literature.

The hybrid layer would be more important for adhesion to apical dentine than resin tag formation because fewer tags are available for resin penetration in this area.[153] Kielbassa et al. in 2004 demonstrated in an in vitro study that complete infiltration of adhesive into root canal dentine was achieved after conditioning dentine with phosphoric acid. [153] Nevertheless, with the development of the innovative universal resin-cements for the luting of the fiber post inside the root canal dentine, as Josic et al. demonstrated in a recent study [154] good bond strength values developed also with multimode resin-cements without previous etching mode.

Degradation of the hybrid layer

Complete infiltration of monomers into the wet and demineralized dentin is not easily achieved, leaving incompletely infiltrated zones along the bottom of the HL containing denuded collagen fibrils [26-29] surrounded by rinse-water. This has been confirmed by immunohistochemical labeling of acid-etched, resin-infiltrated dentin, after staining with anti-type I collagen antibodies. This revealed a weak labeling of collagen fibrils at the top half of the HL, but an intense labeling of collagen fibrils in the deepest part of the HL [29,30]. These results suggest that resin penetrates the top half of the HL, but not the bottom half.

In the SE strategy, a separate acid-etching step is not required since the adhesive co-monomers simultaneously demineralize and infiltrate the dentinal substrate, decreasing the discrepancy between the depth of demineralization and the depth of resin infiltration, creating a more homogenous resin infiltration of demineralized collagen fibrils when compared to E&R systems [29-32]. The stability of the SE adhesive bonding technique depends on the effectiveness of the coupling between the collagen fibril substrate and the comonomers [29-33]. Some studies reported a reduced amount of porosities and more homogenous resin infiltration and a better protection of collagen fibrils in SE adhesives compared to the E&R technique [29,34]. However, the efficacy of SE bonding on enamel without separate acid etching is still questionable [29,35,36].

About the hybrid layer created by using UA, it has been confirmed that this new type of adhesives cannot infiltrate to the full depth of demineralized dentin created by phosphoric acid in the E&R strategy [37]. In contrast, the HL of UA with the SE technique seems to be shallower and more durable, since this adhesive contains functional monomers capable of chemically interacting with hydroxyapatite and maintaining the collagen fibrils protected over time [29,37-40].

The degradation of the HL remains an ongoing issue, fundamental to improve the longevity of the resin-dentin interface. Experimental strategies are developed by different research groups to extend the longevity of resin-dentin bonds: increasing the degree of conversion and esterase resistance of

hydrophilic adhesives; inhibitors of collagenolytic enzymes; use of collagen cross-linking agents; biomimetic remineralization of resin-dentin bonds; Calcium-chelation dry bonding; and finally also ethanol wet-bonding with hydrophobic resins [29,33].

MMPs Enzimatic activity

Dentin is a collagen-based mineralized tissue consisting of inorganic apatite crystallites embedded in an extracellular matrix (ECM). Type I collagen is the main component of the ECM compartment of dentin, representing up to 90% of the organic material, in addition, several proteins constitute approximately 10% of the matrix. The non-collagenous dentin proteins include proteoglycans, phospholipids, and enzymes. Among the dentin enzymes, matrix metalloproteinases (MMPs) have the roles in several physiological and pathological processes in dentin.

Metalloproteinases are a family of more than 20 proteolytic enzymes; a class of zinc and calcium dependent endopeptidases capable of degrading extracellular matrix proteins as well as coagulation factors, lipoproteins, growth factors, chemotactic and cell adhesion molecules [43].

They are usually secreted as pro-enzymes in an inactive form to be then activated by other proteinases (including active MMPs) or by chemical agents (including reactive oxygen species); even a low pH would seem to be able to activate them, interrupting the bridge between the Zn2+ ion and the cysteine residue [43].

Human dentin contains at least five types of MMPs including MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-20 [41,44-49, 156]. They are trapped within the mineralized dentin matrix during tooth development [45] and dentinal caries [48].

The majority of them are produced as latent zymogens (pro-MMPs). Disruption of MMP-collagen binding due to exposure to mild acids (pH1⁄42.3-5) [48] converts the pro-MMPs to active MMPs through splitting the low-molecular- weight peptides [41,50,51]. Over time, auto activation of further pro-MMPs may result in increasing the enzymatic activity [41, 42].

MMPs consist of a prodomain, a catalytic domain, as well as other domains governing factors such as substrate specificity, recognition, and interaction [52, 53]. They are usually expressed as inactive zymogens, and the prodomain must be dissociated from the catalytic one for its activation [53,54]. In nonactivated MMPs, the unpaired cysteine in the prodomain forms a bridge with the catalytic zinc (referred to as the “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 [53, 55].

MMPs can be kept inactive by specific endogenous molecules called “tissue inhibitors of metalloproteinases” (TIMP) [52], these molecules are involved in local control of MMP activities in tissues and consists of 4 members (TIMP1-4) that collectively inhibit MMP activities and restrict ECM breakdown [53, 56,57].

The balance between MMP and TIMP is responsible for the remodeling of the extracellular matrix of the tissues, an important feature for its development and repair.

The first evidence of collagenolytic activity in dentin was reported in the early 1980s both in carious and intact dentin [53]. More recently, MMPs were identified as being responsible for that activity [48,53].

Although the physiological roles of MMPs in dentin are not well understood, they have been suggested to participate in peritubular and tertiary dentin formation and in the release of dentinal growth factors [53, 54, 58, 59].

Since the evidence of bacterial input to the degradation of the organic matrix of carious dentin is lacking, it has more recently been thought to be mediated mainly by host-derived MMPs [43, 48, 54]

The MMPs present in dentin are produced by odontoblasts [56] during secretion of dentin matrix and are suggested to be involved in dentin formation. After mineralization of the collagen matrix, the inactive proforms of MMPs remain trapped within the calcified matrix [54], where they can be re-exposed and potentially activated during the dentin caries process. The acidic environment created by bacterial acids can facilitate the activation of endogenous MMPs. Low pH leads to the cleavage of prodomain and thus facilitates the functional activity of MMPs [48, 60].

Being activated by acidic agents, MMPs are activated during adhesive procedures; self-etching adhesive systems and universal adhesive have mild acidity, that provides a low pH environment that may activate latent endogenous matrix metalloproteinase (MMP) enzymes [41,42]. If the collagen matrix is not completely infiltrated by the resins, they can slowly degrade the collagen fibers of the hybrid layer, resulting in a reduction of longevity of the restoration. They are therefore involved not only in the processes of autolytic degeneration of the dental tissues but also in the degradation of the hybrid layer. Particularly two types of MMPs are involved in this process: MMP-2 and MMP-9.

Several non-specific synthetic inhibitors able to act on different MMPs have been used, currently the most used are: chlorhexidine, benzalkonium chloride and quaternary ammonium methacrylates [61,62].

As mentioned before, adhesion on radicular dentine can be achieved by the use of adhesive system and resin-cements or by the most up-to-date system of the self-adhesive resin-cement. With the use of separate adhesive system the hybrid layer is created, by the elimination of the smear layer and the

penetration of the bonding resin in the radicular tubules. Also in this substrate HL is subject to the degradation by the endogenous dentinal enzymes, such as matrix metalloproteinases (MMPs). 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.

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. Hydrolysis play a fundamental role in this process. (157) In an attempt to overcome this problem, contemporary adhesive systems contain a mixture of hydrophilic resin monomers, such as two-hydroxyethyl methacrylate (HEMA), 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. (158) Still, the mentioned hydrophilic resin monomers in adhesives formulations cause high water sorption by the resin systems and generate a HL which acts like a ruptured membrane after curing, which allows water to move across the bonded interface (159).

Consequently, resin-infiltrated collagen matrix is solubilized, the underlying insoluble collagen fibrils become exposed and enzyme, such as matrix metalloproteinases (MMPs), attact them. (29) 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. (160) 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. (161)

Solvent role in adhesive system

The addition of resin solvents to the composition of adhesives is fundamental since they need to bond to dental tissue. The wet nature of dentine only allows good wetting when a hydrophilic bonding is applied [26]. By adding hydrophilic monomers on one hand, and a solvent on the other hand, the wetting behavior of the adhesive is drastically improved [61]. The low viscosity of primers and/or adhesive resins is partly due to the dissolution of the monomers in a solvent and will improve its diffusion ability in the micro-retentive tooth surface. In E&Rs, the main function of the solvent, present within the primer of 3-step E&Rs, and within the combined primer-adhesive resin (‘one-bottle systems’) in 2-step E&Rs, is to promote good penetration of the monomers in the collagen network

of the demineralized dentin [62]. In case of bonding to air-dried dentin, the solvent should also be capable of reexpanding the collapsed network [63,64]. In self-etch adhesives (SEAs), the use of water as a solvent is indispensable to ensure ionization of the acidic monomers [65,66]. Solvents are substances that are capable of dissolving or dispersing one or more other substances [67]. When a solvent dissolves a solid or a liquid, the molecules (or ions) become separated from each other and the spaces in between become occupied by solvent molecules. The energy required to break the bonds between solute molecules is supplied by the formation of bonds between the solute particles and the solvent molecules: the old intermolecular forces are replaced by new ones. The solubility characteristics of molecules are determined chiefly by their polarity. Non-polar or weakly polar compounds dissolve in nonpolar or weakly polar solvents; highly polar compounds dissolve in highly polar solvents ('alike dissolves alike'). The polarity of solvents is determined by both the dipole moment and the dielectric constant [67]. Chemists have classified solvents into three categories according to their polarity: polar protic, dipolar aprotic and apolar solvents. Polar protic solvents consist of a hydroxyl-group that can form strong hydrogen bonds. Examples are water and ethanol. Polar aprotic solvents do not have the required hydroxyl-group to form hydrogen bonds but do have a large dipole moment. They usually also contain a keton group. Typical example is acetone. Apolar solvents have both a low dielectric constant and dipole moment. The polarity of a solvent is also important to predict the shelf life of adhesives, as apolar solvents will more easily pass-through traditional polyethylene packaging.

In adhesives, water, ethanol and acetone are the most commonly used solvents. Other polyvalent alcohol solvents have been evaluated but are not used commercially [68]. The use of these organic solvents in adhesives must be explained by their inexpensiveness, their wide availability, and their good biocompatibility. Most other typical solvents are toxic. MMA and HEMA, both small monomer compounds have also been described as diluents for other monomers and can therefore also be called solvents. Moreover, the hydroxyl-group of HEMA also provides in hydrogen bonds [69]. However, the H-bonding capacity of HEMA is limited. DENTSPLY added tert-butanol to a recent 2-step E&R, because of its similar vapor pressure as ethanol, but better stability towards chemical reaction with monomers. Most important characteristics of a solvent are its dipole moment, dielectric constant, boiling point, vapor pressure and H-bonding capacity. The vapor pressure of a solvent is important to ensure good evaporation of the solvent after application of the adhesive onto tooth tissue [70,71]. Air-drying after application also facilitates the removal of remaining solvent from the adhesive [72]. In addition, air-drying will decrease the thickness of the adhesive layer, which has been shown to promote further solvent removal [73]. Complete evaporation is however difficult to achieve and is hampered by the short clinical air-blowing time [45, 71]. Remaining solvent in the adhesive may

jeopardize polymerization due to dilution of the monomers and may result in voids and hence permeability of the adhesive layer [64, 74,75]. Instructions for air-blowing solvent-free adhesive resins of course do not envisage solvent evaporation but intend to render the adhesive layer uniform and even. The H-bonding capacity of a solvent has been shown to be important to re-expand the shrunken demineralized collagen network after dehydration [69, 76]. Solvents that have higher affinity to form H-bonds, will be able to break stabilizing H-bonds and other forces that keep the collagen in shrunken state [14].

Evaporation mode

Removal of excess of water and/or solvent from dentin as apply the adhesive system and keeping the dentin itself moist, remains a delicate point.

About organic solvents, they act as carriers of the monomers into the collagen interfibrillar spaces and as diluents to lower the resin viscosity. Additionally, these solvents enhance infiltration of resins into the microporosities created onto by the etchant or by the conditioner [14].

Solvent volatilization can facilitate the polymerization reaction because the distance among monomers is reduced, increasing the degree of conversion [77]. Ideally, solvents should be completely volatilized from the applied mixture prior to polymerization. The choice of solvents impacts the polymerization in several manners. Solvent type affects the diffusion of the polymer chains, the viscosity, the intermolecular termination rate, the primary chain length, the gel point conversion, among others [78]. The evaporation of solvents with compressed air is a technique-sensitive step difficult to accomplish using current clinical techniques. Some reports have suggested that solvents may take up to 20 min to almost evaporate completely [8,79,80]. Solvent can be evaporated also by suction, that means by the application of a negative pressure, by the so-called “suction drying”, a techninque less operator- dependent, and that may guarantee a reduction of collagen fibrils collapse. In 2008 Magne et al. analyzed this technique, about which the data in the literature are scarce or non-existent, and concluded that there are no difference between the two evaporation technique on the microtensile bond strength to dentin. (162)

Acetone has a higher vapor pressure than ethanol and water, which may reduce the time required for evaporation compared to ethanol [81].

As for ethanol solvent, with its evaporation the monomer concentration increases dramatically reducing the vapor pressure of the remaining ethanol [82]. This increase in monomer concentration prevents further solvent evaporation, resulting in residual ethanol being trapped inside the adhesive layer [81].

Changes in solvent concentration and solvent type affect the quality of cross-linking and polymer network [78]. Excess solvent in the cured adhesive may result in a porous structure at the adhesive/dentin interface [82,83]. This situation may be more relevant for the most hydrophilic simplified 1-step adhesives or universal ones, as the amount of residual solvent/water retention is correlated with the hydrophilicity of the adhesive solution [84].
It was shown that ethanol-based adhesives present tensile strength and modulus of elasticity decrease with an increase in ethanol content [36], which may explain the increased bond strength with extended evaporation times [8].

Pulpal pressure

There are various structural components and dentin properties that can directly influence adhesive bonding: biological and clinical factors including dentin permeability and pulpal pressure [85].
Dentin is a very permeable (due to the presence of dentinal tubules) and moist (due to the dentinal fluid contained within the tubules) tissue.
Several adhesive systems today, especially the universal ones, contain hydrophilic monomers and water for greater compatibility with dentin. Given this characteristic, however, over time they are much more susceptible to hydrolytic degradation with a consequent reduction in the bond strength [64].
Pulpal pressure increases the water supply to the hybrid layer via the dentinal fluid flowing within the tubules. Absorption of water at this level promotes degradation of the adhesive. This phenomenon is so influential that many studies describe attempts to plug the tubules to optimize the seal and adhesive bond [86].
It is fundamental to reproduce in vitro the simulated pulpal pressure, in order to have a similar in vivo condition.
There are three different technique in order to reproduce the pulpal pressure in an extracted tooth:

1) Hydrostatic intra-pulpal pressure. The pulpal tissue was removed with a tweezer taking care not to touch the walls of the pulpal chamber. This technique was validated by Feitosa et al [65] immediately after bonding and restorative procedures, two layers of nail polish were applied around the adhesive interface to avoid water penetration at this site. Each specimen was then fixed with wax inside the lid of a cylindrical container , from the dentin side, avoiding to touch the pulp chamber. Subsequently, the vessel was filled with distilled water to a height of 20 cm, the lid with the fixed samples closed and turned upside down. In this way the samples had a water column of 20 cm on them and the pressure inside the pulp

chamber was 20 hPa, according to the hydrostatic pressure equation: P = g x d x h; where P is hydrostatic pressure, g is gravity, d is liquid density and h liquid height.

2) Microsyringe method: crown segments, each with a minimal remaining dentin thickness of 0.7–0.8 mm, were obtained by first removing the roots at 1 mm beneath the cementoenamel junction (CEJ). The occlusal enamel of each crown segment was subsequently removed with a parallel cut at 1.5 mm above the CEJ to expose the dentine. The exposed dentine was polished with 180 grit silicon carbide papers to create a standard bonding substrate in deep dentin. Pulpal tissue was removed from the exposed pulp chamber without altering the predentin surface. A pincer-type caliper was used for measurement of the remaining dentin thickness (RDT) that was between 0.7 and 0.8 mm. Each tooth section was attached to a Plexiglas platform (2 cm × 2 cm × 0.5 cm) that was perforated by an 18 gauge stainless steel tube using cyanocrylate adhesive . Each specimen was connected to a hydraulic pressure device that delivered 20 cm water pressure during the measurement of the dentine permeability (P). [163]
3) Dynamic intra-pulpal pressure simulation (modified protocol of Pashley and Depew with the addiction of an infusion pump [164]): to obtain the crown segment, the occlusal enamel was removed and expose flat mid-coronal dentin and the roots were cut off 1 mm below the cemento-enamel junction. Two cuts were made parallel to each other and perpendicular to the long axis of each tooth, accessing the pulp chamber at the furcation level . The pulp tissue was carefully removed using tweezers, without touching the surrounding predentin walls. The pulp chamber was irrigated with 2.5% NaOCl for 30 seconds, followed by immersion in distilled water for 30 minutes to neutralize the effects of NaOCl .

   Each crown segment was attached to a plexiglass plate on the pulp side using a cyanoacrylate adhesive (Model Repair II Blue; Dentsply-Sankin, Tochigi, Japan) and the pulp chamber was penetrated by an 18-gauge stainless steel needle. A transparent capillary tube was connected to the stainless steel needle, and the other end of this tube was inserted into a pressure reservoir to maintain an airtight seal and then filled with distilled water. To generate an intra-pulpal pressure of 15 cmH2O, the level of distilled water in the container was adjusted to 15 cm above the flat dentin surface of each crown segment. The pump was set at 0.36 μL/min to simulate the outward fluid flow rate .

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 indirect restorations made of metal, whereas today esthetic restorations are usually cemented with resin-based cements, which provide adhesion to tooth tissues [65].

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) [87].

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 [88]. 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 [89]. 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 [89].

When dealing with cases of thick indirect restorations or luting of fiber posts where light transmission is relatively limited, dual-cure resin cements are the material of choice [88, 90, 91]. Like with the 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 [92,93]. Interestingly, even though they are meant to polymerize well in the absence of light, lower degree of conversion was seen when dual-cure cements were light-cured through thicker ceramics, and resin cement shade and light-exposure time also had an effect on the degree of conversion on this group of cements [94]. 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 [65]. Another in vitro study found superior results in terms of post-gel shrinkage when delaying photopolymerization for 5 minutes [95]. 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

with 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 [96].

Another classification of resin cements is based on the number of steps used during their application and their interaction with dentin [65]. 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 [97].

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 [98]. 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 [85, 98]. 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) [98]. 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 [98]. 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 [98]. 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 [99].

Is clinical behavior of composite restorations placed in non-carious cervical lesions influenced by the application mode of universal adhesives? A systematic review and meta-analysis.

Aim:

To answer the following PICOS question: "Is the risk of retention loss, marginal discoloration, marginal adaptation and postoperative sensitivity (POS) equal for etch-and-rinse (EAR) compared to self-etch (SE) or selective-enamel etch (SEE) mode when restoring non carious cervical lesions (NCCLs) with universal adhesives?".

Materials & Methods:

Study protocol and registration

This study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database under the number CRD42020184666. The reporting of this systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [100].

Eligibility criteria and search strategy

The PICOS [101] strategy that guided the choice of the inclusion criteria and the search strategy, is described herein:
Population (P) - adult patients with the need of NCCL restoration;
Intervention (I) – composite restoration placed using universal adhesive in EAR mode;
Comparison (C) - composite restoration placed using universal adhesive in SE or SEE mode;
Outcome (O) - clinical parameters used to evaluate direct composite restorations (retention, marginal adaptation/discoloration, POS) for different follow-up periods;
Study design (S) – randomized controlled clinical trials.
A comprehensive literature search was performed with no language restriction through several international and national databases. To identify relevant RCTs investigating the clinical behavior of NCCL composite restorations placed using universal adhesives in EAR, SE, or SEE modes, Clarivate

Analytics' Web of Science (including Web of Science Core Collection—WoS, Korean Journal Database — KJD, Russian Science Citation Index — RSCI, SciELO Citation Index — SciELO) [1980-2021], Scopus [1960-2021], PubMed [1964-2021], Cochrane Central Register of Controlled Trials (CENTRAL) [1996-2021], and Latin American & Caribbean Health Sciences Literature (LILACS) through the Virtual Health Library (VHL) portal [1982-2021], were explored up to January 11, 2021. Preliminary searches of mentioned key sources were conducted to identify potential previously published systematic reviews and relevant RCTs in the field, as well as terms and synonyms related to the main concepts of interest (non-carious cervical lesions and universal adhesives). Test searches were also used to develop and evaluate various information retrieval strategies, maximize sensitivity, and obtain the most optimal search structure. Various combinations of previously identified free keywords, relevant controlled vocabulary terms (Medical Subject Headings — MeSH descriptors, https://www.ncbi.nlm.nih.gov/mesh), Boolean, truncation, and proximity operators were used, depending on the database being searched. Details on the number of identified articles and complete representation of applied strategies for all searched databases, including the search terms employed, are given in Supplementary Table 1. Furthermore, complementary searches through OpenGrey, Google Scholar™ (first 100 returns), and other available digital repositories (e.g., Networked Digital Library of Theses and Dissertations, Open Access Theses and Dissertations, DART-Europe E-theses Portal – DEEP, Opening access to UK theses – EThOS) were performed to identify unpublished manuscripts, research reports, conference papers, doctoral dissertations, and other grey literature. Finally, reference lists of included studies and relevant reviews were also examined to assure the reliability of obtained data and inclusion of relevant studies that may not have been identified through database and grey literature searches. Additional search during the final drafting of the paper performed up to July 12, 2021, indicated no new relevant studies had been published after completion of the literature search.

The exclusion criteria were as follows: (1) In vitro or ex vivo studies; (2) reviews (narrative or systematic); (3) case reports; (4) conference abstracts; (5) studies that did not involve at least two groups of direct restorations within the same patient comparing EAR with SE or SEE mode; (6) studies that compared outcomes between vital and non-vital teeth; (7) studies on primary dentition; (8) experiments carried out on animal subjects; (9) materials other than resin composite used as restorative material; (10) cavities other than NCCLs. No minimum follow-up period threshold was established for this systematic review and meta-analysis, since POS, which is very likely to occur in the first hours or days after the restorative procedure, was one of the main outcomes of interest.

