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Influence of eugenol-based materials and cleaning procedures previously to resin composite restoration on microleakage after 1-year storage

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Abstract

The interference of eugenol on the adhesive systems and composite polymerization could promote a higher marginal microleakage. This could be reduced by mechanical and chemical cleansing. The objective of this study was to evaluate the influence of the previous use of eugenol-based materials to a permanent restoration on the marginal microleakage. The influence of mechanical cleansing associated with chemical cleansing with chlorhexidine gel was also evaluated. Non-eugenol endodontic sealer and temporary material and mechanical cleansing was used as a control. Eighty bovine incisors were endodontic treated and divided into eight groups ($n = 10$) according to the sealer, temporary material and removal method. The specimens were temporarily restored and stored for 1 week at 37 °C and 100 % relative humidity, followed by definitive restoration and one-year storage at the same environment. The teeth were then sealed, immersed in silver nitrate at 50 % for 2 h and sectioned to have the microleakage qualitatively and quantitatively visually analyzed under a stereomicroscope at 45×. The percentage data were submitted to an ANOVA and Tukey's test, and the scores were submitted to a Kruskal–Wallis and Dunn's test at 5 % of the significance level. There was no difference between the groups when mechanical removal was made ($p > 0.05$). When chlorhexidine was used, the group that used endodontic eugenol based material as a sealer and the resin-based material as a temporary restoration showed less microleakage than the other ($p < 0.05$). All groups showed less microleakage when chlorhexidine was used instead of solely using mechanical removal ($p < 0.05$). For scores, when the eugenol based sealer was combined with resin-based temporary material, and when the resin sealer was combined with eugenol based temporary material using chlorhexidine, a lower median was found ($p < 0.05$) compared to all other groups. Eugenol showed no influence, and chlorhexidine gel showed to be effective in reducing microleakage.

Keywords: Microleakage, Chlorhexidine, Eugenol, Composite resin

Background

The objective of a temporary restoration is to protect the dentin against bacteria and toxins to avoid a series of pulpar tissue reactions [5]. The temporary restoration should perfectly seal the interfaces during the time it stays in place [13]. The restoration should have appropriate wear resistance, dimensional stability and adequate retention in the cavity. These characteristics are related to the setting contraction, thermal coefficient, water absorption and mechanical stress over the restoration, besides the dimensional changes of the dental structures [39].

There are several temporary filling materials with different microstructures, compositions and setting mechanisms on the market. A resin-based material that sets upon light-curing, characteristically presents volumetric shrinkage during polymerization [36]. This contraction, however, is usually followed by expansion due to water sorption [11]. The expansion of resinous temporary materials could compensate the contraction and this is sufficient to adequately seal the cavity.

The success of restoration depends on the selection of appropriate root canal sealers and core build-up materials. Therefore, the compatibility of the chemistries of these different materials (root canal sealer and composite core build-up restoratives) is an important factor for a successful restoration [10]. Resin-luting agents have had a profound effect in the way endodontically treated teeth are restored [32]. Because these surfaces may be contaminated with a eugenol-containing endodontic sealer during root canal therapy, there is concern that such contamination may interfere with the polymerization of the resin [23] thereby weakening the adhesion of the core material to the dentin [32].

Several sealers and temporary cement are eugenol-based. Eugenol is a phenol derivative widely used in combination with zinc oxide as pulp capping agents, temporary cement and root canal filling cement. Eugenol reacts with free radicals, thereby inhibiting the polymerization of methacrylate monomers [18]. Thus, it is incompatible with the resin restorative system. Although eugenol inhibits the polymerization of methyl methacrylate (MMA), a fast polymerization probably occurs when eugenol is consumed. Despite the numerous studies on the inhibitory effects of eugenol [21], the dynamics and mechanism of eugenol inhibition are not well understood [18].

Acrylic and bisacrylate resins are the most indicated to temporarily seal the fixed prosthodontics preparations; however, for partial preparations on posterior teeth, the temporary composite resin is also indicated [2]. These materials are basically composed of UDMA and microparticles, which can be directly applied to the cavity preparation without the need for acid etch or any previous treatment. They are also photoactivated materials that facilitate the use by the clinicians, thus reducing the chair time and promoting a better sculpture, according to the manufacturers.

In several cases, after a root canal filling, the tooth is not permanently restored in the same session, thus jeopardizing the recently filled root canal due to oral exposure [16]. The same could be said about conventional endodontic therapy, where temporary restorative materials are extensively used to perform root sealing between sessions [9]. Therefore, the placement of a temporary restoration with adequate properties is indispensable to prevent marginal microleakage [27, 44].

Researchers have pointed to the potential advantage of chlorhexidine gluconate (CHX) as an antimicrobial medicament in endodontic therapy [8]. CHX is a broad-spectrum antimicrobial agent [8] that can be effectively used as an irrigant [8], to disinfect the dentinal tubules [33] and to adsorb onto the dentin [33]. Root dentin treated with CHX seems to acquire antimicrobial substantivity [25, 43]. This effect has been confirmed to extend at least 7 days; when CHX was used as an intracanal medicament in bovine roots for 1 week, the root dentin demonstrated an inhibition of colonization by *E. faecalis* for a period of 7 days [43].