Supplementary Table 1. Electronic Databases and Search Strategy

Database (n)	Search strategy #1 AND #2	
WoS (n=80) KJD (n=0) RSCI (n=0) SCIELO (n=2)	#1	TOPIC: ((“non-carious cervical” OR “noncarious cervical” OR “Class V” OR “Class 5”) NEAR/0 lesion$) OR NCCL$ OR ((cervical OR tooth) AND (erosion OR abrasion)) OR abfraction (n=7,670)
	#2	TOPIC: (((universal OR multimod* OR multi-mod*) NEAR/2 adhesive$) OR “one-bottle” OR “same bottle” OR “all-in-one”) AND (self-etch OR etch-and-rinse OR total-etch OR (selective AND enamel AND etch*)) (n=743)
Scopus (n=57)	#1	TITLE-ABS-KEY ((“non-carious cervical” OR “noncarious cervical” OR “Class V” OR “Class 5”) W/0 lesion) OR NCCL OR ((cervical OR tooth) AND (erosion OR abrasion)) OR abfraction (n=11,641)
	#2	TITLE-ABS-KEY (((universal OR multimod* OR multi-mod*) W/2 adhesive) OR “one-bottle” OR “same bottle” OR “all-in-one”) AND (self-etch OR etch-and-rinse OR total-etch OR (selective AND enamel AND etch*)) (n=574)
PubMed (n=53)	#1	(("non-carious cervical"[Title/Abstract] OR "noncarious cervical"[Title/Abstract] OR "Class V"[Title/Abstract] OR "Class 5"[Title/Abstract]) AND "lesion*"[Title/Abstract]) OR "NCCL"[Title/Abstract] OR (("cervical"[Title/Abstract] OR "tooth"[Title/Abstract]) AND ("erosion"[Title/Abstract] OR "abrasion"[Title/Abstract])) OR "Tooth Erosion"[Mesh] OR "Tooth Abrasion"[Mesh] OR "abfraction"[Title/Abstract] (n= 7,309)
	#2	((("universal"[Title/Abstract] OR "multimodal"[Title/Abstract] OR "multimode"[Title/Abstract] OR "multi-modal"[Title/Abstract] OR "multi-mode"[Title/Abstract]) AND "adhesive*"[Title/Abstract]) OR "one-bottle"[Title/Abstract] OR "same bottle"[Title/Abstract] OR "all-in-one"[Title/Abstract]) AND ("self-etch"[Title/Abstract] OR "etch-and-rinse"[Title/Abstract] OR "total-etch"[Title/Abstract] OR ("selective"[Title/Abstract] AND "enamel"[Title/Abstract] AND "etch*"[Title/Abstract])) (n=801)

CENTRAL (n=10)	#1	((“non-carious cervical” OR “noncarious cervical” OR “Class V” OR “Class 5”) NEAR/0 lesion?) OR NCCL? OR ((cervical OR tooth) AND (erosion OR abrasion)) OR abfraction OR [mh “Tooth Erosion”] OR [mh “Tooth Abrasion”] (n= 848)
	#2	(((universal OR multimod* OR multi-mod*) NEAR/2 adhesive?) OR “one-bottle” OR “same bottle” OR “all-in-one”) AND (“self-etch” OR “etch-and-rinse” OR “total-etch” OR (selective AND enamel AND etch*)) (n=24)
LILACS (n=231)	#1	TITLE-ABS-SUBJECT ((((“non-carious cervical" OR "lesiones cervicales no cariosas" OR "lesões cervicais não cariosas" OR "noncarious cervical" OR "Class V" OR "clase V" OR "classe V" OR "Class 5" OR "clase 5" OR "classe 5") AND (lesion$ OR cavidade$)) OR NCCL OR ((cervical OR tooth OR dental OR dentaria OR dente) AND (erosion OR Erosão OR Erosión OR abrasion OR Abrasão OR Abrasión)) OR mh:’Tooth Erosion’ OR mh:C07.793.818.500 OR mh:’Tooth Abrasion’ OR mh:C07.793.818.124 OR (abfraction OR abfração OR abfracción))) (n=50,723)
	#2	TITLE-ABS-SUBJECT ((((universal OR multimod$ OR multi-mod$) AND (adhesiv$ OR adesivo$)) OR one-bottle OR same bottle OR (adesivo$ de frasco único) OR all-in-one OR (adesivo$ de passo único) OR (adhesivo$ de paso unico)) AND (self-etch OR autocondicionante$ OR autograbado$ OR etch-and-rinse OR total-etch OR (condicionamento ácido total) OR (adhesivo$ de grabado total) OR (selective AND enamel AND etch$))) (n=2,070)

n - number of hits; WoS - Web of Science Core Collection; KJD - Korean Journal Database; RSCI - Russian Science Citation Index; SCIELO - SciELO Citation Index; CENTRAL - Cochrane Central Register of Controlled Trials; LILACS - Latin American & Caribbean Health Sciences Literature; TOPIC - Article title, abstract and keywords

Study selection and data extraction

All literature search results were imported into the Rayyan QCRI environment [76] for duplicate removal and further analysis. In this systematic review, the study selection process was performed in two stages. To select studies eligible for inclusion, two independent investigators (U.J. and F.D.B.) completed the initial screening of titles and abstracts. Articles that did not meet the

eligibility criteria were excluded and full texts of initially selected studies were retrieved for further evaluation. In the second stage, three investigators (U.J., C.M. and T.M.) independently assessed full texts of studies identified as possibly being relevant in the initial screening stage. All disagreements were resolved by consensus or discussion with a senior investigator (L.B.).

Data extraction was performed by three independent investigators (U.J., C.M. and T.M.) using customized extraction forms in MS Word. We extracted details of the study (author, year, location, and study design), participants (number and age range), direct restoration (number, type, and material used for indirect restorations, and type of teeth restored), adhesive strategy (type of adhesive system used during restorative procedures, number of restorations placed with EAR, SE or SEE approach), methodology (evaluation criteria, follow-up periods), and results (success and failure rates, as well as statistical analyses). If essential data were not reported in a certain study, the corresponding author of that paper was contacted by e-mail in an attempt to retrieve the necessary information.

When more than one universal adhesive was used in a trial, the data were combined and assigned to the adhesive strategy investigated in the study. Since an earlier systematic review [102] found that the isolation method (rubber dam or cotton rolls) and enamel bevel [103] did not influence retention and marginal discoloration, we collected data from all the studies, regardless of these two variables. However, since roughening of dentin can lead to improved retention [102], the data from the studies which had groups with roughened dentin was not considered suitable to be included in the meta-analysis. Similarly, the data from the groups that used nanoparticle-doped universal adhesives, as well as studies in which more than one layer of adhesive was applied during adhesive procedure and where dentin was pretreated with a primer (i.e. cross-linking agents), were not included in quantitative synthesis. Since the study results were reported in several periods of follow-ups, the data for 18/24 months was pooled in order to obtain sufficient data to run the meta-analysis. Lastly, when multiple publications with different follow-up periods were detected, the data from the latest publication were taken into consideration for performing the meta-analysis.

Risk of bias assessment

Two independent reviewers (I.R. and U.J.) performed the risk of bias assessment of the trials using the Cochrane Collaboration's tool for assessing risk of bias in RCTs. [104] Six domains of bias were evaluated: selection bias - random sequence generation and allocation concealment; performance bias - blinding of participants and personnel; detection bias - blinding of outcome assessment; attrition bias - incomplete outcome data; reporting bias - selective outcome reporting;

other possible sources of bias. In case of disagreements between the reviewers, a consensus was reached through discussion, and if needed, by consulting a third reviewer (A.M.).

At the study level, the study was at “low” risk of bias if the two domains considered most relevant for clinical studies in dentistry (selection and detection bias) were at “low” risk of bias. If one or more key domains were judged as at “unclear” risk, the study was considered at “unclear” risk of bias. Finally, if at least one domain was judged at “high” risk of bias, the study was considered at “high” risk of bias.

Meta-analysis

The extracted data were analyzed using Revman (Review Manager 5.4, The Cochrane Collaboration, Copenhagen, Denmark). Data for all outcomes (retention, marginal discoloration, marginal adaptation, POS) of the eligible studies were dichotomous. To summarize the risk of the mentioned outcomes for each study, the relative risk with a 95% confidence interval (CI) was calculated. Random-effects models were applied, and heterogeneity was tested using the I^2 index.

Certainty of evidence assessment

The overall quality of clinical evidence (certainty in the estimates of effect) for each of the outcomes was critically assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework [105], evaluating individual risk for bias, inconsistency, indirectness, imprecision, and publication bias. Based on these indicators, the certainty of the estimated effect was rated as high quality of evidence (the true effect lies close to that of the effect estimate), moderate quality of evidence (the true effect is likely to be close to the effect estimate, but there is a possibility that it is substantially different), low quality of evidence (the true effect may be substantially different from the effect estimate), and very low quality of evidence (the true effect is likely to be substantially different from the effect estimate) [106]. The quality assessment was

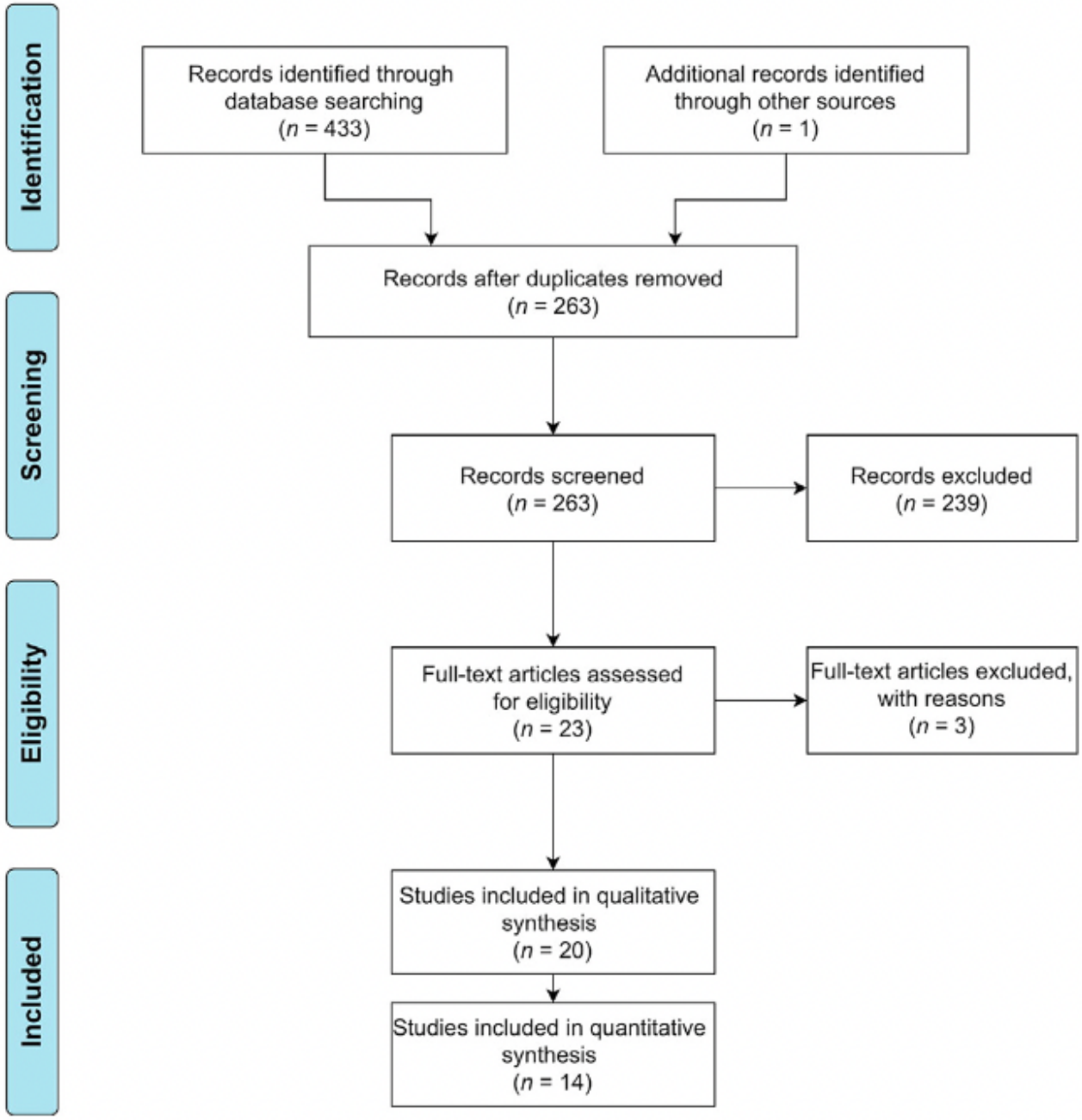
conducted by two independent investigators (U.J. and A.M.) and any disagreements were resolved through discussion.

Results:

Study selection

Figure 1 shows a PRISMA flow diagram of the study selection process based on the presented eligibility criteria. The initial search of the chosen databases and other relevant sources retrieved 434 references for potential inclusion in this systematic review. In the next step, 171 duplicates were identified and removed from the database. Following the initial screening of titles and abstracts, 240 records did not satisfy the inclusion criteria and were therefore excluded, while 23 studies were eligible for full-text assessment. In total, 3 studies were excluded after the full-text examination due to the missing EAR group [107, 108] or data reported only in percentages, and no answer was

obtained after writing to the authors for additional information [70]. Finally, 20 RCTs were included in this systematic review.



Descriptive analysis of the selected studies

Detailed information about 20 articles selected for this review is shown in Supplementary Table 2. All studies that were included were conducted as RCTs with split-mouth design in University settings, with majority of them carried out in Brazil [67, 109-119], followed by Turkey [120, 121], USA [61], Spain [62], Germany [63] and Portugal [64]. The studies were published between 2013 and 2020 and included a total number of 1.890 NCCL restorations placed in both anterior and

posterior teeth of 527 patients older than 18 years. The follow-up periods included 1-week, 6-, 12-, 18-, 24- to 36-months for most of the studies, and only one study [109] evaluated the restorations after 5 years of clinical service.

Before placing composite restorations, prophylaxis was performed on NCCLs with pumice and water, whereas in only two studies a cervical bevel was created [61]. Several studies [67, 109,110, 114, 115] reported using rubber dam during restorative procedures, while no information on NCCL pretreatment or rubber dam placement was available in one study [121]. A universal adhesive was modified by adding Cu nanoparticles in one study [110] and in 3 publications two different brands of universal adhesives that were used for restoration of the lesions were compared [116-118]. The clinical outcomes were assessed using either the FDI World Dental Federation (FDI) or modified United Stated Public Health Service (USPHS) criteria. Interestingly, POS, which was one of the main outcomes analyzed in this review, was assessed in two ways: by applying a stimulus in dental office [61, 63, 66, 68, 109, 110, 111, 113, 115, 120] or via questionnaire (asking the patient if he/she experienced any pain within the week following the restorative procedure) [110, 113]. One study [62] employed both methods in assessing POS, whereas two studies did not asses POS [117, 121]. In 2 studies the method of POS evaluation was not reported, and after writing to the authors it was not possible to obtain this information [68, 119].

Risk of bias of the included studies

Figure 2 summarizes the risk of bias judgment for each of the included studies. Overall, the reviewed studies had no major problems regarding the study design and reporting of results. The raised concerns were related to: selection bias – not clearly stated if the allocation concealment was kept hidden until the moment of restorative procedure [61, 63, 66, 111, 119]; performance bias – not reported if the participants were blinded [69, 116, 121]; detection bias – not mentioned if the evaluators were blinded [62, 117, 121]; attrition bias – patient drop out led to the loss of follow up greater than 20% [62, 117, 118]. Consequently, eight studies [111, 117-119, 61-63, 66] were

considered to be at “unclear” risk of bias, while the remaining twelve were judged as “low” risk of bias.

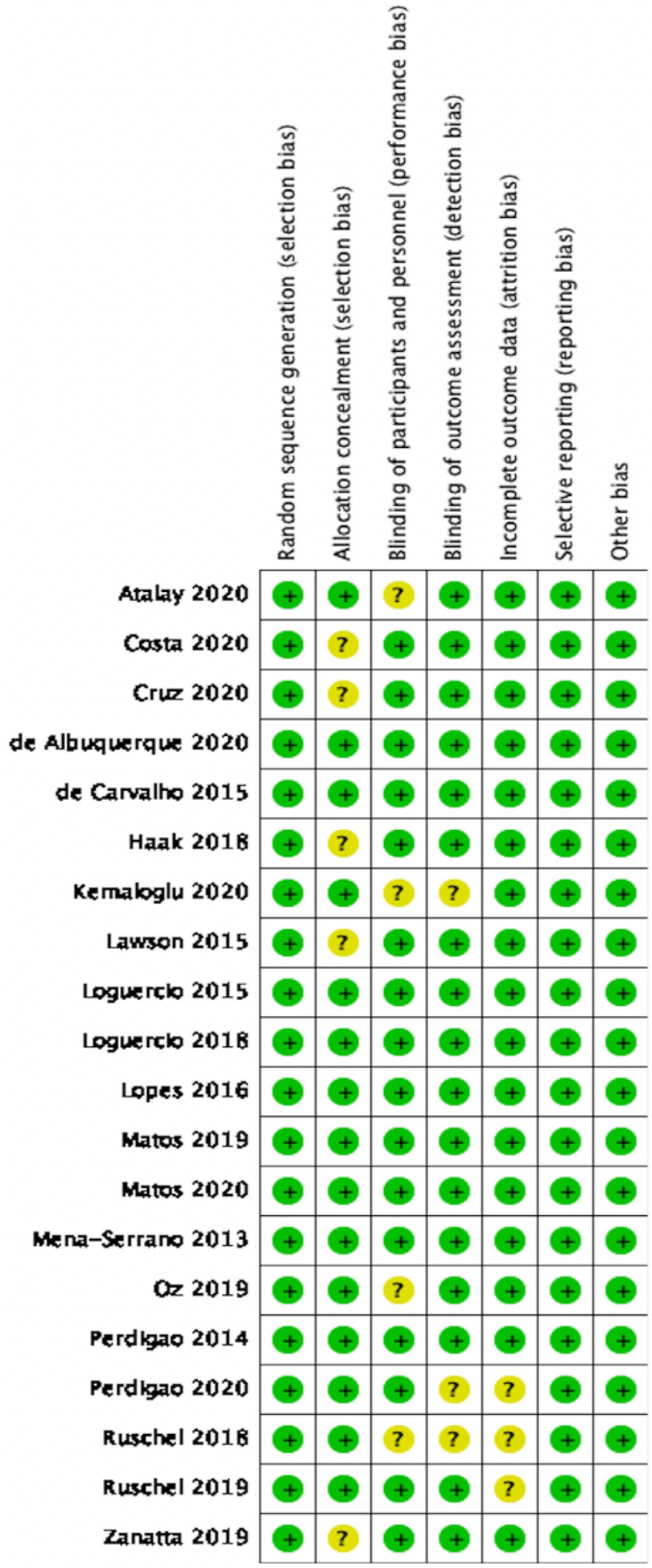


Fig. 2 – Risk of bias of the included studies.

Quantitative synthesis: meta-analyses

Based on data extraction, 14 studies [61, 63, 66, 67, 109, 110, 111, 112, 114, 115, 117, 119] were suitable for the inclusion in the meta-analyses for the outcomes of interest. The data from some studies [70] could not be used for meta-analysis since the authors reported their results in percentages, and we received no response after contacting the corresponding author.

Loss of retention

The forest plots of meta-analyses for loss of retention at different follow-up periods for EAR and SE mode are shown in Figures 3 - 6. No significant differences between the groups were observed at 6- and 36- months (p=0.36; p=0.14, respectively) recall (Figures 3 and 6). However, there was a statistically significant difference for 12- (p=0.005; RR=0.22, 95% CI [0.08, 0.63]) and 18/24- (p= 0.0002; RR=0.32, 95% CI [0.17, 0.58]) months follow-up between the two groups, favoring the EAR groups (Figures 4 and 5). Data from 12- and 18/24-months follow up were not heterogeneous (I^2=0%), while the data from 6- (chi^2 test; p=0.02; I^2=66%) and 36-months (chi^2 test; p=0.13, I^2=56%) follow-up showed substantial heterogeneity.

Figures 7 - 10 illustrate the forest-plots for meta-analyses for loss of retention at different follow-up periods for EAR and SEE mode. No statistically significant difference was observed at 6-, 12-, 18/24- and 36- months follow-up (p=0.97; p=0.15; p=0.49; p=0.99, respectively). The data for 6- (chi^2 test; p=0.68, I^2=0%), 12- (chi^2 test; p=0.56, I^2=0%), 18/24- (chi^2 test; p=0.44, I^2=0%) and 36-months (chi^2 test; p=0.98, I^2=0%) follow-up were not heterogeneous.

Marginal discoloration

Forest plots of the meta-analyses for risk of marginal discoloration for EAR and SE groups are presented in Figures 11 - 13. No statistically significant differences were seen at 6-, 12- and 18/24-months follow-up period (p=0.40; p=0.34; p=0.73, respectively). The data for 6- and 18/24- months

follow up showed no heterogeneity, while substantial heterogeneity was observed at 12- months (chi^2 test; p=0.07, I^2=70%).

No events were observed when comparing EAR with SEE adhesive strategy and therefore the meta-analyses could not be performed.

Marginal adaptation

Forest plots of the meta-analyses for marginal adaptation for EAR and SE groups are seen in Figures 14 - 16. No statistically significant differences were seen at 6-, 12- and 18/24-months follow up periods (p=0.88; p=0.21; p=0.34, respectively). The data for 6- (chi^2 test; p=0.59, I^2=0%), 12- (chi^2 test; p=0.83, I^2=0%), 18/24- months (chi^2 test; p=0.43, I^2=0%) were not heterogeneous.

Similar to marginal discoloration, no events were observed when comparing EAR to SEE strategy.

POS

Three meta-analyses were performed for POS, taking into account the method of the assessment and the adhesive strategy for this clinical outcome. Figure 17 demonstrates the forest plot for the risk of POS for EAR and SE modes, analyzed Based on the data derived from questionnaires (subjective POS) which was given to patients one week within the restorative procedure, no significant difference was seen for subjective POS (p=0.55, Figure 17). The second meta-analysis (Figure 18), which included studies that assessed POS by applying stimuli during recall (objective

POS) after one week of the restorative procedure demonstrated significantly increased likelihood for POS occurring in the EAR groups (p=0.007, RR=2.12, 95% CI [1.23, 3.64]).

Lastly, no significant difference was observed (p=0.80) when comparing EAR to SEE groups in terms of stimulated POS (Figure 19).

Certainty of evidence assessment

The certainty of evidence for each of the outcomes evaluated in our meta-analyses was assessed by the GRADE tool [105].

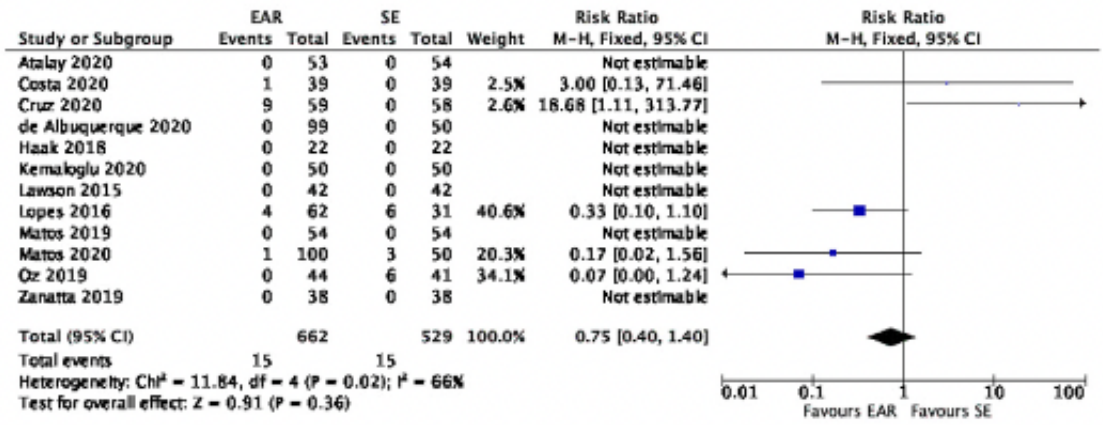


Fig 3. Forest plot for retention at 6-months follow-up (E&R vs SE)

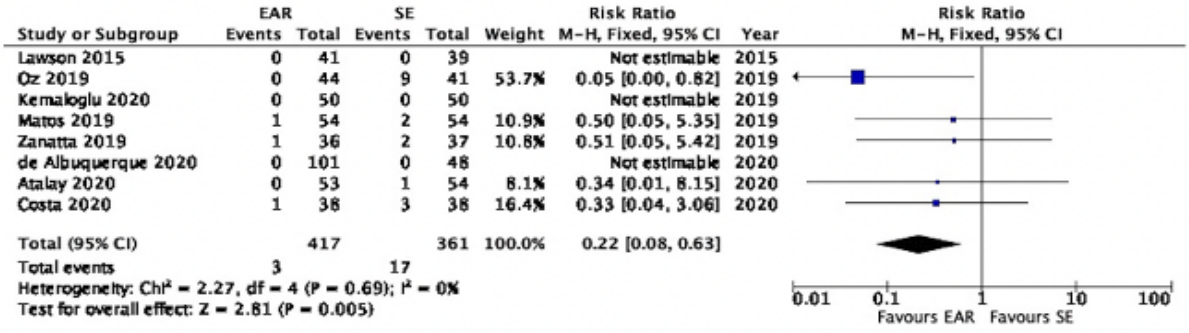


Fig 4. Forest plot for retention at 12-months follow-up (E&R vs SE)

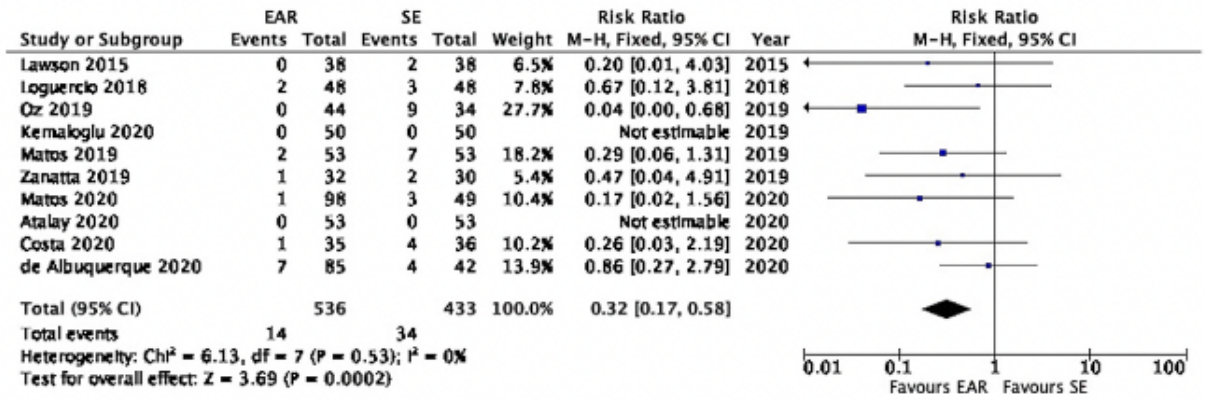


Fig 5. Forest plot for retention at 18/24-months follow-up (E&R vs SE)

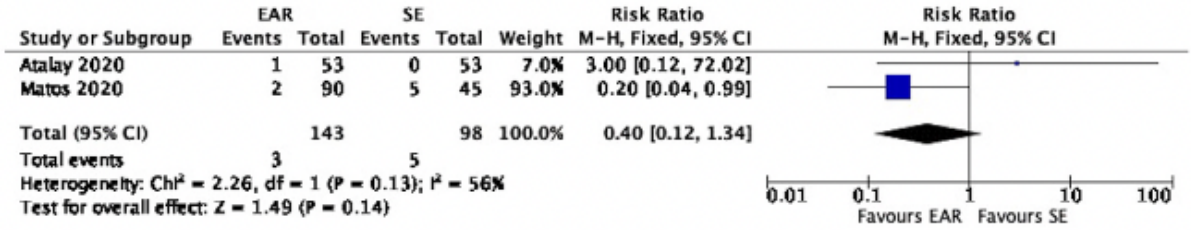


Fig 6. Forest plot for retention at 36-months follow-up (E&R vs SE)

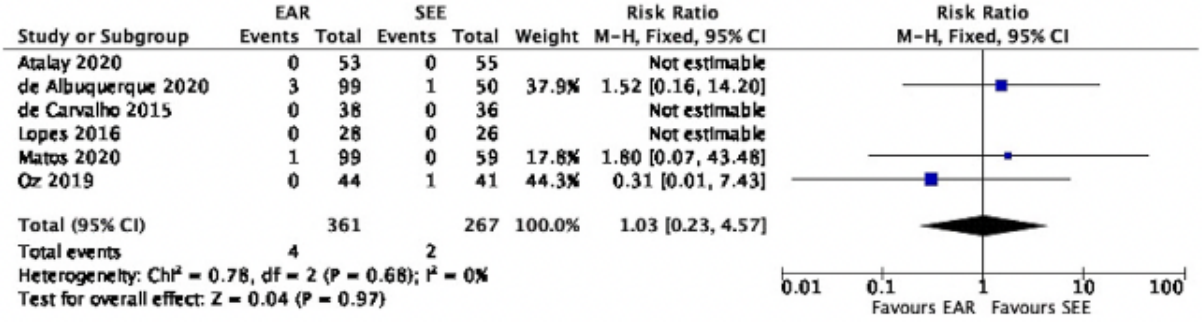


Fig 7. Forest plot for retention at 6-months follow-up (E&R vs SEE)

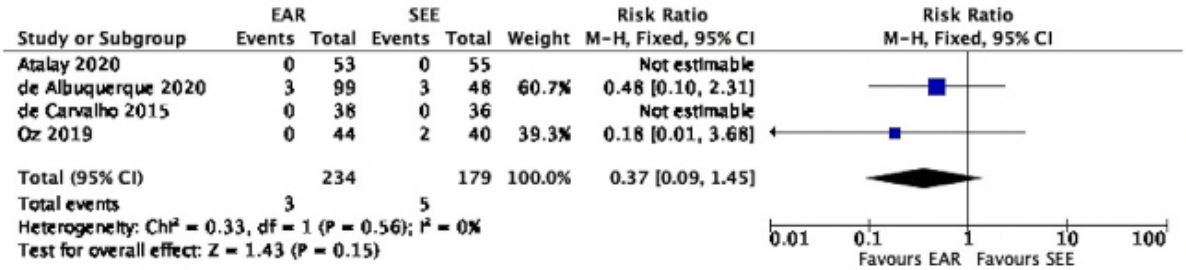


Fig 8. Forest plot for retention at 12-months follow-up (E&R vs SEE)

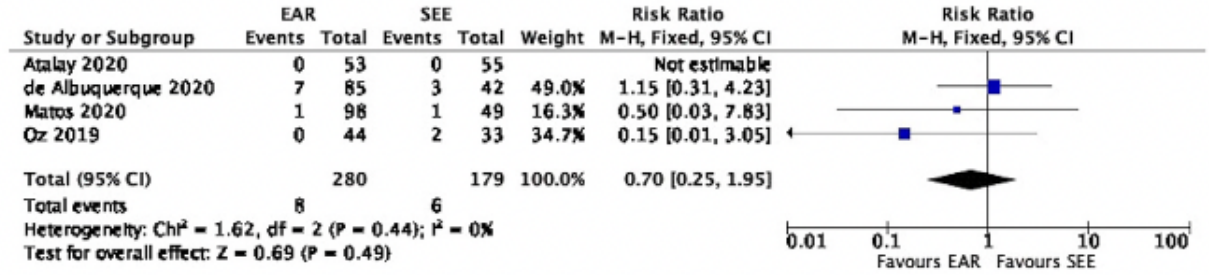


Fig 9. Forest plot for retention at 18/24-months follow-up (E&R vs SEE)

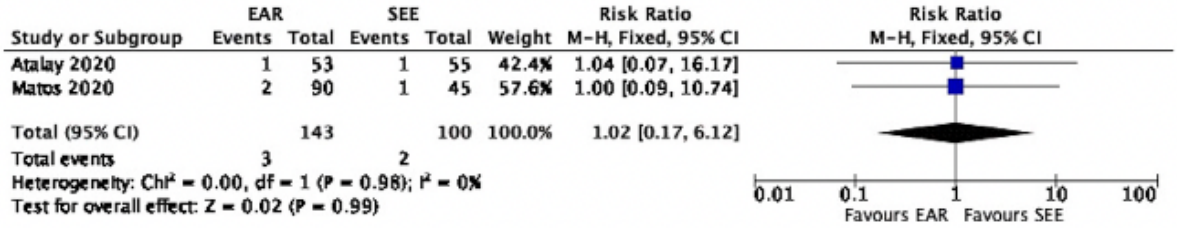


Fig 10. Forest plot for retention at 36-months follow-up (E&R vs SEE)

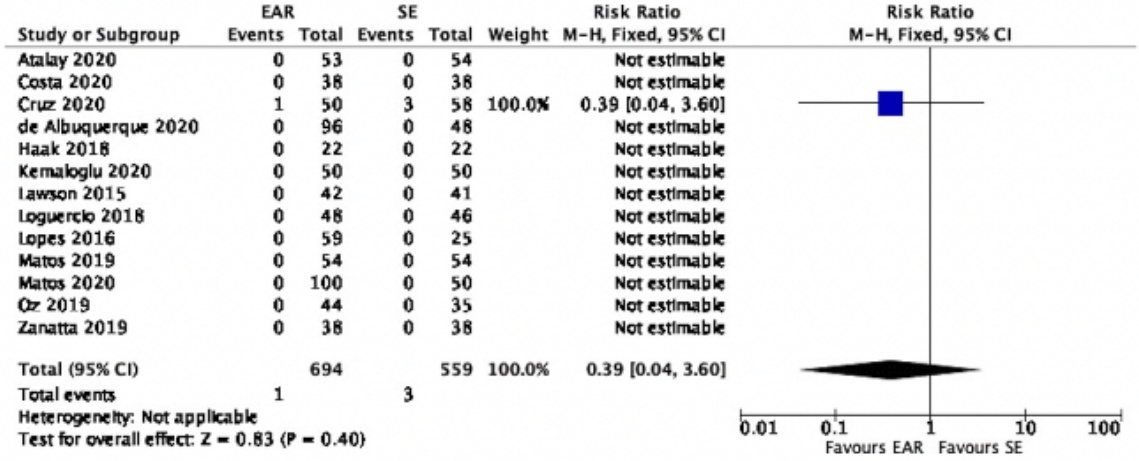


Fig 11. Forest plot for discoloration at 6-months follow-up (E&R vs SE)

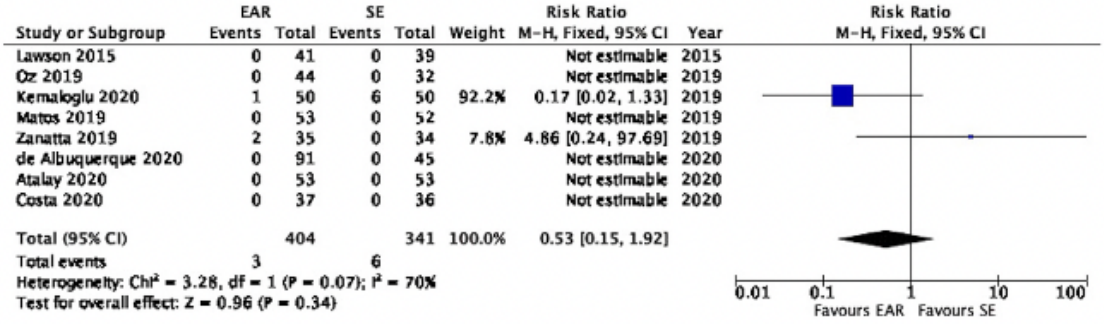


Fig 12. Forest plot for discoloration at 12-months follow-up (E&R vs SE)

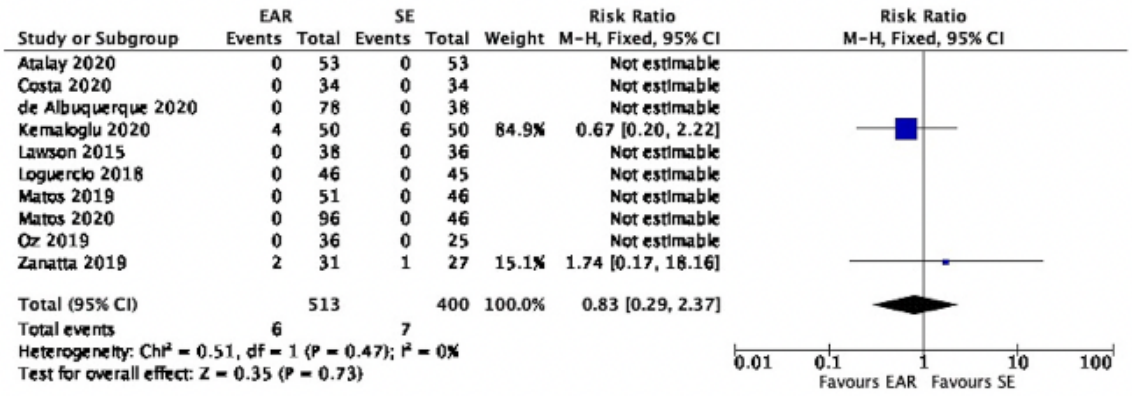


Fig 13. Forest plot for discoloration at 18/24-months follow-up (E&R vs SE)

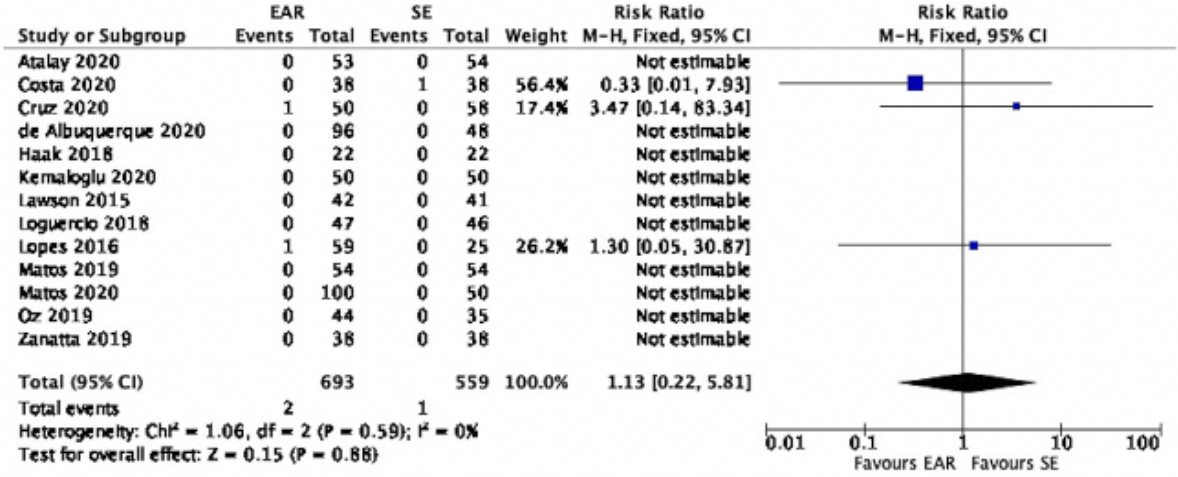


Fig 14. Forest plot for marginal adaptation at 6-months follow-up (E&R vs SE)

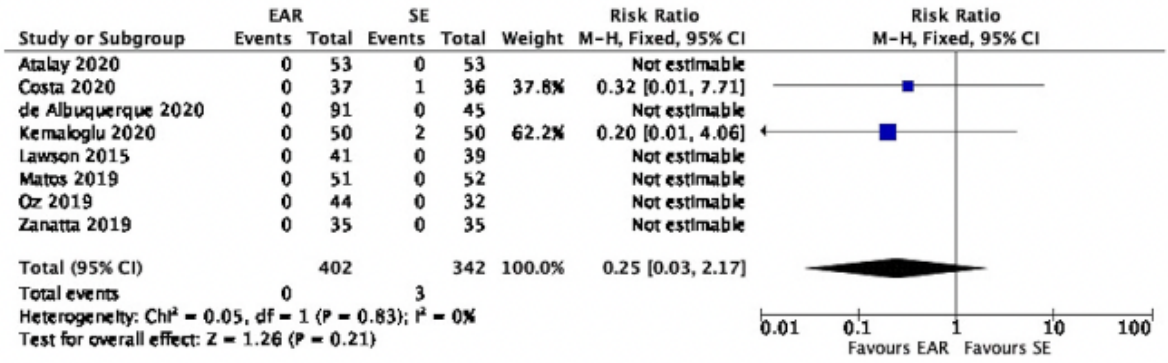


Fig 15. Forest plot for marginal adaptation at 12-months follow-up (E&R vs SE)

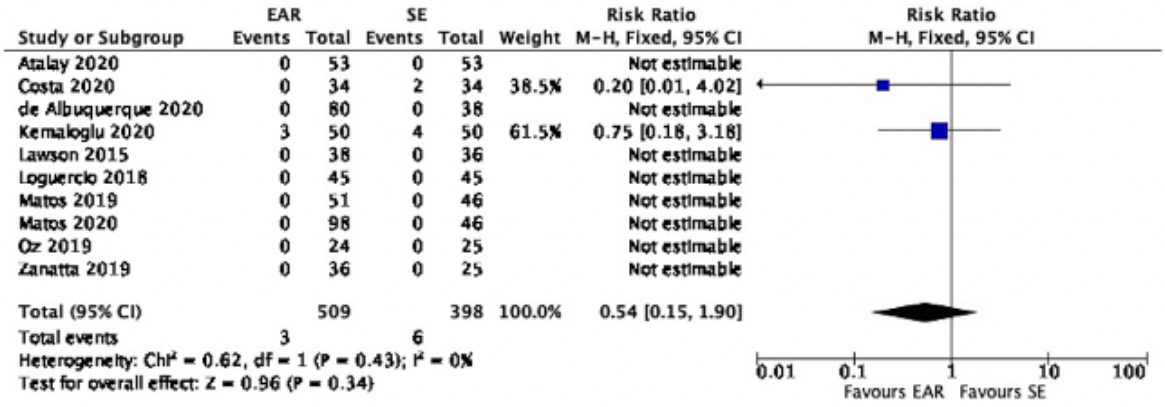


Fig 16. Forest plot for marginal adaptation at 18/24-months follow-up (E&R vs SE)

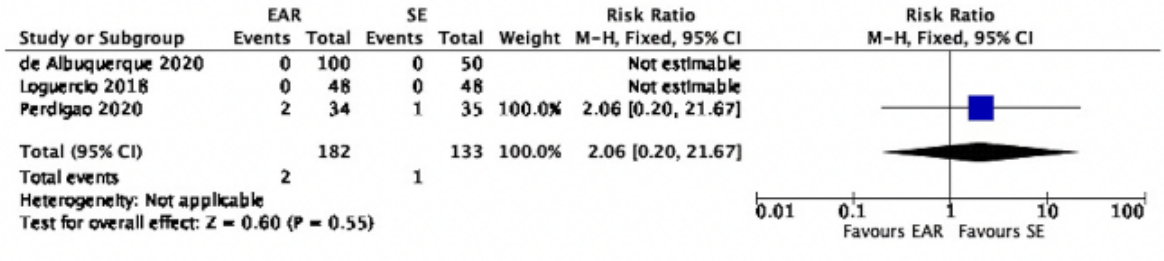


Fig 17. Forest plot for subjective POS at baseline (E&R vs SE)

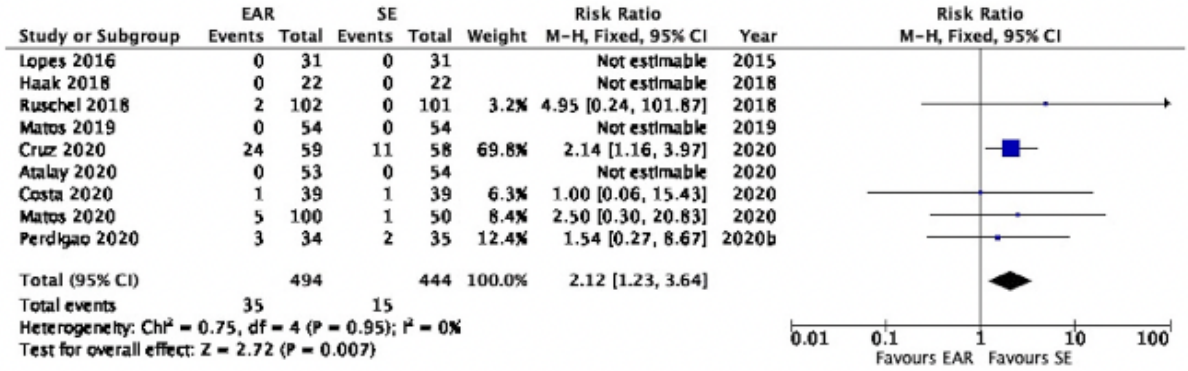


Fig 18. Forest plot for objective POS at baseline (E&R vs SE)

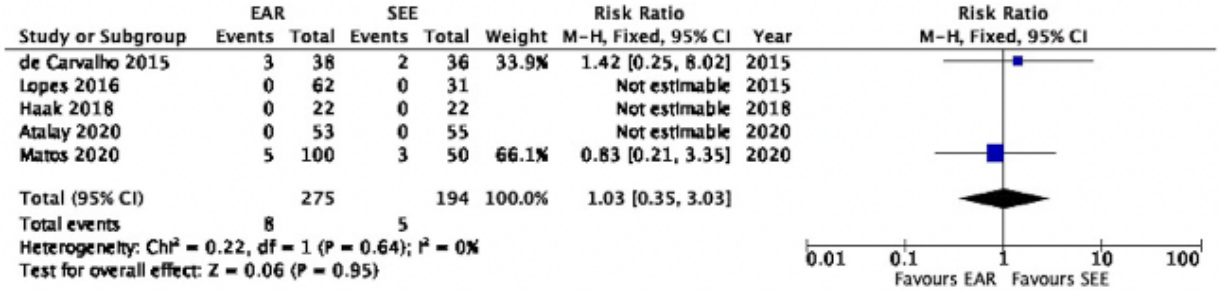


Fig 19. Forest plot for objective POS at baseline (E&R vs SEE)

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SE	Relative (95% CI)	Absolute (95% CI)		
Retention 6 months												
12	randomized trials	not serious	serious [a]	not serious	serious [b]	none	15/662 (2.3%)	15/529 (2.8%)	**RR 0.75** (0.40 to 1.40)	**7 fewer per 1,000** (from 17 fewer to 11 more)	⨁⨁◯◯ LOW	
Retention 12 months												
8	randomized trials	not serious	not serious	not serious	serious [c]	none	3/417 (0.7%)	17/361 (4.7%)	**RR 0.22** (0.08 to 0.63)	**37 fewer per 1,000** (from 43 fewer to 17 fewer)	⨁⨁⨁◯ MODERATE	
Retention 18/24 months												
10	randomized trials	not serious	not serious	not serious	serious [c]	none	14/536 (2.6%)	34/433 (7.9%)	**RR 0.32** (0.17 to 0.58)	**53 fewer per 1,000** (from 65 fewer to 33 fewer)	⨁⨁⨁◯ MODERATE	
Retention 36 months												
2	randomized trials	not serious	serious [a]	not serious	serious [d]	none	3/143 (2.1%)	5/98 (5.1%)	**RR 0.40** (0.12 to 1.34)	**31 fewer per 1,000** (from 45 fewer to 17 more)	⨁⨁◯◯ LOW	

Table 1: **CI:** Confidence interval; **RR:** Risk ratio. Explanations

a. Confidence intervals do not overlap; substantial heterogeneity

b. 95% CI includes appreciable benefit of harm (RR > 1.25)

c. Narrow confidence intervals, but few events.