Chlorhexidine (CHX) gluconate is a cationic biguanide that seems to act by adsorbing onto the cell wall of a microorganism, resulting in the leakage of intracellular components. At low concentration, it has a bacteriostatic effect. Although it is at a high concentration, CHX is bactericidal because of precipitation and/or coagulation of intracellular constituents [4]. Its optimal antimicrobial activity is at pH 5.5–7.0 [41]. CHX has a broad-spectrum antimicrobial activity, targeting both gram-positive and gram-negative microbes [4]. The objective of this study was to evaluate the influence of the previous use of eugenol-based materials to a permanent restoration on the marginal microleakage. It will also evaluate if mechanical cleansing associated or not to chemical cleansing with chlorhexidine gel can reduce eugenol influence on the marginal microleakage. The null hypothesis tested was that there is no difference in the performance of the different materials and cleaning protocols.

Methods

The composition of the tested materials was at Table 1.

Initial procedures

Eighty bovine incisor teeth with similar dimensions and no root defects were cleaned from surface-adhered debris using periodontal curettes and stored in distilled water. The roots were embedded in acrylic resin (Dencrilay; Dencril, Caieiras, SP, Brazil) cylinders, the coronal portion of the canals was opened, following the convenience form with a 1016 bur, and the canal was prepared with Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) sizes two, three and four. Next, a size 15 K-file (Dentsply Maillefer)

Table 1 Tested materials, composition, manufacturers and lot number

Type	Eugenol	Materials	Composition	Manufacturer	Lot
Zinc oxide based endodontic sealer	YES	Endofill	Zinc oxide, hydrogenated resin, bismuth subcarbonate, barium sulfate, sodium borate. Eugenol and oil of sweet almonds	Dentsply Ind. e Com. Ltda. Petrópolis, RJ, Brazil	288,028
Resin based endodontic sealer	NO	Sealer 26	Bismuth trioxide, calcium hydroxide, hexamethylenetetramine and titanium dioxide. Bisphenol epoxy resin	Dentsply Ind. e Com. Ltda. Petrópolis, RJ, Brazil	079941H
Zinc oxide based temporary restorative material	YES	IRM	Zinc Oxide, polymethylmethacrylate. Eugenol	Dentsply DeTrey GmbH Konstanz Germany	005677G
Resin based temporary restorative material	NO	Bioplic	Dimethacrylate groups, organic filler, silicium dioxide, catalysts and sodium fluoride	Biodinâmica química e farmacêutica ltda. Londrina, PR, Brazil	79,108

was inserted, and the canals were prepared according to a stepback technique up to a size 60 K-file. The canals were irrigated with 1 mL of 1.0 % NaOCl at each change of the file during chemomechanical preparation and then filled with 2 mL of 17 % EDTA for 3 min, flushed with 2 mL of 1 % NaOCl and dried with absorbent paper points (Dentsply Maillefer) [26].

Test groups

The teeth were randomly allocated into 2 groups (n = 40) according to the type of endodontic sealer: zinc oxide eugenol-based sealer (Endofill, Dentsply Maillefer); and Epoxy resin-based sealer (Sealer 26, Dentsply Maillefer) (Fig. 1; Table 2).

Specimens were filled with a size 60 master gutta-percha cone and accessory gutta-percha cones (Dentsply Maillefer). The cones at the root canal orifice were removed with a hot plugger, and the filling material was left inside the canal up to the amelodentinal junction. Root canal sealers were handled according to the manufacturers' instructions (Fig. 1).

Each group was divided into two subgroups (n = 20) according to the temporary restorative material. The specimens were temporarily restored with a zinc oxide eugenol-based sealer (IRM, Dentsply Maillefer) or flexible resin (Bioplic, Biodinâmica, Londrina, PR, Brazil) (Fig. 1 and Table 2). The specimens were stored at 100 % humidity and 37 °C for 1 week.

Thereafter, the temporary restorative was removed and the specimens were restored with composite resin (Filtek Z350, 3 M Espe, Saint Paul, MN, USA). In this process, the specimens were subdivided into two groups (n = 10) related to mechanical (Curette, Hu-Friedy Mfg. Co, Chicago, IL, USA) and mechanical and chemical cleaning (Gel Chlorhexidine gluconate 2 %, Biodinâmica) before the adhesive procedure (Fig. 1 and Table 2).

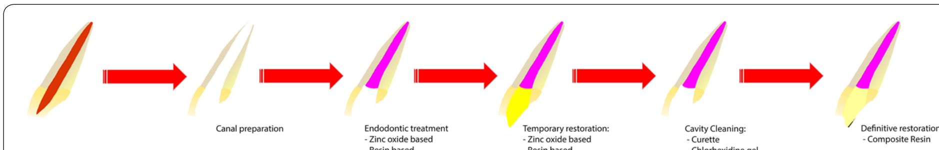

Fig. 1 Sequence of tooth treatment

Table 2 Distribution of groups

Groups	Sealer	Temporary material	Removal method
1	Zinc oxide	Zinc oxide	Mechanical
2	Zinc oxide	Zinc oxide	Mechanical + Chlorhexidine
3	Zinc oxide	Resin based	Mechanical
4	Zinc oxide	Resin based	Mechanical + Chlorhexidine
5	Resin based	Zinc oxide	Mechanical
6	Resin based	Zinc oxide	Mechanical + Chlorhexidine
7	Resin based	Resin based	Mechanical
8	Resin based	Resin based	Mechanical + Chlorhexidine

All specimens have the temporary restorations removed with a curette by applying a moderate pressure. Then the tooth structure was washed with water to remove the excess temporary material and was dried with mild air blow (Fig. 1 and Table 2).