d. 95% CI includes appreciable benefit of harm (RR>1.25); fairly small sample size;

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SE	Relative (95% CI)	Absolute (95% CI)		
Marginal discoloration 6 months												
13	randomized trials	not serious	not serious	not serious	very serious [c]	none	1/694 (0.1%)	3/559 (0.5%)	**RR 0.39** (0.04 to 3.60)	**3 fewer per 1,000** (from 5 fewer to 14 more)	⨁⨁◯◯ LOW	
Marginal discoloration 12 months												
8	randomized trials	not serious	serious [a]	not serious	serious [b]	none	3/404 (0.7%)	6/341 (1.8%)	**RR 0.53** (0.15 to 1.92)	**8 fewer per 1,000** (from 15 fewer to 16 more)	⨁⨁◯◯ LOW	
Marginal discoloration 18/24 months												
10	randomized trials	not serious	not serious	not serious	serious [d]	none	6/513 (1.2%)	7/400 (1.8%)	**RR 0.83** (0.29 to 2.37)	**3 fewer per 1,000** (from 12 fewer to 24 more)	⨁⨁⨁◯ MODERATE	

Table 2: CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Confidence intervals do not overlap; substantial heterogeneity

b. 95% CI includes appreciable benefit of harm (RR > 1.25)

c. Very wide 95% CI; few events

d. 95% CI includes appreciable benefit of harm (RR> 1.25); few events

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SE	Relative (95% CI)	Absolute (95% CI)		
Marginal adaptation 6 months												

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SE	Relative (95% CI)	Absolute (95% CI)		
13	randomized trials	not serious	not serious	not serious	serious [a]	none	2/693 (0.3%)	1/559 (0.2%)	**RR 1.13** (0.22 to 5.81)	**0 fewer per 1,000** (from 1 fewer to 9 more)	⨁⨁⨁◯ MODERATE	
Marginal adaptation 12 months												
8	randomized trials	not serious	not serious	not serious	serious [b]	none	0/402 (0.0%)	3/342 (0.9%)	**RR 0.25** (0.03 to 2.17)	**7 fewer per 1,000** (from 9 fewer to 10 more)	⨁⨁⨁◯ MODERATE	
Marginal adaptation 18/24 months												
10	randomized trials	not serious	not serious	not serious	serious [b]	none	3/509 (0.6%)	6/398 (1.5%)	**RR 0.54** (0.15 to 1.90)	**7 fewer per 1,000** (from 13 fewer to 14 more)	⨁⨁⨁◯ MODERATE	

Table 3: CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Very wide 95% CI; few events

b. 95% CI includes appreciable benefit of harm (RR> 1.25); few events

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SE	Relative (95% CI)	Absolute (95% CI)		
Postoperative sensitivity (baseline, subjective)												

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SE	Relative (95% CI)	Absolute (95% CI)		
3	randomized trials	not serious	not serious	not serious	very serious [a]	none	2/182 (1.1%)	1/133 (0.8%)	**RR 2.06** (0.20 to 21.67)	**8 more per 1,000** (from 6 fewer to 155 more)	⨁⨁◯◯ LOW	
Postoperative sensitivity (objective, baseline)												
9	randomized trials	not serious	not serious	not serious	serious [b]	none	35/494 (7.1%)	15/444 (3.4%)	**RR 2.12** (1.23 to 3.64)	**38 more per 1,000** (from 8 more to 89 more)	⨁⨁⨁◯ MODERATE	

Table 4: CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Very wide 95% CI; few events

b. Few events

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SEE	Relative (95% CI)	Absolute (95% CI)		
Retention 6 months												
6	randomized trials	not serious	not serious	not serious	serious [a]	none	4/361 (1.1%)	2/267 (0.7%)	**RR 1.03** (0.23 to 4.57)	**0 fewer per 1,000** (from 6 fewer to 27 more)	⨁⨁⨁◯ MODERATE	
Retention 12 months												
4	randomized trials	not serious	not serious	not serious	serious [b]	none	3/234 (1.3%)	5/179 (2.8%)	**RR 0.37** (0.09 to 1.45)	**18 fewer per 1,000** (from 25 fewer to 13 more)	-	

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SEE	Relative (95% CI)	Absolute (95% CI)		
Retention 18/24 months												
4	randomized trials	not serious	not serious	not serious	serious [c]	none	8/280 (2.9%)	6/179 (3.4%)	**RR 0.70** (0.25 to 1.95)	**10 fewer per 1,000** (from 25 fewer to 32 more)	⨁⨁⨁◯ MODERATE	
Retention 36 months												
2	randomized trials	not serious	not serious	not serious	very serious [d]	none	3/143 (2.1%)	2/100 (2.0%)	**RR 1.02** (0.17 to 6.12)	**0 fewer per 1,000** (from 17 fewer to 102 more)	⨁⨁◯◯ LOW	

Table 5: CI: Confidence interval; **RR:** Risk ratio

Explanations

a. 95% CI includes appreciable benefit of harm (RR>1.25); wide 95% CI; few events

b. 95% CI is wide; few events

c. 95% CI includes appreciable benefit of harm (RR>1.25); few events

d. 95% CI is wide; small sample size; few events

Certainty assessment							№ of restorations		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EAR	SEE	Relative (95% CI)	Absolute (95% CI)		
Postoperative senisitivty (baseline objective)												
5	randomized trials	not serious	not serious	not serious	serious [a]	none	8/275 (2.9%)	5/194 (2.6%)	**RR 1.03** (0.35 to 3.03)	**1 more per 1,000** (from 17 fewer to 52 more)	⨁⨁⨁◯ MODERATE	

Table 6: **CI:** Confidence interval; **RR:** Risk ratio

Explanations

a. 95% CI includes appreciable benefit of harm (RR>1.25); wide 95% CI; few events

EAR versus SE groups

Low certainty of evidence was observed for retention at 6- and 36-months follow-up with serious inconsistency and imprecision, while moderate certainty was seen at 12-, 18/24- months follow-up (Table 1). Similarly, low certainty with serious imprecision was seen for marginal discoloration at 6- and 12-months follow-up, while moderate certainty was observed for 18/24-months (Table 2). Moderate certainty was observed for marginal adaptation for all follow-up periods (Table 3). As for POS, low certainty with very serious imprecision was seen for subjective POS, whereas moderate certainty of evidence was observed for objective POS evaluation (Table 4).

EAR versus SEE groups

Moderate certainty of evidence was noted for retention at 6-, 12- and 18/24- months, while low certainty with very serious imprecision was detected at 36-months follow-up (Table 5). Our assessment revealed moderate certainty of evidence for the outcome POS when comparing EAR to SEE groups (Table 6).

The influence of selective enamel etch and self-etch mode of universal adhesives' application on clinical behavior of composite restorations placed on non-carious cervical lesions: A systematic review and meta-analysis.

Aim:

To answer the PICOS question: "Is the risk of retention loss equal for SEE and SE approach when universal adhesives and composite restorations are indicated for restoring NCCLs?"

Materials and methods

Study protocol and registration :

This study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database under the number CRD42020184666 and represents a continuation of a recently conducted systematic review by the same group of authors [71]. The reporting of this systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [72].

Eligibility criteria and search strategy:

The PICOS strategy [101] was as follows:

Population (P) - permanent teeth with NCCLs in adult patients (>18 years of age);

Intervention (I) – direct composite restoration placed using universal adhesive in SEE mode;

Comparison (C) – direct composite restoration placed using universal adhesive in SE mode;

Outcome (O) - clinical parameters relevant for direct composite restorations (retention, POS, marginal adaptation/discoloration) at various follow-up periods;

Study design (S) – randomized controlled clinical trials (RCTs).

The literature search was performed through several international and national databases, with no language filters, and reported in accordance with the PRISMA-S guidelines [73]. To identify potential studies of interest, Clarivate Analytics' Web of Science (including Web of Science Core Collection—WoS, Korean Journal Database — KJD, Russian Science Citation Index — RSCI, SciELO Citation

Index — SciELO), Scopus, PubMed (including MEDLINE), Cochrane Central Register of Controlled Trials (CENTRAL) [Cochrane Library], and Latin American & Caribbean Health Sciences Literature (LILACS) [the Virtual Health Library (VHL) portal], were explored up to September 2021, utilizing the search strategy collectively developed by the experienced medical librarian and information specialist (J.J.) and the review team. In order to optimize the search strategy and maximize sensitivity, test searches were used. Details on the number of identified articles and complete representation of applied strategies for all searched databases, including the search terms employed, are shown in Supplementary Table 1. Following the Peer Review of Electronic Search Strategies (PRESS) guideline [74], electronic literature search strategies were peer-reviewed by a second information specialist, whose feedback was incorporated in the definitive database search. Additional searches through OpenGrey (http://www.opengrey.eu), Google Scholar™ (first 100 hits), and other available digital repositories (e.g., Networked Digital Library of Theses and Dissertations (http://www.ndltd.org), Open Access Theses and Dissertations (https://oatd.org), DART-Europe E-theses Portal – DEEP (https://www.dart-europe.org/basic-search.php), Opening access to UK theses – EThOS (https://ethos.bl.uk)) were conducted to find unpublished manuscripts, research reports, conference papers, doctoral dissertations, and other grey literature. Finally, forward snowballing and screening of the reference lists of included trials and relevant previously published reviews were also completed using citation indexes (WoS, Scopus, or Google Scholar). During the final drafting of the paper on November 26, 2021, conducted searches were rerun, and no new relevant trials had been identified after the conclusion of the literature search.

The exclusion criteria were: (1) conference abstracts; (2) studies that did not involve at least two groups of direct composite restorations within the same patient comparing SEE with SE mode; (3) studies that compared outcomes between vital and endodontically treated teeth. We established no minimum follow-up period threshold for this systematic review and meta-analysis, since POS, which usually occurs immediately after the restorative procedure [75], was one of the outcomes of interest.

Study selection and data extraction :

All literature search results were imported into the Rayyan QCRI platform [76] for duplicate removal and further screening. The study selection process was conducted in two stages. To select studies eligible for inclusion, two independent investigators (U.J. and F.F.) completed the initial screening of titles and abstracts. Papers that did not meet the eligibility criteria were excluded, and full texts of initially selected studies were retrieved for further reading. In the next stage, three investigators (C.M., T.M., and U.J.) independently assessed full texts of studies in order to select the articles of interest. All disagreements were resolved by consensus or discussion with a senior investigator (A.M.).

Three independent investigators (U.J., C.M., and T.M.) did the data extraction using custom-made extraction forms in MS Word. The following data were extracted: details of the study (author, year, location, and study design), participants (number and age range), direct restoration (number, type, and material used for indirect restorations, and type of teeth restored), adhesive strategy (type of adhesive system used during restorative procedures, number of restorations placed with SE and SEE approach), methodology (evaluation criteria, follow-up periods), and results (success and failure rates, as well as statistical analyses). If data were missing, the corresponding author of the paper was contacted by e-mail in an attempt to retrieve the information of interest.

When more than one brand of universal adhesive was used in a trial [116], the data belonging to a certain adhesive strategy, regardless of the adhesive brand used, was combined in order to make a single entry for the statistical analysis. Since the study results were reported in several periods of follow-ups, the data from similar follow-up periods (for 18- and 24 months) were united, thus observing 18/24- month as a unique follow-up period. When multiple publications with different follow-up periods were observed, the data from the latest publication (with the longest follow-up period) were taken into consideration for performing meta-analyses.

Assessment of the Risk of Bias:

Two reviewers (U.J. and I.R.), independently from each other, performed the risk of bias assessment of the trials using the Cochrane Collaboration's tool for assessing the risk of bias in RCTs [104]. Six domains of bias were evaluated: selection bias - random sequence generation and allocation concealment; performance bias - blinding of participants and personnel; detection bias - blinding of outcome assessment; attrition bias - incomplete outcome data; reporting bias - selective outcome reporting; other possible sources of bias. In case of disagreements between the reviewers, a consensus was reached through discussion, and if needed, by consulting a third reviewer (A.M.).

Meta-analysis:

The data which had previously been extracted from the selected studies were analyzed using Revman (Review Manager 5.4, The Cochrane Collaboration, Copenhagen, Denmark). Data for all outcomes (retention, POS, marginal discoloration, marginal adaptation) of the eligible studies were dichotomous. The odds ratio with a 95% confidence interval (CI) was calculated. Fixed-effects models were applied, and heterogeneity was tested using Cochran Q test and the I2 index. Before choosing fixed-effects models, random effects analysis was carried out as a sensitivity analysis, which

produced very similar summary estimate. The follow-up periods were considered as short- (6 months), medium- (from 12 up to 36 months) or long-term (over 3 years) [110].

Certainty of evidence assessment :

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework [121] was used to rate the overall quality of clinical evidence (certainty in the estimates of effect) for each of the outcomes, evaluating individual risk for bias, inconsistency, indirectness, imprecision, and publication bias. Based on the mentioned indicators, the certainty of the estimated effect was rated as high quality of evidence (the true effect lies close to that of the effect estimate), moderate quality of evidence (the true effect is likely to be close to the effect estimate, but there is a possibility that it is substantially different), low quality of evidence (the true effect may be substantially different from the effect estimate), and very low quality of evidence (the true effect is likely to be substantially different from the effect estimate) [106].

Certainty assessment							**№ of patients**		**Effect**		
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	**Certainty**
Retention 6 months											
5	randomised trials	serious [a]	serious[b]	not serious	serious[c]	publication bias undetected	8/222 (3.6%)	18/222 (8.1%)	**OR 0.42** (0.18 to 0.99)	**45 fewer per 1,000** (from 65 fewer to 1 fewer)	⊕◯◯◯ Very low

Table 7. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Two out of five studies rated as “unclear” risk of bias

b. 95% CI do not entirely overlap

c. Wide 95% CI

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Retention 12 months											
4	randomised trials	very serious[a]	serious[b]	not serious	serious[c]	none	6/192 (3.1%)	12/191 (6.3%)	**OR 0.52** (0.20 to 1.34)	**29 fewer per 1,000** (from 50 fewer to 20 more)	⊕◯◯◯ Very low

Table 8. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Three out of four studies considered as "unclear" risk of bias

b. 95% CI do not overlap

c. Wide 95% CI with few events

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Retention 18/24 months											
5	randomised trials	serious[a]	not serious	not serious	serious[b]	publication bias undetected	7/204 (3.4%)	22/207 (10.6%)	**OR 0.31** (0.13 to 0.72)	**71 fewer per 1,000** (from 91 fewer to 27 fewer)	⊕⊕◯◯ Low

Table 9. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Three out of five studies considered as "unclear" risk of bias

b. Wide 95% CI

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Retention 36 months											
3	randomised trials	very serious[a]	serious[b]	not serious	serious[c]	none	3/142 (2.1%)	10/140 (7.1%)	**OR 0.31** (0.09 to 1.07)	**48 fewer per 1,000** (from 65 fewer to 5 more)	⊕◯◯◯ Very low

Table 10. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Two out of three studies considered as "unclear" risk of bias

b. 95% CI do not overlap

c. Wide 95% CI with few events

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Marginal discoloration 6 months											
2	randomised trials	very serious[a]	not serious	not serious	very serious[b]	none	4/81 (4.9%)	4/83 (4.8%)	**OR 1.02** (0.24 to 4.22)	**1 more per 1,000** (from 36 fewer to 128 more)	⊕◯◯◯ Very low

Table 11. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. The included studies were considered as "unclear" risk of bias

b. Very wide 95% CI with few events

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Marginal discoloration 18/24 months											
2	randomised trials	very serious[a]	serious[b]	not serious	serious[c]	none	11/79 (13.9%)	15/78 (19.2%)	**OR 0.66** (0.28 to 1.56)	**57 fewer per 1,000** (from 130 fewer to 79 more)	⊕◯◯◯ Very low

Table 12. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. The included studies were considered as "unclear" risk of bias

b. 95% CI do not overlap

c. Few events observed

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Marginal adaptataion 6 months											
2	randomised trials	very serious[a]	serious[b]	not serious	serious[c]	none	9/81 (11.1%)	6/83 (7.2%)	**OR 1.60** (0.55 to 4.71)	**39 more per 1,000** (from 31 fewer to 196 more)	⊕◯◯◯ Very low

Table 13. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. The included studies considered as "unclear" risk of bias

b. 95% CI do not overlap entirely

c. Few events observed

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Marginal adaptataion 12 months											
2	randomised trials	very serious[a]	not serious	not serious	serious[b]	none	8/80 (10.0%)	14/81 (17.3%)	**OR 0.53** (0.21 to 1.34)	**73 fewer per 1,000** (from 131 fewer to 46 more)	⊕◯◯◯ Very low

Table 14. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. The included studies considered as "unclear" risk of bias

b. Few events observed

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**SEE**	**SE analyses**	**Relative (95% CI)**	**Absolute (95% CI)**	
Marginal adaptataion 18/24 months											
2	randomised trials	very serious[a]	serious[b]	not serious	serious[c]	none	15/79 (19.0%)	15/78 (19.2%)	**OR 0.99** (0.45 to 2.19)	**2 fewer per 1,000** (from 96 fewer to 150 more)	⊕◯◯◯ Very low

Table 15. CI: Confidence interval; **OR:** Odds ratio

Explanations

a. The included studies considered as "unclear" risk of bias

b. 95% CI do not entirely overlap

c. Few events observed

Results:

Study selection

Figure 20 shows a PRISMA flow diagram of the study selection process based on the eligibility criteria. 429 references for potential inclusion in this systematic review were found in the initial search. In the next step, 168 duplicates were identified and removed from the database. After screening of titles and abstracts, 237 records did not satisfy the inclusion criteria and were excluded, while 25 articles were eligible for full-text assessment. Subsequently, ten articles were excluded after full-text examination due to the missing SEE or SE group, leaving 15 articles to be included in this systematic review.

Descriptive analysis of the selected studies:

Details about 15 articles selected for this review are shown in Supplementary Table 2. All studies that were included were conducted as RCTs with split-mouth design in university settings, and they were carried out in Brazil [67, 109, 110, 113, 115,116, 122-125], Turkey [120], Germany [63, 107], the USA [108] and India [126].

The studies were published between 2013-2021, involving a total of 384 adult patients who received 853 composite restorations placed with universal adhesives used in SEE or SE modes. The longest follow-up period was ararars [109]. One study used two different brands of universal adhesives during restorative procedures [116]. In most studies, clinical outcomes were assessed using Modified United States Public Health Service (USPHS) criteria or FDI World Dental Federation (FDI) criteria, or a combination of both. Only one study [108] used custom-made criteria for the evaluation of composite restorations. POS, which was one of the main outcomes of interest of this systematic review, was assessed by applying a stimulus during dental check-ups in the first week of restorations' placement. In one study, the first evaluation of POS took place after 6 months of a restorative procedure, and therefore the data from the mentioned study [108] could not be considered for running the meta-analysis. Some studies [107, 126] reported the data in percentages, and since we were not successful in retrieving the original data from the authors, the studies were not included in the meta-analyses.

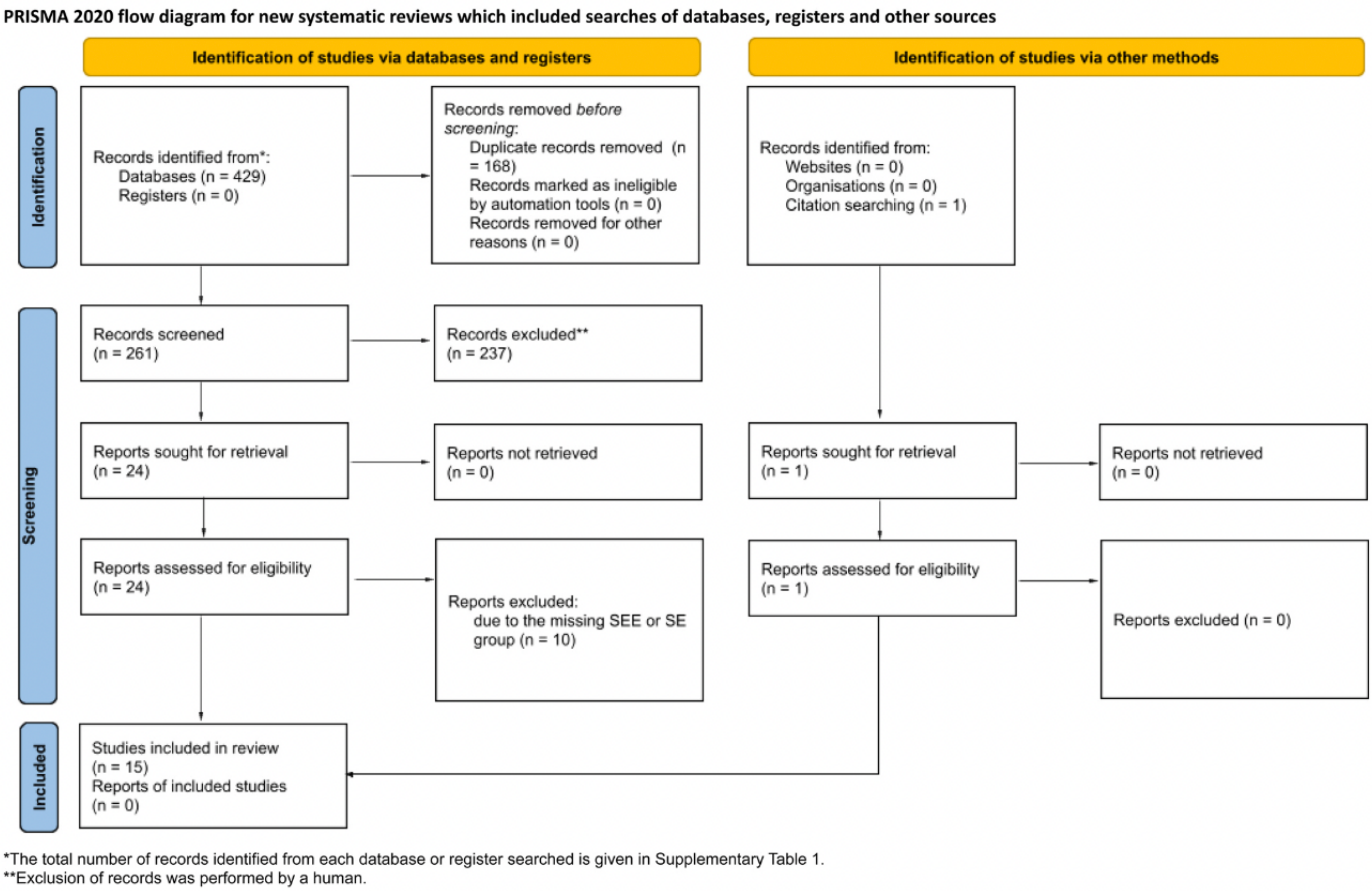


Fig 20. PRISMA flowchart of study identifications.

Risk of bias of the included studies

Figure 21 summarizes the risk of bias judgment for each of the included studies. Generally, the studies included in this review had no major problems regarding the study design and reporting of the results. The raised concerns were related to: selection bias – not clearly stated if the allocation concealment was kept hidden until the intervention [107, 63]; performance bias – not reported if the participants were blinded [108, 116, 120, 124, 125]; detection bias – not clearly reported if the evaluators were blinded to the restorative procedure [108, 126].

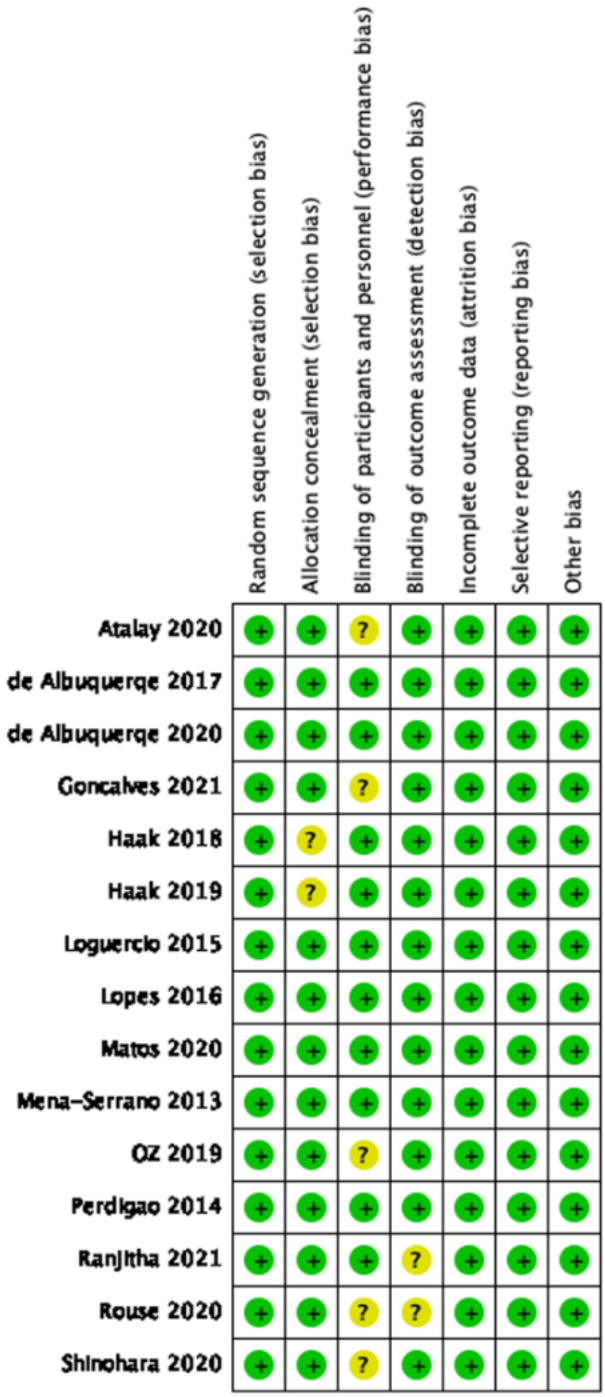


Fig. 21. Authors' risk of bias assessment of the included studies.

Quantitative synthesis: meta-analyses:

After data extraction, 7 [67, 108, 109, 115, 116, 120, 124] studies presented suitable for running meta-analyses for the outcomes of interest. Since four articles [109, 110, 113, 122] were publications derived from the same cohort of patients at different follow-up periods, only data from the most recent study [109] were taken into consideration for the meta-analyses. Similarly, we also detected 4

publications with different follow-up periods [67, 123, 124, 125]. The studies with no events in both arms were not included in the meta-analysis, since they did not provide any indication of either the direction or magnitude of the relative treatment effect. Since only one study [109] reported events for the outcome POS, the meta-analysis for this clinical outcome was not performed.

Loss of retention

The forest plots of meta-analyses for retention loss at different follow-up periods for the SEE and SE approach are shown in Figures 22-25. No statistically significant differences were observed at 12- and 36-month follow-up periods (p=0.18; OR= p=0.52, 95% CI [0.20, 1.34] and p=0.06; OR= p=0.31, 95% CI [0.09, 1.07], respectively). There were statistically significant differences at 6- (p=0.05; OR=0.42, 95% CI [0.18, 0.99]) and 18/24- months (p=0.007; OR=0.31, 95% CI [0.13, 0.72]), favoring SEE groups. The data from 6- and 18/24-months were not heterogeneous (I2=0%), while data from 12- and 36-months showed heterogeneity which may not be important (I2=36%; I2=14%, respectively).

Marginal discoloration:

Forest plots of the meta-analyses for marginal discoloration for SEE and SE groups are presented in Figures 26 and 27. No statistically significant differences were observed at 6- and 18/24- months (p=0.98; OR=1.02, 95% CI [0.24, 4.22] and p=0.34; OR=0.66, 95% CI [0.28, 1.56]). The data from 6-months was not heterogeneous (I2=0%), while the data from 18/24-months showed substantial heterogeneity (I2=61%). No events in any of the groups were observed at other follow-ups, and consequently, meta-analysis was not run.

Marginal adaptation:

Forest plots of the meta-analyses for marginal adaptation for SEE and SE groups are shown in Figures 28-29. No statistically significant differences were observed at 6-, 12- and 18/24- months (p=0.39; OR=1.60, 95% CI [0.55, 4.71] and p=0.18; OR=0.53, 95% CI [0.21, 1.34] and p=0.98; OR=0.99, 95% CI [0.45, 2.19], respectively). The data was not heterogeneous (I2<1%). No events in SEE and SE groups were observed at the 36-month follow-up period.

Certainty of evidence assessment:

GRADE tool [120] was used to assess the certainty of the evidence for each of the outcomes evaluated in our meta-analyses.

"Very low" certainty of the evidence was observed for the outcome Retention at 6-, 12- and 36- month follow-up period, while "low" certainty was reported at 18/24- month follow-up (Tables 1, 2 and 3, respectively).

For the outcome Marginal discoloration, "very low" certainty of the evidence was observed at 6- and 18/24- months (Tables 5 and 6, respectively). Similarly, "very low" certainty of the evidence was seen at 6-, 12-, 18/24- months for the outcome Marginal adaptation (Tables 7, 8 and 9, respectively).

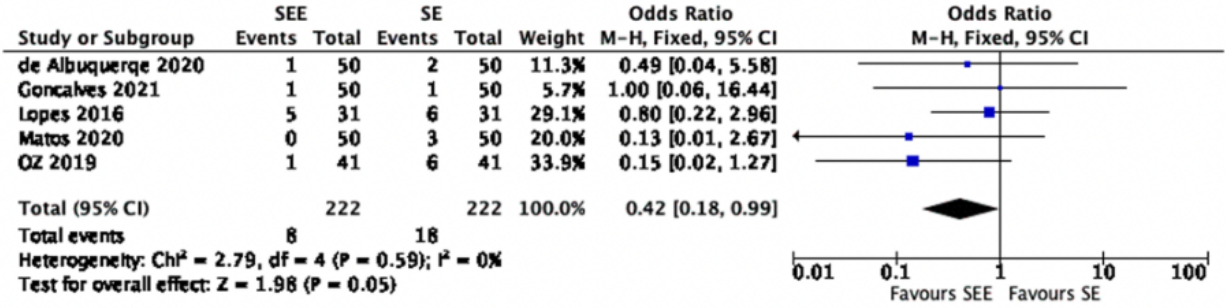


Fig. 22. Forest plot for retention at 6- months follow-up

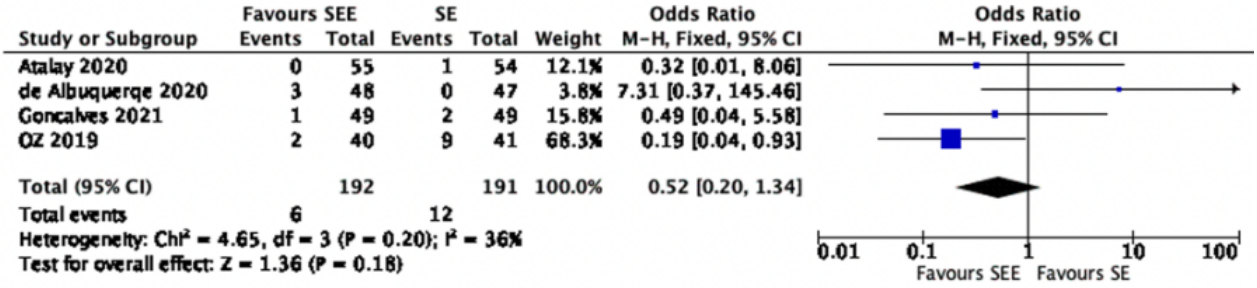


Fig. 23. Forest plot for retention at 12- months follow-up

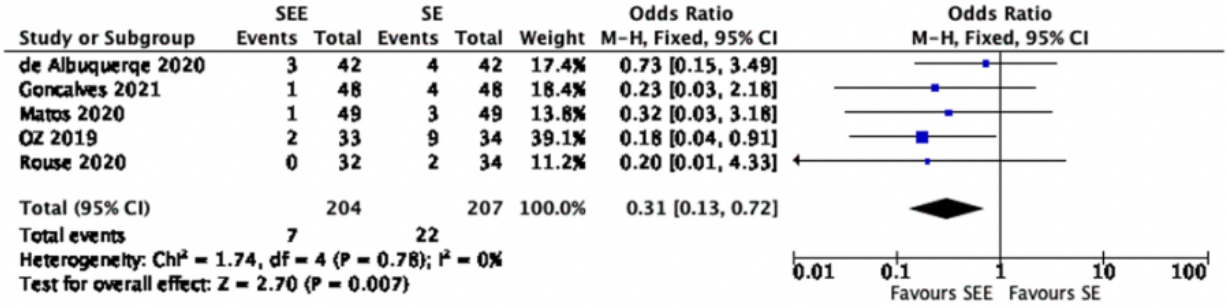


Fig. 24. Forest plot for retention at 18/24- months follow-up

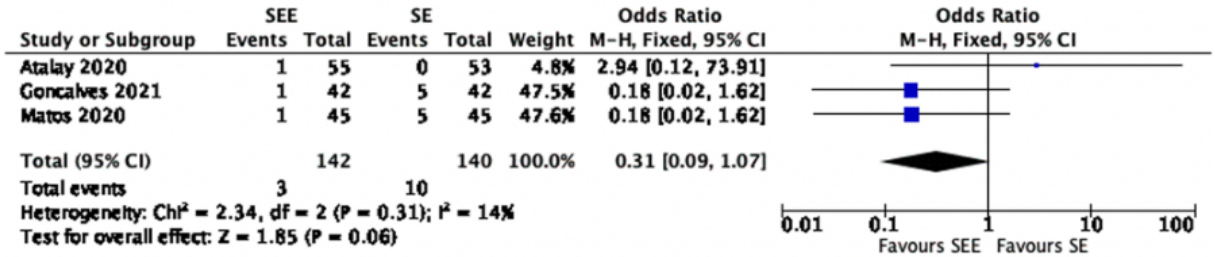


Fig. 25. Forest plot for retention at 36- months follow-up

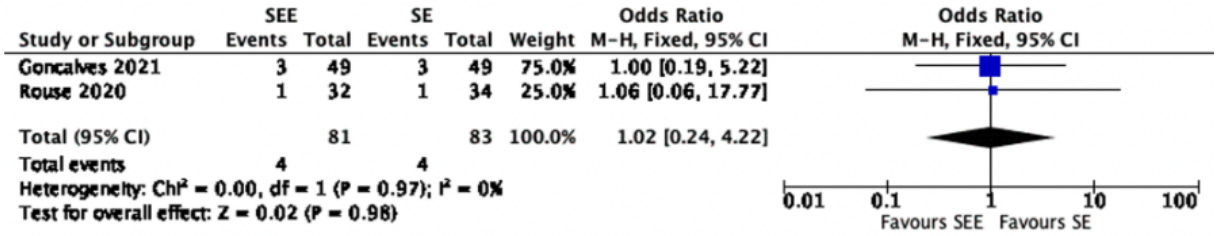


Fig. 26. Forest plot for marginal discoloration at 6- months follow-up

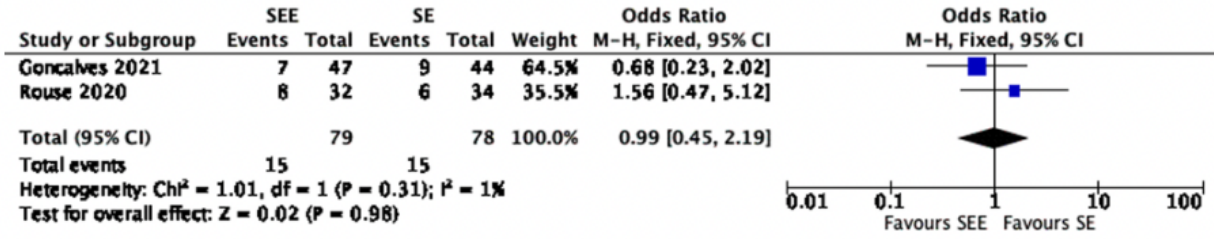


Fig. 26. Forest plot for marginal discoloration at 18/24- months follow-up

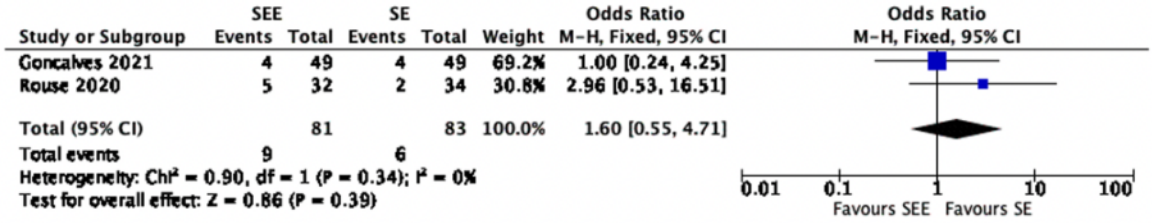


Fig. 27. Forest plot for marginal adaptation at 6- months follow-up

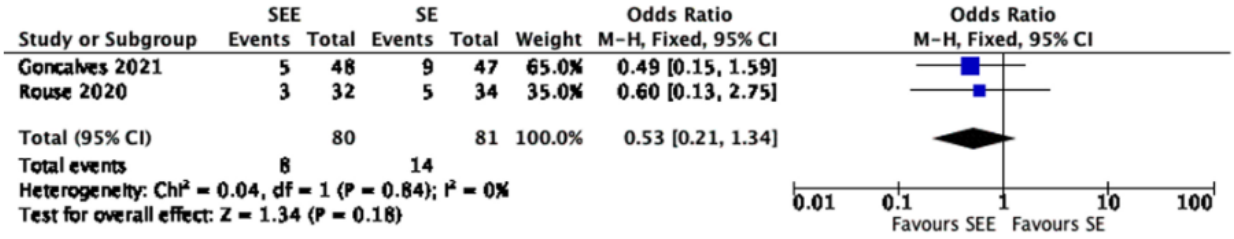


Fig. 28. Forest plot for marginal adaptation at 12- months follow-up

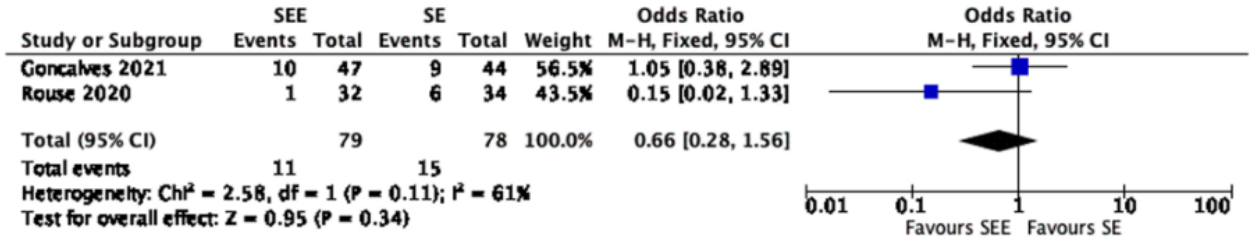


Fig. 29. Forest plot for marginal adaptation at 18/24- months follow-up

The influence of adhesive evaporation on bonding performances to dentin and on enzymatic activity to dentin in presence or not of pulpal pressure

Aim:

To evaluate the influence of two adhesive evaporation techniques on the microtensile bond strength (μTBS) of a universal adhesive to dentin, and the activity of dentinal matrix metalloproteinases (MMPs) in presence or not of simulated pulpal pressure.

Materials and methods:

Specimens' preparation

Eighty non carious, sound human molars were collected and stored in water until use, no longer than 1 month. The roots were removed and each molar was decoronated with a diamond blade under water cooling as to expose the middle/deep dentin. A standardized smear layer was created using a 180-grit silicon-carbide (SiC) paper.

A universal adhesive (Clearfil Universal Bond Quick, Kuraray Noritake, Hattersheim, Germany) was used according to the manufacturer's instruction and applied in the etch-and-rinse (E&R) or self-etch (SE) mode, according to the group.

The dentin blocks were randomly divided into 6 groups, according to the evaporation technique of the adhesive and the presence or not of the intra-pulpal pressure (n=5): G1) the universal adhesive was used in the E&R mode and air-dried with the disposable air/water syringe; G2) the universal adhesive was used in the E&R mode and evaporated by means of a disposable suction device; G3) the universal adhesive was used in the SE mode and air-dried with the disposable air/water syringe ; G4) the universal adhesive was used in the SE mode and evaporated with a disposable suction device. The same groups were repeated (G5-G8) in presence of a hydrostatic intra-pulpal pressure. For that purpose, the pulpal tissue was removed with a tweezer taking care not to touch the walls of the pulpal chamber. In order to simulated in vitro the intra-pulpal pressure conditions, the method previously

validated by Feitosa et al (4) was used. Briefly, immediately after bonding and restorative procedures, two layers of nail polish were applied around the adhesive interface to avoid water penetration at this site. Each specimen was then fixed with wax inside the lid of a cylindrical container , from the dentin side, avoiding to touch the pulp chamber. Subsequently, the vessel was filled with distilled water to a height of 20 cm, the lid with the fixed samples closed and turned upside down. In this way the samples had a water column of 20 cm on them and the pressure inside the pulp chamber was 20 hPa, according to the hydrostatic pressure equation: P = g x d x h; where P is hydrostatic pressure, g is gravity, d is liquid density and h liquid height.

Polymerization procedures were performed with a light-emitting diode (LED) lamp (1.470 mW/cm2, Elipar Deep cure-L, 3M, Saint Paul, Minnesota, Stati Uniti). After adhesive polymerization, the coronal restoration was performed with two 2 mm layers of resin composite (Clearfil Majesty ES-2, Kuraray Noritake, Hattersheim, Germany).

Microtensile bond strength test (μTBS)

Microtensile sticks was prepared according to the non-trimming method of the microtensile bond strength test and submitted to tensile strength immediately or after 6 month of artificial storage: specimens were cut into resin-dentin sticks with cross- sectional area of ~ 0.9 mm. The sticks were equally and randomly divided into two groups and stored in an incubator at 37 °C in artificial saliva for 24 h (T0) or 6 months (T6). After aging, each stick was measured with a digital caliper and stressed to failure under tension using a simplified universal testing machine (Bisco Inc., Schaumburg, IL, USA; crosshead speed: 1 mm/min). After testing, the sticks were observed under optical microscopy (Stemi 2000- C; Carl Zeiss Jena GmbH) at 50x to evaluate the fracture modes, so divided: adhesive (A) at the dentin side, cohesive (C) within the resin or mixed (M) a combination of the previously mentioned failures.

Scanning electron microscopy (SEM) evaluation

Two representative sticks per each group were prepared for scanning electron microscopy (SEM, Nova NanoSEM 450. Thermo Fisher Scientific, Waltham, MA, USA) evaluation. As to do so, the dentin side of the fractured sticks was mounted on metal stubs and gold sputter coated before evaluation at different magnifications (accelerating voltage of 10.00 kV and magnifications at 200 × and 500 ×).

In situ Zymography

Additional 3 intact molars per group were used for the in situ Zymography analysis. The specimens were prepared according to previously published studies (32).

Briefly, the specimens were cut vertically into 1mm-thick slabs to expose the treated dentin surface or the hybrid layer using a slow speed saw. Half of the obtained slabs was tested immediately while the other half was aged in artificial saliva at 37°C for 6 months and then tested as follows.

Each bonded slab with exposed hybrid layer was glued to a microscope slide and ground down to obtain 50µm-thick specimens. In situ zymography was performed using quenched fluorescein-conjugated gelatin as the MMP substrate. The gelatin stock solution (diluted 1:8 with the dilution buffer and an anti-fading agent) was placed on top of each slab and covered with a coverslip. Slides was light-protected and incubated in humidified chambers at 37°C. Hydrolysis of quenched fluorescein-conjugated gelatin substrate, indicative of endogenous gelatinolytic enzyme activity, was assessed by examination under a confocal microscope, ex:488nm and em:lp530nm.

Statistical analysis

After checking the normality (Shapiro-Wilk test) and equal distribution (Brown-Forsythe) of the data, the three-way analysis of variance (ANOVA) was run to examine the effects at, baseline and after 6 months of storage in artificial saliva at 37 °C, of the variables “application mode” (E&R vs SE), “evaporation mode” (air syringe vs suction) and “pulpal pressure” (presence or not), and the interaction of these factors on the uTBS values. Pairwise comparisons were performed using Tukey’s post-hoc test. At T0 the evaporation of the adhesive using an air-water syringe led to significantly higher µTBS values than suction ($p<0.05$) , aging doesn’t show significant difference between the evaporation mode used (0,367), but only in the etching mode (<0,001).

Results:

Microtensile bond strength test (uTBS)

The mean (standard deviations) results of the microtensile bond strength test are presented in Table 1. the statistical analysis revealed that differences exist according to the application mode of the universal adhesive, the evaporation mode and the presence or not of pulpal pressure. Moreover, the interactions between these variables were also significant.

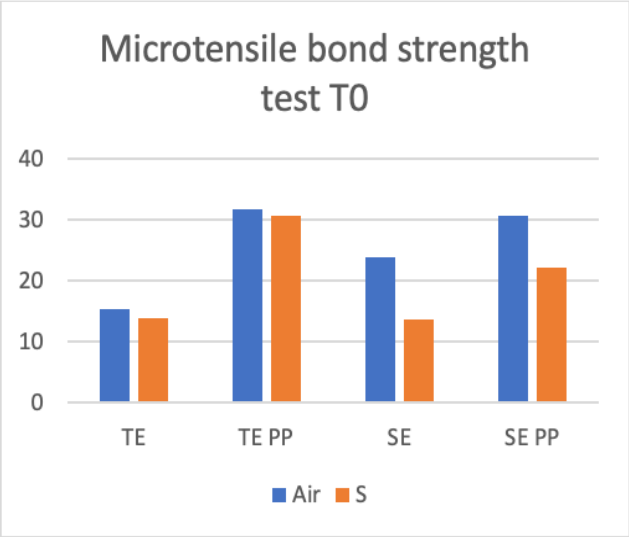
At baseline, when no pulpal pressure was present, the interaction between etching mode and evaporation was statistically significant (P = 0,006), as follows; G2=G1>G4>G1.

According to the results obtained at T0, μTBS showed statistically significant difference in samples with pulpal pressure (P = 0,028).

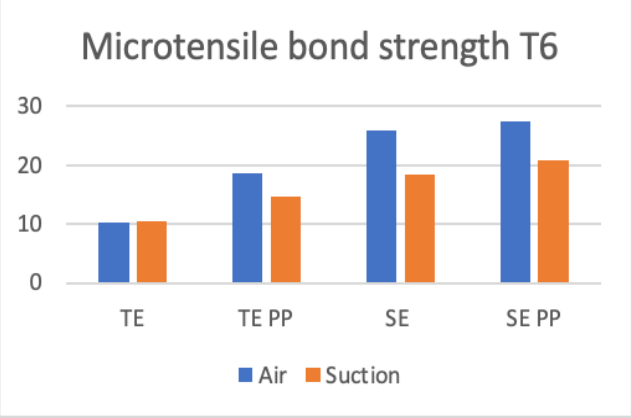
The presence of pulpal pressure significantly influenced the bond strength values ($p<0.05$), regardless of the method of evaporation the adhesive (P = 0.720).

	air (A)		suction (S)	
	TE	SE	TE	SE
without PP	15,34 ± 1,72	23,87±1,68	13,81±1,91	13,58±1,62
PP	31,80±1,66	30,70±1,42	27,01±1,36	22,02±1,58

Table 16: μTBS values in MPa (Mean±)



Graphic 1: μTBS values in MPa at T0.



Graphic 2: μTBS values in MPa at T6.

	air (A)		suction (S)	
	TE	SE	TE	SE
without PP	10,4±13,78	26±10,03	10,6±10,19	18,5±10,59
PP	18,6±11,4	27,5±4,15	14,7±7,48	20,9±6,57

Tab 17: μTBS values in MPa at T6

At T6 evaporation approach did not influenced the results (P=0.367), meanwhile the results showed statistically significant differences between the etching mode used (P = <0.001). SE groups performed significantly better than TE groups (P = <0.001) and also about the presence of PP (=0,028).

The effect of different levels of etching mode does not depend on what evaporation is used at T6. There is not a statistically significant interaction between etching mode at T6 and evaporation at T6 (P = 0,544), contrary on what obtained at the baseline.

At T6, the SE groups maintained the same bond strength level as at baseline (p>0.05), while in the ER groups aging significantly decreased bond strength (p<0.05).

About the simulation of pulpal pressure, as seen at the baseline, it positively influenced the uTBS (0,028).

Moreover results show that the effect of different etching mode with PP depends on what level of Aging is present. There is a statistically significant interaction between this two factors (P = <0,001)

The effect of different levels of Evap just PP does not depend on what level of Aging just PP is present. There is not a statistically significant interaction between Evap just PP and Aging just PP. (P = 0,072)

Finally graphics and tables show that air-drying resulted in significantly higher μTBS values than suction, regardless of the aging and the adhesive application mode (p<0.05).

A predominance of mixed fractures was observed when no pulpal pressure was present, followed by adhesive debondings (ranging from 3-20%), except for G2 that did not exhibit adhesive fractures.

In the presence of pulp pressure, mixed fractures were also prevalent in all groups. Adhesive-type fractures did not exceed 2% incidence.

Scanning electron microscope (SEM) evaluation

SEM images showed the presence of sparse water-tree formations at the adhesive interface in the Suction groups, irrespective of the application mode. Suction-evaporation, aging and ER mode increased MMPs activity (p<0.05). (Fig. 30)

In situ zymography analysis

At the baseline when the universal adhesive was applied in the E&R mode without PP, (Fig.31) higher enzymatic activity was observed (p<0.05). A greater enzymatic activity was also found in the presence of pulpal pressure (p<0.05). The values are shown below in Tab. 17.

No statistically significant differences were found regarding the different method of evaporation of the adhesive ($p>0.05$).

After 6 months of storage in artificial saliva in presence of pulpal pressure higher enzymatic activity was observed in correlation of the etching mode (0,006) . The confocal evaluations shows also no significant correlation between evaporation mode and pulpal pressure (0,720).

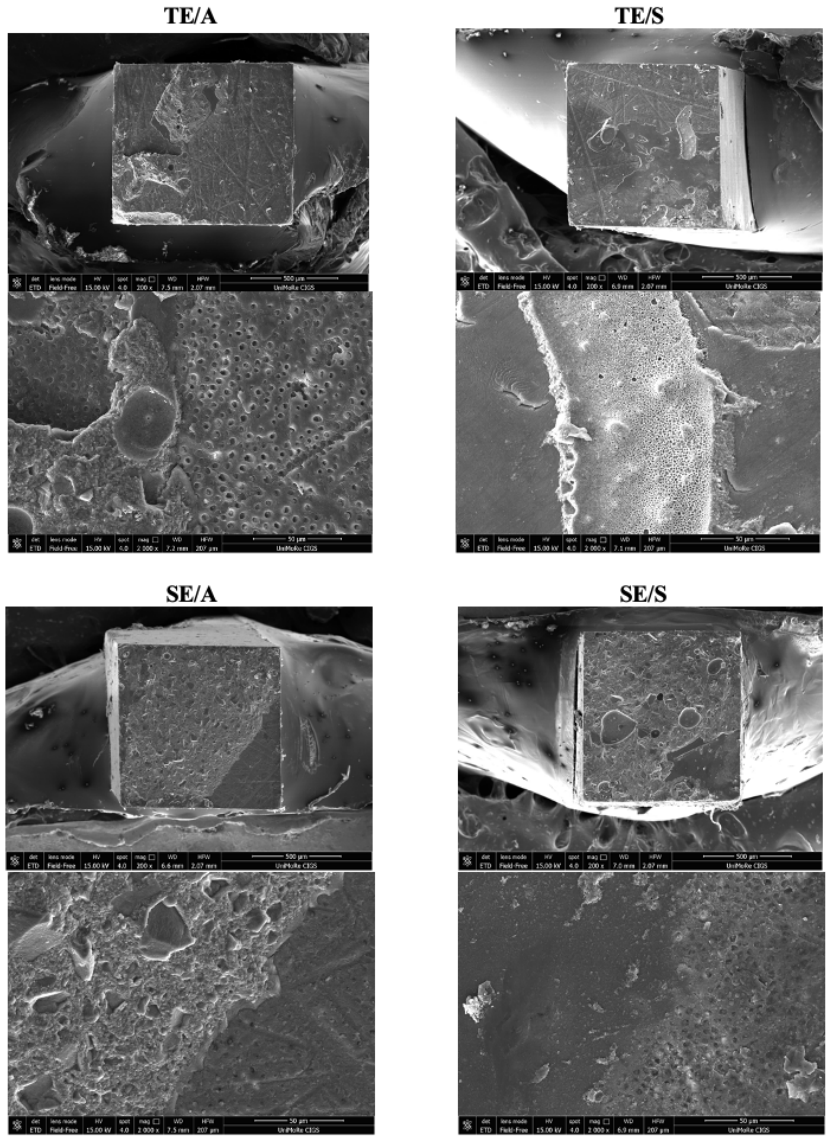


Fig 30: The images above depict the adhesive surface of the sticks of each group following detachment following the microtensile bond strength test observed by SEM, mag:200x (above); 2000x (low).

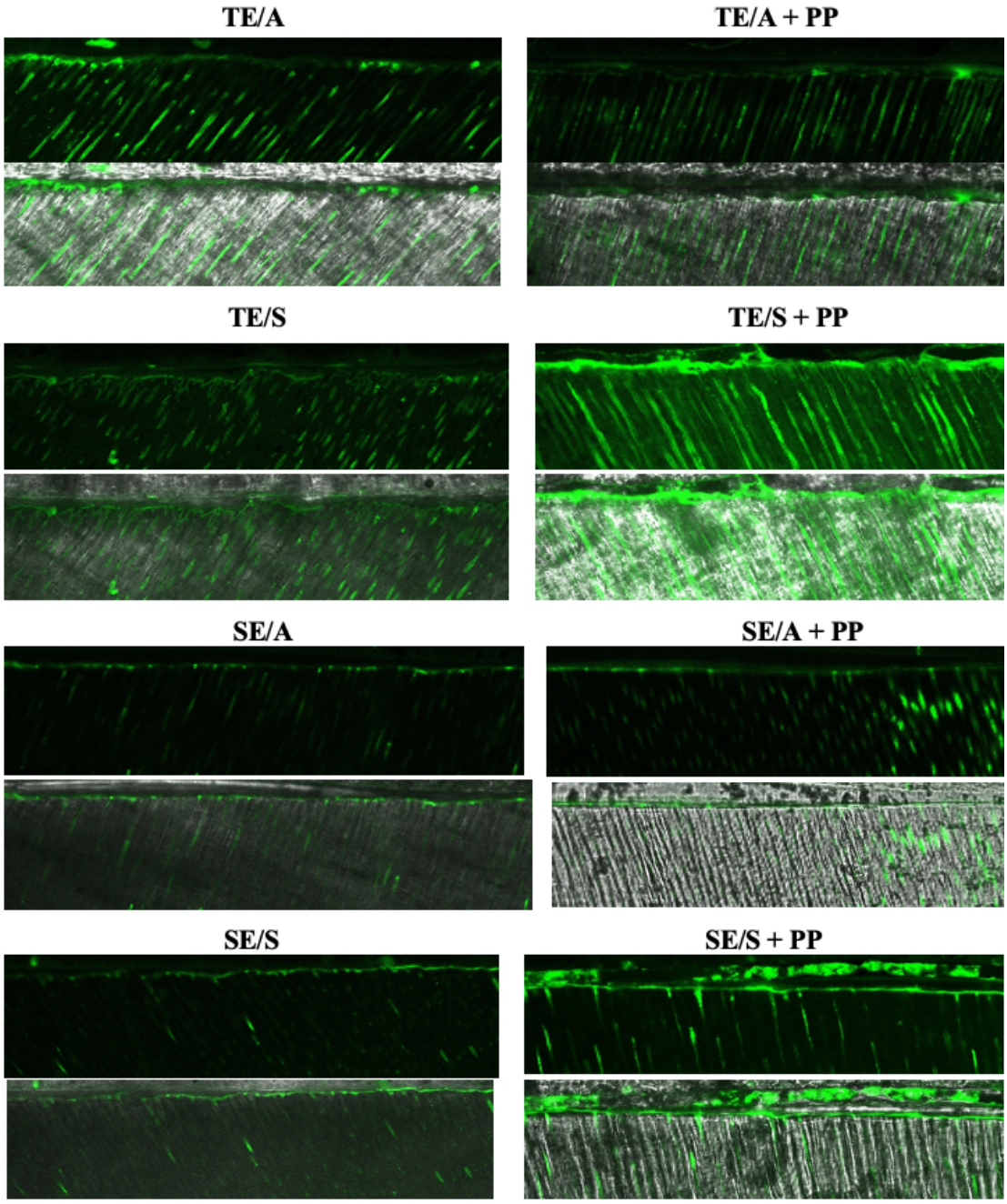


Fig 31: Results of in situ zymography. Each group shows a green fluorescence image indicative of enzymatic activity (above) and another image combining fluorescence with dentin morphology (below).

Evaluation of fiber post adhesion to root dentin achieved with different resin cements: 1-year in vitro results

Aim:

To evaluate push-out bond strength (PBS) and interfacial nanoleakage (NL) of adhesively luted fiber posts using different resin cements and polymerization protocols.

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).

One-hundred extracted, caries-free, mandibular premolars were stored in 0.5% chloramine solution at 4°C for no longer than 2 months after extraction. 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 (RelyX^{TM}, 3M, Neuss, Germany) was inserted into the canal to check if it reached the intended 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=10):

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);

MAX and CAL are self-adhesive resin cements. RXU is defined as universal resin cement that, in the present study, was used in the self-adhesive modality (no previous application of Scotchbond Universal Plus adhesive). 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 etch-and-rinse (E&R) bonding system. The details of fiber post surface pretreatments, chemical compositions and application modes of the cements are shown in Table 18.

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	Self-adhesive resin cement: Dispense in the post space and insert the post.	Clean with alcohol and air-dry for 5 s. Priming with adhesive not

	initiator system, radiopaque fillers and rheological additives, pigments		required for 3M RelyX fiber posts.
Maxcem Elite Chroma, Kerr (LOT 71887933)	HEMA, GDM, UDMA, 1,1,3,3-tetramethylbutyl hydroperoxide TEGDMA, fluoroaluminosilicate glass, GPDM, barium glass filler, fumed silica (69 wt %)	Self-adhesive resin cement: 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.
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 %)	Self-adhesive resin cement: 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	Self-etch 1-step adhesive resin cement: 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	Etch-and rinse 3-step adhesive resin cement: 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 the cement 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.

Table 18. The details of fiber post surface pretreatments, chemical compositions and application modes of the cements.

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, in order to prevent dehydration [127, 128], 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. Six slices from each root were obtained, with first three slices being considered as coronal part, while the last three slices were considered to belong to the apical part. Half of the specimens from each group (N=5) were immediately processed for PBS test (T_0). The other half was stored at 37˚C for 12 months (T_{12}) in 2 ml Eppendorf tubes filled with artificial saliva that was regularly changed every 2 weeks.

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. 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 (8).

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=4 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. NL analysis was performed at the baseline (T_0) and after 12 months (T_{12}) storage in artificial saliva at 37 °C. 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. [129] . 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 [. Briefly, interfacial nanoleakage was scored based on the percentage of the adhesive surface showing silver nitrate deposition: 0, no nanoleakage; 1, < 25% with nanoleakage; 2, 25% to 50% with nanoleakage; 3, 50% to 75% with nanoleakage; and 4, > 75% with nanoleakage [130].

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", "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. 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 or T_{12} are presented in Tables 19 and 20 for the coronal and apical root regions, respectively. The statistical

analysis revealed that the “cement” and “aging” significantly influenced the PBS ($p<0.05$), but not the variables “polymerization protocol” and “root region” ($p>0.05$). The interactions between the variables were not statistically significant ($p>0.05$). 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 ($p<0.05$). This was particularly evident at T_{12} in the SC mode, where RXU showed higher bond strength compared to the other investigated systems, especially in the apical root region ($p<0.05$). After artificial aging, PBS values increased in the majority of the investigated groups, irrespective of the root region ($p<0.05$).

The percentage of the types of failure mode within each group is presented in Table 21. 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 at T_0 for MAX SC, CAL SC e MUL SC, and at T_{12} for RXU both in the LC and SC groups. No cohesive fractures were detected.

Groups	**T_0**		**T_{12}**	
	LC	**SC**	**LC**	**SC**
RXU	16.5±3.7 ^{bA}	15.0±4.3 ^{bA}	23.3±6.0 ^{aA}	28.9±6.5 ^{aA}
MAX	15.6±4.6 ^{aA}	19.6±3.1 ^{aA}	16.1±6.0 ^{aB}	19.7±8.7 ^{aB}
CAL	8.6±4.5 ^{cB}	14.7±6.1 ^{bAB}	24.5±3.6 ^{aA}	16.4±7.0 ^{bB}
LUX	17.4±5.4 ^{bA}	12.6±3.0 ^{bcB}	30.2±9.5 ^{aA}	21.6±5.9 ^{bAB}
MUL	18.4±6.2 ^{aA}	20.4±7.3 ^{aA}	25.0±8.6 ^{aA}	22.8±6.5 ^{aAB}

Table 19: Push-out bond strength values with standard deviations (MPa) in coronal section at T0 and T12. Different superscript letters indicate significant differences. Lower case letters refer to differences within the rows, upper case letters refer to differences within the columns. LC – light cure; SC – self-cure.

Groups	**T_0**		**T_{12}**	
	LC	**SC**	**LC**	**SC**
RXU	17.7±6.6 ^{bA}	19.9±4.8 ^{bA}	27.7±10.9 ^{aA}	30.3±7.8 ^{aA}
MAX	13.1±7.3 ^{bB}	23.3±3.3 ^{aA}	26.2±5.1 ^{aA}	20.9±7.3 ^{aB}
CAL	5.9±3.9 ^{bC}	8.1±2.6 ^{bB}	16.5±6.3 ^{aB}	14.8±8.9 ^{aC}
LUX	18.7±6.7 ^{bA}	20.4±3.3^{bA}	31.4±8.3 ^{aA}	21.6±12.0 ^{bB}
MUL	19.0±4.5 ^{bA}	19.9±6.2 ^{bA}	26.2±10.9 ^{aA}	16.8±5.9 ^{bB}

Table 20: Push-out bond strength values ± standard deviations (MPa) in apical section at T0 and T12. Different superscript letters indicate significant differences. Lower case letters refer to differences within the rows, upper case letters refer to differences within the columns. LC – light cure; SC – self-cure.

Groups	**T_0**		**T_{12}**	
	LC	**SC**	**LC**	**SC**
RXU	M: 52 AP: 48 AD: 0 CC: 0 CP: 0	M: 60 AP: 40 AD: 0 CC: 0 CP: 0	M: 50 AP: 16.6 AD: 33.4 CC: 0 CP: 0	M: 36.3 AP: 9 AD: 54.7 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	M: 54.5 AP: 45.5 AD: 0 CC: 0 CP: 0	M: 70 AP: 30 AD: 0 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	M: 85.5 AP: 14.5 AD: 0 CC: 0 CP: 0	M: 65 AP: 35 AD: 0 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	M: 36.3 AP: 63.7 AD: 0 CC: 0 CP: 0	M: 66.6 AP: 33.4 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	M: 53.3 AP: 46.7 AD: 0 CC: 0 CP: 0	M: 76.9 AP: 23.1 AD: 0 CC: 0 CP: 0

Table 21: Failure mode of the dislodged specimens from five experimental groups at baseline (24h) and after one year of aging in artificial saliva. Data are expressed as percentages (%) of the total number of specimens tested for each group. Abbreviations: AD - adhesive, between dentin and the

cement; AP - adhesive between the cement and the post; CC - cohesive within the cement; CP - cohesive within the post and M-mixed.

Interfacial nanoleakage expression

Descriptive statistics of interfacial NL scores within the groups in the experimental conditions are presented in Figures 32 and 33 at T_0 and T_{12}, respectively. The statistical analysis showed differences in the interfacial silver deposition among the tested groups, and this was material-dependent ($p<0.05$). At T_0, 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 ($p<0.05$).

After 12 months of artificial aging, differences in marginal infiltration among the tested groups were still present ($p<0.05$). In general, the aging process produced an increase in marginal infiltration and results were as follows: MAX = CAL > MUL = LUX > RXU ($p < 0.05$), irrespective of the polymerization condition and root region. Representative images of NL expression analyses are shown in Figures 34 and 35 (at baseline and after 12 months, respectively).

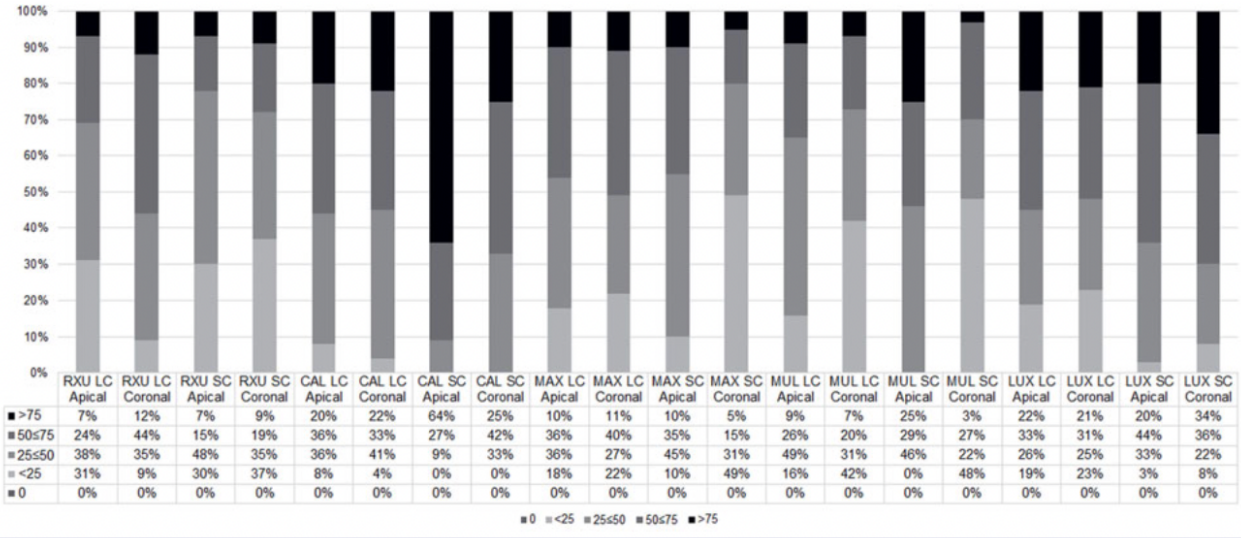


Fig. 32. Percentage of interfacial nanoleakage expression in resin-dentin interfaces created in radicular dentin at T0.

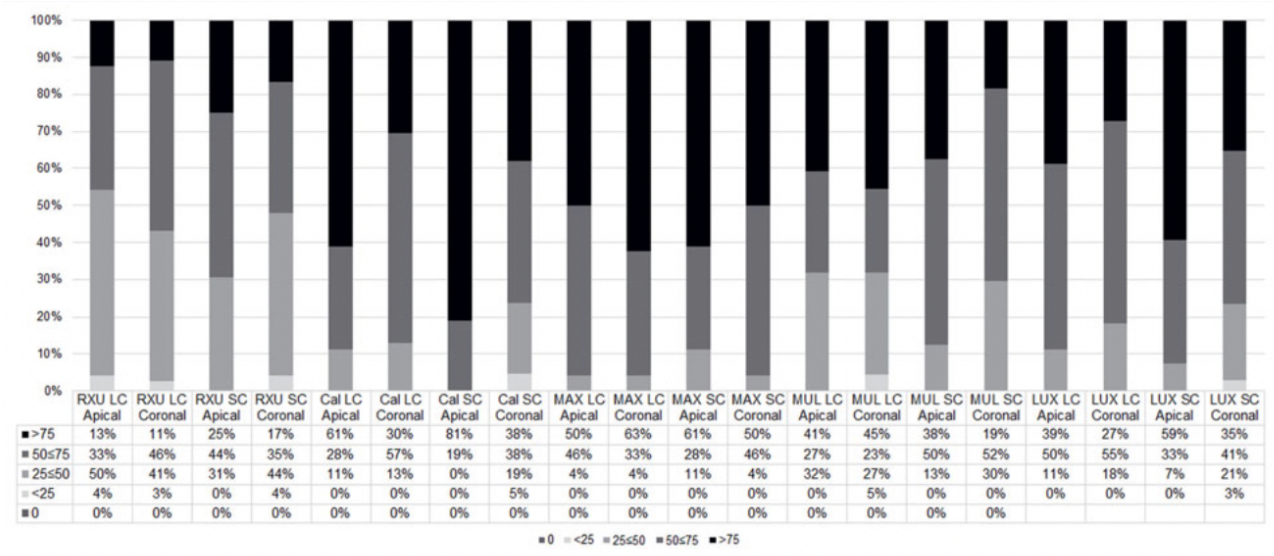


Fig. 33. Percentage of interfacial nanoleakage expression in resin-dentin interfaces created in radicular dentin at T6.

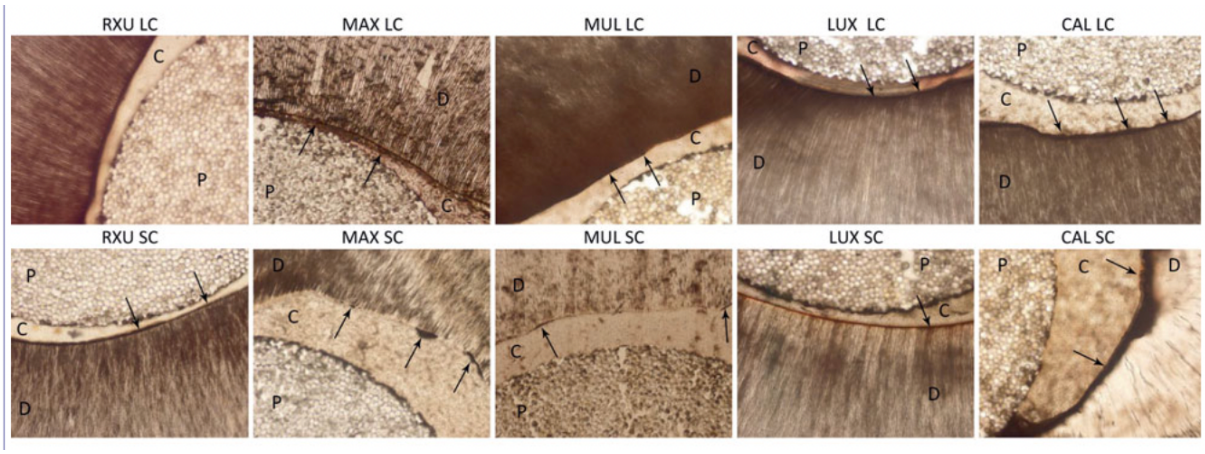


Fig. 34. Light micrographs showing adhesive interface created by different composite cements at T0. Top row: coronal root slices; bottom row: apical root slices. Arrows indicate silver nitrate deposition; D, dentin; P, post; C, cement.

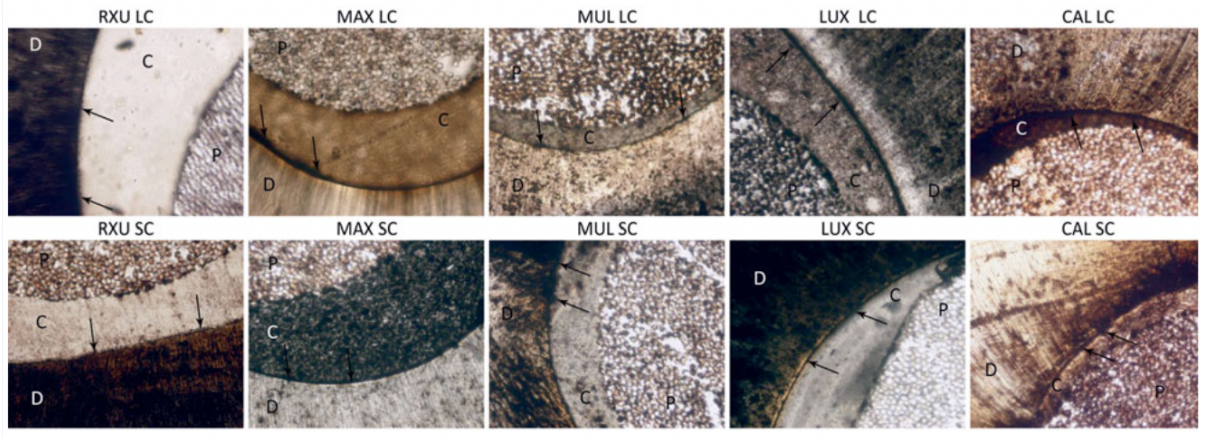


Fig. 35. Light micrographs showing the adhesive interface created by different composite cements at T12. Top row: coronal root slices; bottom row: apical root slices. Arrows indicate silver nitrate deposition; D, dentin; P, post; C, cement.

Effect of resin cement strategy on bonding performances of orthodontic bracket and tooth color changes.

Aim:

To evaluate the shear bond strength (SBS) of different cementation techniques for luting orthodontic brackets. Additionally, the amount remaining resin cement after testing and different material removal techniques were compared.

Matherials and metods:

Collection, storage and preparation of the teeth:

Forty sound human premolars were collected for the study. The criteria for tooth selection included intact enamel with no fractures due to extraction (visible under 4x magnifications), no previous restorations and no carious lesions. After extraction, the teeth were washed under running water, stored in distilled water at 4°C and used within six months after extraction. The water was replaced every two weeks.

Specimens' preparation:

Before the bonding procedures, the selected teeth were polished with non-fluoridate pumice (Super Polish, Kerrhawe, Bioggio, Switzerland) and a prophylaxis brush mounted on a low-speed handpiece for 20 s, rinsed with water for 20 s and air-dried for 10 s. One type of orthodontic bracket was used: a maxillary premolar stainless steel bracket of size 0.022" slot (Mini Diagonali MBT system, width 3.4 mm x height 2.9 mm o surface area 9.86mm2, Leone S.p.A., Florence, Italy). Each specimen was numbered from 1 to 40 with an incision made with a diamond bur. A digital three-dimensional scan (T0) of each tooth was taken in STL format (TRIOS 3 basic, 3shape, Copenhagen, Denmark) (Fig. 36. a). At this point, teeth were randomly divided into 4 groups (N=10).

TXT group: Control group. Brackets were bonded with a traditional orthodontic cement: enamel surfaces were treated with 37% H3PO4 (Scotchbond Universal Etchant, 3M ESPE, Saint Paul, MN, USA) for 30 s ,rinsed with water for 30 s and air-dried. The adhesive was applied with a microbrush (Transbond XT Light Cure adhesive primer, 3M) and brackets cemented with Trasbond

XT (3M). The bracket was placed on the tooth, pressed firmly on the enamel surface , the excesses were gently removed with a spatula and then it was light cured with a LED lamp (Ortholux Luminous Curing Light, 3M) for 40 s (20 s on the mesial side and 20 s on the distal side).

RXU E&R group: Brackets were bonded using a universal resin cement (RelyX Universal, 3M) with a universal adhesive used in the etch-and-rinse mode (Scotchbond Universal Plus Adhesive, 3M) applied with a microbrush. The enamel surfaces were treated with 37% H3PO4 (Scotchbond Universal Etchant, 3M) for 15 s, rinsed with water for 30 s and air-dried. Brackets were then light cured with a LED lamp (Ortholux Luminous Curing Light, 3M) for 40 s (20 s on the mesial side and 20 s on the distal side).

RXU SE group: Brackets were luted with RelyX Universal used in combination with the universal adhesive used in the self-etch mode.

RXU group: Brackets were luted with RelyX Universal used in the self-adhesive mode.

A single operator performed the luting procedures.

After bonding, the specimens were stored in artificial saliva at 37 °C for 24 h, before being submitted to the shear bond strength test (SBS).

After 24 h, each tooth was mounted on a light cured tray material (Impression Tray Resin LC, Henry Schein, Melville, NY, USA) incorporated into a PVC jig to block the position of the dental elements with the horizontal lines of the bracket parallel to the ground and the bracket base perpendicular to the worktop.

Shear bond strength test (SBS):

The SBS test was conducted with an Instron Universal Testing machine (Instron, Norwood, MA, USA). Each specimen was positioned in the machine to secure the bracket base parallel to the force direction. A chisel-shaped blade was placed between the bracket base and the vestibular surface of the tooth in its maximum curvature. The shear force was exerted through the chisel-shaped blade in an occlusal-gingival direction. The shear force was applied at a crosshead speed of 1mm per min until bracket failure (in accordance with the ISO protocol which provides a crosshead speed of 0.75 ± 0.3 mm per min or a load of 50 ± 2 N per min).

The force required to detach the bracket was recorded in Newtons (N) and then converted in MPa. After debonding, each tooth surface and each bracket base was examined under an optical microscope at 20× magnification. The amount of adhesive remnant was assessed with the aid of a digital image processing software ImageJ (National Institutes of Health, USA) using the adhesive remnant index (ARItooth).

The ARItooth was ranked from 0 to 3, as follows:

-0: no adhesive present on the enamel surface.

-1: less than 50% adhesive on enamel.

-2: more than 50% adhesive on enamel.

-3: 100% adhesive on enamel (the mark of the bracket's mesh is clearly visible).

A second digital three-dimensional scan (T1) of each model was taken in STL format (TRIOS 3 basic, 3shape, Copenhagen, Denmark) (Fig. 36. b) to superimpose them with the scans performed at T0.

Additional 2 teeth per group were randomly selected to be evaluated under a Scanning Electron Microscopy (SEM) to evaluate the enamel surface morphology after testing.

Adhesive removal:

Teeth were randomly divided into two subgroups to evaluate two different adhesive removal techniques (n=5): A) contouring and polishing coarse discs (Sof-Lex, 3M) rotating on low-speed contraangle handpiece; and B) tungsten carbide multi-laminated high-rotation drill. In group A, the disc was positioned perpendicular to the long axis of the tooth and lateral movements in the mesiodistal direction of the crown were performed. In group B the same movements were performed but the drill was positioned parallel to the long axis of the tooth.

After removal of the adhesive remnant from the enamel surface, a third digital three-dimensional scan (T2) of each model was taken in STL format (TRIOS 3 basic) (Fig. 36. c).

Once STL 0-2 were obtained, a geomorphometric software (3D Geomagic Capture Wrap, 3D Systems©, Rock Hill, SC, USA) was used to superimpose the scans.

A Scanning Electron Microscopy (SEM) evaluation was performed at T2 to evaluate the effects of polishing methods on the enamel surface morphology.

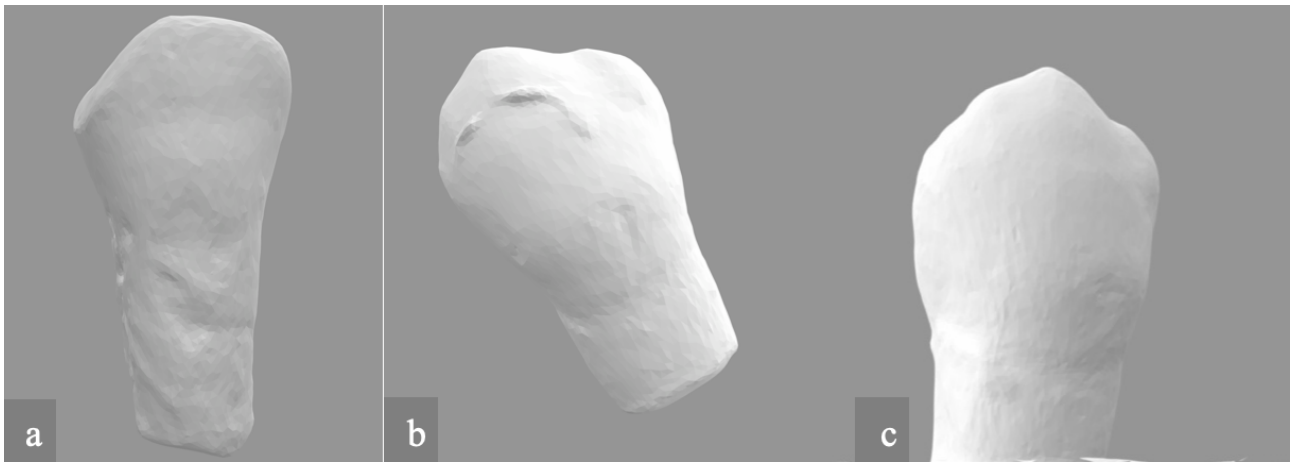


Fig. 36. a): digital three-dimensional scan at T0; b): digital three-dimensional scan at T1; c): digital three-dimensional scan at T2.

Colorimetric Analysis:

The color stability of enamel during the different operative stages was analyzed with the SpectroShade Micro spectrophotometer (Medical High Technologies, Verona, VR, Italy). The analysis was carried out at room temperature under natural light. The spectrophotometer device used for colorimetric analysis was calibrated before each color measurement for every tooth. Each tooth was analyzed before and after debonding of brackets and after the polishing procedures. All color measurements were done by one operator and repeated three times per surface. The CIELAB analysis was used to calculate color differences among the groups. The output of the colorimetric analysis were the following data: L*, a* and b*. Inserting these data in the following formula, a value demonstrating the difference between the two investigated moments was obtained (deltaE).

Statistical analysis:

The data on shear bond strength, adhesive removal techniques' efficiency and on the color changes after the bracket debonding procedure and after cement removal have been tested for normality (Shapiro-Wilk test), and homogeneity (Brown-Forsythe test). Since the bond strength and color change data did not pass one or both of these requirements, they were analyzed using Kruskal-Wallis and post-hoc Dunn's test. The data regarding the efficiency of cement removal techniques were normally distributed and homogenous and were therefore analyzed using the two-way ANOVA test (main factors "Cementation technique" and "Removal technique") and pairwise multiple comparison test (Holm-Sidak). All the analyses were performed using the SigmaPlot software v. 14.0 (Systat Software Inc., Berkshire, UK) and the level of significance was set at $\alpha=0.05$.

Results:

The mean bracket bond strengths and standard deviations of the four groups are presented in Table 1. The differences in the median values among the treatment groups was greater than would be expected by chance; there was a statistically significant difference ($P < 0.001$). The highest bond strength was achieved in the RXU E&R group with significant differences from RXU SE and RXU groups (respectively $P=0.020$ and $P<0.001$) but statistically similar to TXT group ($P=0.979$). The lowest strength was obtained in RXU in self-adhesive mode with no significant difference from RXU

SE group (P=0.651). The control group (TXT) yielded a significantly higher strength compared to the RXU group used in the self-adhesive mode (P=0.001), but no significant differences were observed from RXU SE group (P=0.062).

The distribution of mean values and standard deviations of the superimposition of the scans of the four groups after the cement removal using two different techniques is shown in Table 2.

In T1, the differences in the mean values among the treatment groups were greater than would be expected by chance, there was a statistically significant difference (P = 0,016). The highest amount of adhesive remnant on the enamel surface was shown in RXU E&R group while the lowest one in RXU SE group with a significant difference between them (P = 0,023). There was a significant difference also between RXU E&R group and RXU group (P = 0,045). No significant difference was observed between control TXT group and RXU E&R group (P = 0,169), RXU SE group (P = 0,653), or RXU group (P = 0,794). No significant difference was found between RXU SE and RXU groups (P = 0,624).

In T2, the difference in the mean values among the different levels of cementation techniques was not great enough to exclude the possibility that the difference was just due to random sampling variability after allowing for the effects of differences in removal techniques. There was not a statistically significant difference (P = 0,094). Conversely, the difference in the mean values among the different types of removal techniques was statistically significant (P = 0,002). Finally, there was not a statistically significant difference between the interaction of cementation techniques and removal techniques (P = 0,809).

Mean values and standard deviation of the tooth color change after bracket removal is presented in Table 4, while the same values about the tooth color change after bracket removal and after the adhesive remnant removal were reported in Table 5. The data was analyzed statistically ($P < 0.05$) and no statistically significant difference was found between cementation, removal techniques and enamel color changes ($P > 0.05$).

Group	Mean	Standard Deviation
TXT	134.9[a, b]	±71.2
RXU E&R	156.0[a]	±81.8
RXU SE	54.3[b,c]	±29.7
RXU	33.4[c]	±26.7

Table 19 – Mean shear bond strength and standard deviations among the groups. TXT: phosphoric acid etching for 30s + Trasbond XT adhesive primer and cement; RXU E&R: phosphoric acid etching for 15s + Scotchbond Universal Plus adhesive + RelyX Universal cement; RXU SE: Scotchbond Universal Plus adhesive in self-etch mode + RelyX Universal cement; RXU: RelyX Universal cement

in self-adhesive mode. Different superscript letters indicate statistically significant differences among the groups ($p<0,05$).

Group	T1	
	Mean	Std Dev
TXT	$0.812^{a,b}$	±0.871
RXU E&R	1.426^{a}	±0.556
RXU SE	0.482^{b}	±0.345
RXU	0.633^{b}	±0.657

Table 20 – Superimposition of tooth scans at baseline and after debonding (T1).

Group	Removal technique			
	Sof-Lex		Tungsten	
	Mean	Std. Dev	Mean	Std. Dev
TXT	0.304^{a}	±0.275	0.161^{b}	±0.171
RXU E&R	0.346^{a}	±0.222	0.147^{b}	±0.0349
RXU SE	0.399^{a}	±0.196	0.0705^{b}	±0.00493
RXU	0.583^{a}	±0.301	0.315^{b}	±0.199

Table 21 – Superimposition of tooth scans at baseline and after removal with two different techniques (T2)

Group	Mean	Standard Deviation
TXT	4.385	±3.148
RXU E&R	5.070	±2.205
RXU SE	2.537	±1.457
RXU	3.215	±1.017

Table 22 – Tooth color changes after debonding (deltaE). TXT: phosphoric acid etching for 30s + Trasbond XT adhesive primer and cement; RXU E&R: phosphoric acid etching for 15s + Scotchbond Universal Plus adhesive + RelyX Universal cement; RXU SE: Scotchbond Universal Plus adhesive in self-etch mode + RelyX Universal cement; RXU: RelyX Universal cement in self-adhesive mode.

Group	Mean	Standard Deviation
TXT + SF	4.027	±2.075
TXT + Tung	6.612	±3.613
RXU E&R + SF	4.803	±2.638
RXU E&R + Tung	5.336	±1.950
RXU SE + SF	6.350	±3.901
RXU SE + Tung	5.236	±4.338
RXU + SF	7.297	±5.537
RXU + Tung	5.718	±4.397

Table 23 – Tooth color changes after adhesive removal procedures . TXT: phosphoric acid etching for 30s + Trasbond XT adhesive primer and cement; RXU E&R: phosphoric acid etching for 15s + Scotchbond Universal Plus adhesive + RelyX Universal cement; RXU SE: Scotchbond Universal Plus adhesive in self-etch mode + RelyX Universal cement; RXU: RelyX Universal cement in self-

adhesive mode. SF: Sof-Lex discs rotating on low-speed contra angle handpiece; Tung: tungsten carbide multi-laminated high-rotation drill.

Discussion

Adhesive dentistry considerably change in the last decade, with the aim of assure good bond strength and reproducibility, so less steps and ease of application. Universal adhesive became a very popular system, it can be applied in different ways, so it was necessary in our opinion to enhance the correct protocol to follow when an universal adhesive was used in clinical practice. With the first two systematic reviews we want to clarify the state of the art of the etching mode of universal adhesive in non retentive cavities and we found out that universal adhesives in the EAR mode could lead to better medium-term retention, while the use of the SE adhesives could lead to less immediate POS and therefore better short-term patient satisfaction, the SEE approach was comparable with the EAR approach in terms of retention and POS, finally we can assume that SEE is the best approach to practice. Then we conducted three in vitro studies in order to analize the evaporation mode, the adhesion on radicular dentine, and finally adhesion of brackets. Regarding the evaporation mode, air-drying performed better than suction and the self-etch mode seem to be more reliable choices when an ethanol-based universal adhesive is used. About the adhesion of fiber post on root canals, it can be deduced that the choice of resin cement, rather than polymerization protocol, influenced fiber posts' retention in root canal. Finally for adhesion of brackets on enamel, E&R mode improves bond strength values.

More specifically, organizing RCTs to evaluate clinical behavior of resin-based restorations placed in NCCLs using different adhesive strategies is considered to be state of the art. Earlier systematic reviews analyzed the clinical performance NCCLs restored with EAR or SE adhesive systems and SE adhesives in two different etching modes (SE or SEE). However, to the best of our knowledge, no systematic reviews analyzing clinical trials in which universal adhesives were used for restoring NCCLs have been published so far. Therefore, by conducting a systematic review with meta-analyses, we synthetized the data from the available RCTs and sought to investigate which adhesive strategy should be employed in order to optimize clinical performances of composite restorations placed with this category of adhesive systems.

The results of our study revealed that the loss of retention is not significantly influenced by the adhesive strategy at 6-months follow-up (low certainty of evidence). On the contrary, significant difference was observed for 12- and 18/24- months with a moderate certainty of evidence, with SE group being exposed to increased likelihood for loss of retention when compared to EAR group. Even though the trend towards increased risk of retention loss was expected to be found with a longer follow-up period, no difference was observed at 36-months recall. However, this result must be

interpreted with caution since low certainty of evidence was present at 36-months evaluation, meaning that the true effect might be markedly different from the estimated one.

The fact that higher retention rates were observed when universal adhesives were used in EAR compared to SE mode may be explained by the morphology and configuration of NCCLs. The margins, or at least a part of NCCLs is located in enamel, and it is well known that it is easier to achieve predictable bonding to enamel compared with dentin, due to the differences in the composition of these two tissues. Furthermore, our meta-analysis results showed no differences for the risk of retention loss between EAR and SEE groups. This suggests that SEE mode may be an alternative approach to EAR mode, since the application of phosphoric acid is limited to enamel only, therefore leaving behind mineralized dentin. This strategy enables Ca-salts to be embedded within the hybrid layer, and when using adhesives that contain 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) as a functional molecule, common for universal adhesives used in the present systematic review, it may lead to the formation of stable MDP-Ca salts which provide clinical durability of the hybrid layer.

Contrary to what might have been expected, the results of our meta-analysis revealed that the choice of the adhesive strategy (EAR vs. SE) did not have an influence on marginal discoloration at any of the follow-up periods.

Furthermore, although it seems that applying universal adhesives in EAR mode offers no advantage over SE mode, closer look should be given to the certainty of evidence tool and the length of the follow-up periods. Low certainty was seen for 6- and 12- months follow-up, while moderate level with very few events was observed for 18/24 months. Besides low and moderate certainty of evidence observed at these short- and medium-term follow-ups, the literature suggests that it may take more than 5 years to observe a significant number of events between the treatment groups in clinical settings. Unfortunately, we could not run a meta-analysis for long-term follow-ups since only one study [8] evaluated the NCCL restorations after 5 years of clinical function, and found superior clinical performance for EAR and SEE compared to SE strategy. Another factor to be considered is that marginal discoloration, assessed by the FDI and USPHS criteria, was not evaluated separately between dentin and enamel margins, as suggested by Cieplik et al. (2017), thus potentially masking differences between different adhesive strategies [131].

POS is a clinical parameter widely discussed among clinicians since it can cause patients' dissatisfaction and sensibility [132]. Despite the large interest, this clinical parameter has not always been studied in previous systematic reviews that analyzed different types of adhesives employed in resolving the problem of NCCLs [133, 134], and neither was it addressed in a recent systematic review which evaluated the influence of etching mode (SE vs. SEE) for NCCLs restored with SE

adhesives [135]. As far as the authors of this paper are aware, the only systematic review that analyzed POS after placing composite restorations in NCCLs found no differences when EAR were compared to SE adhesive systems [136]. However, unlike the previous review [136] in which dichotomous data from 19 studies, irrespective of the POS assessment method, were used to run a single meta-analysis, we performed separate meta-analyses, distinguishing the data based on the way in which POS was estimated and taking into account the adhesive strategy. We opted to investigate POS only at baseline, since this it clinically most often occurs only within the first week following the intervention. Our results for subjective POS are in line with earlier conclusions [136], since no difference was observed when universal adhesives were employed in the EAR and SE mode for restoration of cervical lesions. However, an interesting finding from our study was that EAR groups had higher risk for objective POS occurrence than SE groups. Contrary, no differences in terms of POS when EAR and SE adhesives were used for restoration of posterior cavities has been reported in the literature [137], and the choice of the adhesive strategy (EAR or SE) seemed to play no role in POS occurrence in NCCLs restorations [136]. Therefore, this may be the first systematic review which reported, with moderate level of evidence, that the choice of adhesive strategy could influence objective POS when universal adhesives are used for NCCLs restoration, suggesting that SE approach could be more appropriate than EAR when aiming to reduce POS sensitivity during NCCLs restoration.

One of the main remarks of evaluating POS by applying a stimulus is that it serves rather as pulp vitality indicator and that the absence of preoperative POS may change due to the adhesive procedure and become detectable on stimulus after the restoration has been placed [138]. However, the primary studies included in our meta-analysis involved (in various percentage) NCCLs which already exhibited baseline preoperative sensitivity, thus it is not likely that the reported POS sensitivity occurred due to the restorative procedure. Regardless of potential drawbacks for POS assessment by applying a stimulus, we observed higher risk for POS occurrence in EAR groups, most probably due to the fact that phosphoric acid partially or even completely dissolved the hypermineralized layer within NCCLs [139].

Generally, RCTs included in this systematic review demonstrated no major concerns considering the risk of bias assessment. The random allocation sequence took place in all reviewed RCTs, but the lack of clear reporting of allocation concealment, blinding of participants and/or evaluators led to classifying some domains as “unclear” (Figure 2). Furthermore, we ranked 3 articles [62, 117, 118] as “unclear” for attrition bias, since more than 20% of patients were lost and no intention-to-treat analysis was reported to had been performed. Traditional understanding suggests that patient drop-out rate higher than 20% may represent a serious threat to study’s validity. [140] Despite this belief, our decision to score attrition bias domain as “unclear” instead of “high” was

based on the fact that the split-mouth design was employed in all RCTs, and consequently, the patient drop-out led to the balanced loss of restorations across the groups [141].

Lastly, one of the novelties of this review compared to the previous ones [135, 136,137] was the implementation of certainty of evidence that was assessed according to the GRADE tool. The benefits of introducing GRADE assessment is that it provides assessments about the quality of evidence for each outcome in a transparent manner, and may differ for the same outcome at various follow-up periods depending on inconsistency, indirectness and imprecision. One of the limitations of this review is that our conclusions are drawn from meta-analysis performed for short- and medium-term follow-up periods (the longest follow-up was 36 months). Another limitation is that direct comparison between SE and SEE strategy was not performed, as it would have led to a less focused PICOS question. The rationale for comparing EAR with SE or SEE mode lies in the fact that when using universal adhesives in EAR mode dentin is etched, while it is left unetched in both SE and SEE strategy. In future, it would be of interest to conduct systematic reviews that compare the influence of SE and SEE strategy on clinical performance of composite restorations placed in NCCLs with universal adhesives and include RCTs with follow-ups longer than 5 years. [142]
Continuing analize NCCLs, due to their non-retentive cavity shape, we conducted a systematic review to answer the question “Is the risk of retention loss equal for SEE and SE approach when universal adhesives and composite restorations are indicated for restoring NCCLs?”

To the best of our knowledge, only one systematic review [71] included RCTs which compared EAR to SEE or SE strategy when universal adhesives and composite restorations are indicated for restoring NCCLs. However, the previous review did not make a distinct comparison between SEE and SE strategy, thus leaving doubts for clinicians who prefer to leave the dentin surface unetched and should decide whether or not enamel should be conditioned with phosphoric acid.

The most important finding of our meta-analyses concerns clinical outcome retention. According to the results, retention of composite restorations at 6- and 18/24- months was improved when universal adhesives were used in SEE mode. The explanation for this may lie in the fact that, when using universal adhesives in SEE mode, an additional etching step with phosphoric acid is performed solely on enamel. It is well known that, in most cases, borders of the cavity of an NCCL are located in enamel [143]. The mechanism responsible for successful bonding on enamel is based on the micro-mechanical interlocking of the adhesive’s resin tags within the micro-sized porosities in the structure of enamel, created by phosphoric acid [18]. Although designed to be used on enamel in both EAR and SE mode, it seems that the enamel demineralization potential of universal adhesives is not sufficient to achieve optimal adhesion without an additional etching step. This conclusion was derived from a systematic review that included in vitro studies investigating the bonding performance

of universal adhesives [144] and can be extrapolated to clinical settings since the greater loss of retention was observed when universal adhesives were used in SE mode.

The common characteristic of the application of universal adhesives in SE and SEE mode is that the dentin surface is left unetched. This is of particular importance for the bonding mechanism of universal adhesives to dentin, due to the specific chemistry of this generation of adhesives. The incorporation of functional monomers, the most widely discussed one being 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), allows chemical interaction with dental tissues [145]. Briefly, 10-MDP is able to ionically interact with hydroxyapatite within dentin and form hydrolytically stable MDP-Ca salts. When using universal adhesives in SE mode, the Ca salts are embedded in the hybrid layer, unlike with the EAR protocol where they are rinsed off. This way the formed MDP-Ca salts can preserve the integrity of hybrid layers and contribute to the longevity of the resin-based restorations. The described process is known as nanolayering [146] and might be responsible for the good clinical performance of adhesives containing 10-MDP [63, 67, 108, 109, 110, 116, 122]. However, it should be underlined that SEE is a technique-sensitive clinical procedure and that, in order to benefit from nanolayering, the etching step should be limited to enamel only. If accidentally applied on dentin and left for longer than 15 s, it can cause over-etching with consequent problems associated with incomplete resin infiltration and POS [29, 147]. On the other hand, if applied insufficiently on the enamel surface (i.e. short etching time or incomplete enamel coverage with etching agent), inferior adhesion can occur [148]. Since all RCTs included in this review were conducted in university settings by well-trained and calibrated operators, it can be presumed that the SEE protocol was performed precisely, thus combining the beneficial effect of applying universal adhesives on etched enamel and unetched dentin.

POS is a clinical parameter widely investigated in clinical trials and discussed among dentists since it can lead to patients' discomfort and dissatisfaction [137]. The studies included in this systematic review reported no differences in terms of POS occurrence when comparing SE to SEE approach. Furthermore, since during data extraction process events were observed in only one study [109], the meta-analysis was not performed. Interestingly, a meta-analysis previously conducted by this research group found a higher risk for POS occurrence in EAR groups compared to SE and SEE groups when universal adhesives were used for restoring NCCLs with composites [71]. It seems that the POS development after the application of universal adhesives is mainly due to the additional etching step of dentin and subsequent removal of smear layer and opening of dentinal tubules, with the risk of incomplete resin penetration or dentin over-drying (EAR mode) [139]. On the other hand, when dentin is left unetched (SE and adequate SEE technique) universal adhesives rely on creating a hybridized complex that consists of the residual smear layer and partly demineralized dentine

collagen matrix, therefore reducing the risk associated to the EAR strategy that can cause POS [149,150].

A surprising finding of our meta-analyses was that no significant differences were observed in terms of marginal discoloration between SEE and SE groups. Contrary to our findings, an earlier systematic review reported reduced marginal discoloration when self-etch adhesives were used in SEE mode [135]. Some authors suggest that universal adhesives are essentially self-etch systems provided in one bottle, with no major improvements in overcoming the challenges associated with previous generations of adhesive systems [149]. Considering this, it was expected to detect more marginal discoloration when universal adhesives were used in SE mode because of the reduced etching capacity to enamel when acid etching step is left out and possible debunking of margins with consequent accumulation of pigments from food sources and bacterial biofilm [151]. However, perhaps the previously explained mechanism of chemical binding of functional monomers to the hydroxyapatite in enamel, (and differences in the composition) could have contributed to a lower level of marginal discoloration when universal adhesive systems were used as compared to the purely self-etch systems. Further, in clinical settings, it would be expected that marginal discoloration or adaptation issues precede loss of retention. However, they cannot be evaluated in cases where there was retention loss at the follow-up. Hence, we can hypothesize that marginal discoloration might have occurred during the 6-months or 1-year follow-up intervals, but was underestimated at the controls in cases where loss of retention already occurred. Additionally, the analyzed follow-up periods can be considered as short- and middle- term, and it is well known that it can take more than five years of clinical service to observe potential differences between the treatment groups [143, 152].

The present systematic review and meta-analysis included the GRADE assessments, often omitted in the published/available literature. The advantage of the inclusion of a GRADE system in a systematic review is that it allows judgment about the quality of evidence for an effect estimate in a systematic and transparent way. GRADE assessments have direct implications for both practice and research, with certainty of evidence assessments highlighting where the evidence base is adequate or where either more or better research is needed. In the GRADE system, RCTs start as high quality, and factors that can lower the quality of evidence are: risk of bias, inconsistency, indirectness, imprecision and publication bias. The most common reason for downgrading the quality of evidence in this systematic review was due to the study design (most of the data included in the meta-analysis came from the studies rated as “unclear” risk of bias, Tables 2, 3, 4, 5, 6, 7, 8 and 9), inconsistency (95% confidence intervals did not overlap, Tables 1, 2, 4, 6, 9, 7) and imprecision (wide 95% confidence intervals with few events, Tables 2, 4, 5, 6, 7, 9). Finally, GRADE is also a transparent process of moving from evidence to recommendations which should answer the initial clinical

question. Considering this, we suggest that, when restoring NCCLs, clinicians might consider applying universal adhesives in SEE mode since it could lead to more predictable retention compared to SE approach up to 2 years of follow-up.

One of the potential limitations of this review is that the meta-analyses were performed for short- (6 months) and medium-term (up to 3 years) follow-up periods. This was due to the fact that only one RCT [109] with a follow-up period of five years was found in the literature. Future RCTs should be performed with follow-ups longer than five years in order to detect possible differences in clinical behavior of composite restorations placed in NCCLs using universal adhesives in various application modes.

After having analyzed the etching mode of universal adhesive, we decided to focus our research work on the evaporation mode and on trying to reproduce the pulp flow of a vital tooth with the simulated pulpal pressure. We didn't find specific information in the literature so we conducted an in vitro sperimental study and we assess this null hypothesis "different type of bonding evaporation don't affect the bond strength and the enzymatic activity of a universal adhesive to dentin; and secondary the presence of pulpal pressure doesn't affect the bond strength and the enzymatic activity of an universal adhesive to dentin."

The main purpose of this study was to evaluate the effect of different evaporation technique of a ethanol-based universal adhesive in terms of bond strength and endogenous enzymatic activity to dentin in presence or not of intrapulpal pressure. The first null hypothesis was in part rejected because different evaporation mode affect the bond strength but not the enzymatic activity. Instead the presence of pulpal pressure affects both the bond strength and the enzymatic activity, therefore the second null hypothesis was completely rejected.

The universal adhesive that we use in this study attributed his quick action to his particular composition: lower HEMA content, higher purity of the functional monomer 10-MDP and the new acrylamide monomer technology, which was claimed to improve the adhesive's infiltration properties, together with the ethanol-based primer. (71, 72) The ethanol-based primer seems to prevent the long- term degradation of resin–dentin bonds: after rinsing the acid-etched cavity preparation with water, the water is replaced with an ethanol-based primer, the purpouse is to replace all water in interfibrillar spaces and in the tops of the dentinal tubules, with ethanol. (19)

During the adhesive procedures one of the main objectives is the control of the humidity to prevent the accumulation of water and solvent residues and decrease the permeability of the hybrid layer, it is fundamental to reduce the water sorption to avoid the hydrolytic degradation of the adhesive-dentin interface. (5) This permeability is the result of a non-correct evaporation of the adhesive solvent, that

is difficult to reach moreover in one-step adhesive, where there is a high concertation of solvent to reduce hydrophilic and hydrophobic monomers. (6,7).

The difference between the air-drying device and the suction device is basically the direction of the air flow (8). By using the air syringe the air is pushed on the tooth with a positive pressure, meanwhile by suction the pressure is negative. The air causes a collapse of the collagen fibers which precludes an optimal infiltration of the adhesive, even if this problem seems of secondary importance compared to a poor evaporation of the adhesive (9,10). The suction limits the collapse of the fibers, but does not seem to exercise sufficient negative pressure to remove the accumulations of water and solvents, contained into the adhesive system. This could explain the lower adhesion values of the suction-only versus air-use groups demonstrated in our study, although this difference isn't statistically significant, this agrees with the results obtained in the study by Magne et al. (8) where there were no statistically significant differences between the different evaporation methodologies.

The indications about the evaporation times are five seconds and ten seconds for polymerization. This quick adhesive ,unlike other adhesives on the market, provides for the application and continuation in the restoration phases without waiting times. This is possible thanks to the presence of a new hydrophilic acrylamide monomer technology , a lower content of HEMA and to the presence of the 10- MDP monomer. These components permit the Bond Quick adhesive to promises a very fast adhesion.

The data obtained in the microtensile bond strength test for groups in the presence of simulated pulpal pressure could be explained precisely by the composition and chemical characteristics of this adhesive. The new monomer contained in Bond Quick is very hydrophilic, more than other monomers already widely used such as, for example, HEMA. This characteristic allows it to penetrate and perform very well in moist dentin conditions and in the presence of water. Moreover, the results of the microtensile bond strength test at T0 show that the groups in the presence of pulp pressure have immediate adhesion values higher than the groups in the absence of pulp pressure, and after 6 months SE values remains higher than TE, and in particular SE without PP seems to remain unchanged.

About the greater enzymatic activity, this leads us to think of a greater degradation over time of the adhesive bond of the groups with pulp pressure simulation.

Furthermore, this study demonstrated increased enzymatic activity associated with TE groups. This result agrees with what has been reported by other studies in the literature such as Mazzoni et al (11,12) and Perdigao et al(13). The etchant has a demineralizing action on the dentin up to a depth of 5 μm, the adhesive, however, cannot reach this depth, leaving uncovered the network of collagen fibers exposed by the action of the acid (14). The collagen fibers not protected by the adhesive are thus subject to the action of hydrolysis and metalloproteinases. It is known that metalloproteinases

are proteolytic enzymes constituents of tissues such as dentin and usually present in inactive form in mineralized dentin. Their activation can be due to already activated enzymes or chemical agents such as etchant and adhesives (12,15). It is also known that a greater enzymatic activation is responsible over time for a greater degradation of the hybrid layer and consequently for a shorter duration of the adhesive bond(16). Therefore, it can be hypothesized that the TE groups will have a shorter duration of the adhesive bond strength over time than the SE groups due to degradation of the hybrid layer by the metallo-proteinases.

We continue our research with a different in vitro study, by focusing our work on a different adhesive surface: bonding performances on radicular cement. This in vitro study aimed to investigate the bonding performance and sealing ability of different resin cements. According to the results obtained, the first working hypothesis was accepted 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 system does not require the pretreatment of the post with silane, further simplifying the clinical cementation procedure. In order to optimize the bond strength between fiber post and resin cement, the surface of posts was pretreated differently among the groups, following the manufacturers' instructions for the specific cement used during luting procedures. However, since a recent systematic review reported that the use of silane alone cannot enhance FRC posts' resistance to dislodgment, we fiber post pretreatment was not considered as an additional variable (19).

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 E&R 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 (2). On the other hand, a superficial dentin demineralization was observed with self-adhesive resin cements with very thin and short resin tags (10). 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 (27).

The formation of a reliable and stable bond is in part related to the resin cement polymerization process (6). Dual-cure resin cements contain a combination of initiation systems present in both light-cure and self-cure systems, and subsequently photoactivation of dual-cure cement activates photoinitiators and starts polymerization of the material. A proper polymerization reaction of the material translates into better physical and chemical properties (6), increased stability and integrity at the adhesive interface (29), reduced water sorption/solubility phenomena and extended durability of the restoration (24). 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 working 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 achieve surface demineralization of enamel and dentin, they contain acidic monomers (10). 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 (20, 28). 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 (22) since the root region did not influence BS and NL expression, requiring the rejection of the third working hypothesis.

One year aging in artificial saliva influenced the bonding performances of the tested materials, which led to the acceptance also of the fourth working hypothesis as the BS values and NL expression significantly increased after artificial storage. The exposure of the root slices to artificial saliva for 12 months may have enabled water molecules to enter the resin cement and fiber posts by diffusion (7, 32). Water diffusion into the material could influenced 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 (11). Interestingly, higher BS values were observed for the new self-adhesive RXU cement at T_{12} compared to the other tested cement systems. Self-adhesive resin cements show different water sorption and solubility characteristics (17). Acidic monomers 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 (23).

Even though a recent systematic review found considerable variations in the design of the push-out test among studies (5), it is considered to be more appropriate and reliable for FRC post testing than microtensile BS tests. 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. Mechanical tests and spectroscopy studies should be performed to better define the mechanical and curing characteristics of the recently introduced self-adhesive universal resin cement, followed by randomized clinical trials.

Finally regarding the adhesion of metallic brackets to enamel' tooth in this study, the SBS of the self-etching primers were lower than that of the convectional ones, but the divergences with the control group were not statistically significant. Trasbond XT was used as control group because it is one of the standard cements in orthodontics (185,189-191). In this context, the highest SBS was yielded by RXU E&R group followed by TXT group so the first null hypothesis that the use of phosphoric acid at 37% concentration prior to luting improved the bond strength had to be accepted. Nevertheless, no significant differences were founded between TXT and RXU in SE mode so it seemed that brackets could be bonded using RelyX Universal as universal resin cement plus Scotchbond Universal Plus Adhesive in self-etch mode. The findings indicated that the shear bond strength of RXU SE was 5.5 ± 3 MPa; successful clinical bonding can be achieved with a SBS of 5-10 MPa. According to Reynolds (166) the minimum SBS for the clinical use of brackets should lay between 5.9 and 7.8 MPa, it meant that the RXU SE mean value was lower than the minimum required. However, the standard deviation of this group was notably high (3 MPa), so the value was sometimes higher than the threshold and sometimes lower. This procedure would eliminate the step of acid etching of the enamel surface and consequently reduce the clinical steps, the risk of enamel decalcification around the brackets and the operator sensibility.

In our study, application of RelyX Universal in self-adhesive mode yielded the lowest SBS value even if no significant difference was observed from RXU SE group. Bracket bonding with this multi-mode composite resin was faster than the other three groups due to the single-step application and had fewer procedural errors but RXU had low viscosity and was highly flowable so, during the

bonding procedures, care must be taken for the bracket not to slip. Also, removal of excess resin was more difficult compared with TXT.

At the end of the orthodontic treatment, brackets must be removed. The ARI method allowed to obtain information about the quality of adhesion between the adhesive and the tooth surface as well as between the adhesive and the bracket base. Penido et al. (192) illustrated that bonding failures more often occurred at the adhesive bracket interface. This type of fracture was preferable because any fracture at the adhesive enamel interface was able to damage the enamel surface. If a greater amount of adhesive was left on the tooth surface, a more secure debonding can be achieved (192). Brauchli et al. (193) demonstrated that higher SBS also resulted in higher ARI scores. This correlation was also shown in our study. There were more cement remnants in the RXU E&R group compared to the self-adhesive RXU technique ($P<0.05$), while differences were not noted between other groups ($P>0.05$). The RXU E&R group had the highest SBS value as well as the highest ARI score.

We also found that there was not a statistically significant interaction between cementation techniques and removal techniques. This means that the mechanical removing of the adhesive remnant and polishing of the tooth's surface was the main responsible for the enamel damage. Many studies (194-197) have demonstrated the presence of adhesive remnant incorporate into the enamel after bracket detachment and enamel surface cleaning. Alessandri Bonetti et al. (198) evidenced that low-speed 12-bladed tungsten carbide bur followed by finishing Sof-Lex discs did not restore the enamel surface to its original condition, even though there was no clinically relevant damage to the tooth. Contrary, Janiszewska-Olszowska et al. (171) conducted a systematic review of the literature and concluded that cleaning procedures caused an irreversible damage to the tooth surface. Recently, Pinzan-Vercelino et al. (199) have demonstrated that both Sof-Lex discs and Spiral Wheel polishing system used after 12-bladed tungsten carbide bur did not appear to significantly damage the enamel surface. Even though there was a lack of consensus about the safest and the least aggressive technique to remove residual adhesive, the use of a tungsten carbide multi-laminated bur was supported by several authors (193,194,200,201). Janiszewska-Olszowska et al. (171) demonstrated that high-rotation tungsten carbide drills were the most commonly use because they were more effective and require shorter working time as compared to other methods. Zarrinia et al. (202) suggested the use of a multi-bladed tungsten carbide bur followed by Sof-Lex discs to produce a smoother enamel surface. Ozer et al. (193) recommend the use of Sof-Lex discs since, when used alone, provided more even enamel surfaces, and restored the enamel the closest to the original enamel surface. In our study, both contouring and polishing coarse discs rotating on low-speed contra angle handpiece and tungsten carbide multi-laminated high-rotation drill successfully cleaned the tooth surface, but also removed a portion of the enamel, particularly SL ($p<0.05$). Therefore, the second null hypothesis that the

methods of cement removal does not influence the remnants index was rejected. Recently, D'Amario et al. (180) suggested the use of magnification systems during adhesive removal procedures to save as much enamel tissue as possible. This aspect will be the object of further studies.

The tooth color might be altered by orthodontic therapy, affecting the dental aesthetic. Eliades et al. (204) observed that the color of enamel was affected by debonding and subsequent cleaning procedures. According to Karamouzos et al. (205) the optical characteristics of enamel changed during orthodontic treatment, with the color change being affected by several factors. External coloring occurred because of superficial absorption of food pigments, while internal coloring developed during aging (i.e., water sorption and hydrolytic deteriorations). Boncuk et al. (206) reported that both the orthodontic adhesive systems and the burs used to remove their residuals, in particularly the etch-and-rinse/tungsten carbide bur, were responsible for discolorations. Conversely, Gorucu-Coskuner et al. (207) and Al Maaitah et al. (208) observed that tooth color changes were detected after orthodontic treatment, but they were independent from the etching nor the adhesive removal techniques. Also, Pinzan-Vercelino et al. (209) confirmed that the multibracket appliance resulted in enamel color changes but regardless of the different polishing procedures. Nevertheless, several of these studies (205, 207, 208) reported that these color changes were significative in the period just after removal of fixed appliance, but they were not consistently clinically discernible. In the present study, the lack of saliva, food coloring, and the inability to simulate the mechanic abrasion caused by brushing were the limitations of this methodology. Moreover, observation of the teeth over time should be made to evaluate the changes in color over a longer period of time. However, in our findings the changes in the tooth color were not affected significantly neither by the cementation mode with the materials tested, independent of the separate etching step, nor by the different cement removal techniques ($p>0.05$). So, the third null hypothesis that the different technique of cementation and debonding of brackets did not influence tooth color changes was accepted.

Conclusions:

- Based on clinical data available so far, we could recommend with a moderate certainty of evidence that the application of universal adhesives in the EAR mode could lead to better medium-term retention of composite restorations of NCCLs compared to the SE application strategy, while the use of the SE adhesives could lead to less immediate POS and therefore better short-term patient satisfaction. The SEE approach was comparable with the EAR approach in terms of retention (moderate level of evidence at 6 and 18/24 months) and POS (moderate level of evidence).
- Further, applying universal adhesives in SEE mode leads to more predictable retention at 6- and 18/24-month of follow-up when compared to self-etch mode (very low and low certainty of evidence, respectively). Our meta-analyses suggest that the choice of adhesive strategy did not influence marginal discoloration and marginal adaptation (very low certainty of the evidence) up to 2 years of follow-up.
- The use of air with a disposable syringe to evaporate the adhesive, and the self-etch mode seem to be more reliable choices when an ethanol-based universal adhesive is used for bonding procedures.
- Aging and the choice of material, influenced the bond-strength between adhesively luted fiber posts and radicular dentin. Polymerization protocol and root region had no effect on posts' retention in root canal.
- Etch-and-rinse cementation systems (separate step of 37% phosphoric acid etching) obtained higher shear bond strength of orthodontic brackets to enamel. However, also self-etching cementation strategy yielded shear bond forces that can be successfully used for bonding orthodontic brackets.
- Cleaning of the remaining cement from the enamel with tungsten-carbide burs seems to be as efficient and less aggressive in terms of healthy tooth tissue preservation compared to the Sof-Lex technique.
- The cementation of orthodontic brackets, as well as their removal and polishing procedures did not influence the change in tooth color at baseline.

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