For the mechanical and chemical removal group, the chlorhexidine gluconate gel 2 % was then applied vigorously for 1 min on a dentin surface with a microbrush and washed with water for 1 min and then dried with mild air blow (Fig. 1 and Table 2).

After this, 35 % phosphoric acid (3 M Espe) and Adper Single Bond 2 (3 M Espe) were applied according to the manufacturer's instruction. The specimens were polymerized using a LED light-curing unit (Radii-cal, SDI Limited, Bayswater, VIC, Australia) followed by incremental placement of resin composite (Filtek Z-350, 3 M Espe), and each layer was light-cured for 20 s. After completing the composite resin restorations, the specimens were stored for 12 months in an oven at 37 °C and 100 % relative humidity.

Microleakage test

The teeth were sealed with nail varnish, leaving 1 mm around the restoration unsealed, immersed in a silver nitrate solution (50 %) pH 8.0 for 2 h and then immersed in an x-ray fixing solution for 8 h. The specimens were sectioned longitudinally and the sections were examined under stereomicroscope at 45× magnification (Kyowa Optical Co. Ltd, Tokyo, Japan), and the appearance receiving scores from 0 to 4, according to the infiltration (Table 3). For quantitative analysis, digital photography were taken and analyzed in a computer by the program Image Tool (v.3, UTHSCSA, San Antonio, TX, USA). The values of coronal marginal leakage were measured in millimeters from the root canal entrance up to the most longitudinal point of dye penetration. The percentage was calculated by dividing the infiltrated length by the total length of the interface restoration-dentin.

All data were submitted to the Kolmogorov–Smirnov normality test. The percentage data were analyzed using three-way analysis of variance (ANOVA) with Tukey's post hoc test at a 5 % significance level. The scores data were submitted to the Kruskal–Wallis and Dunn's test at 5 % of significance.

Results

The mean values in percentage obtained for the coronal marginal leakage using different sealing materials can be seen in Table 4. There was no statistical difference between the groups when mechanical removal was made ($p > 0.05$). When chlorhexidine was used, the group that used eugenol-based material as a sealer and the resin-based material as a temporary restoration showed less microleakage than the other ($p < 0.05$). All groups

Table 3 Score classification

Score	Description
0	Absence of stain at the tooth-restoration interface
1	Stain penetration up to amelodentinal junction
2	Stain penetration up to pulpal wall
3	Stain penetration on pulpal wall
4	Stain penetration beyond pulpal wall

Table 4 Mean values, standard deviations and scores of coronal marginal leakage

Endodontic sealer	Temporary restoration	Removal method			
		Mechanic		Chlorhexidine	
		%	Score	%	Score
Zinc oxide	Zinc oxide	11.50 ± 2.55 Aa	4 ◊	6.09 ± 2.26 Ba	2 ◊
	Resin based	15.00 ± 5.04 Aa	3 ◊	1.85 ± 1.07 Bb	1 +
Resin	Zinc oxide	21.52 ± 11.54 Aa	3 ◊	5.20 ± 2.24 Ba	1 +
	Resin based	19.96 ± 9.01 Aa	3 ◊	8.44 ± 1.98 Ba	1 ◊

The different capital letters in the line, the small letters in the column for percentage indicate the statistically significant differences by ANOVA and Tukey's test at 5 % of significance

The different symbols for score indicate the statistically significant differences by Kruskal–Wallis and Dunn's test at 5 % of significance for all comparisons

showed less microleakage when chlorhexidine was used instead of solely using mechanical removal ($p < 0.05$).

For the scores, when the eugenol based material sealer was combined with the resin based temporary material, and when the resin sealer was combined with eugenol based provisional material using chlorhexidine, the lower median with statistical difference ($p < 0.05$) was found compared to all other groups (Table 4). All the other comparisons have no statistical differences ($p > 0.05$).

Discussion

The null hypothesis should be rejected, although eugenol-based materials and resin-based materials did not have differences on the microleakage, and the chlorhexidine positively affected the microleakage.

Eugenol-containing materials have several advantages as bases for restorations; however, they are believed to interfere with polymerization during the curing of composite restorations [12, 23]. The hydroxyl group of the eugenol molecule tends to protonize the free radicals formed during the polymerization of resin-based materials, thereby blocking their reactivity and reducing the degree of conversion of these materials [3, 18]. Studies indicated that shear bond strengths were reduced when eugenol-based cements were used to lute temporary crowns or inlays [31, 35], although this point has been contested [19]. Some researchers found eugenol to have no effect on the shear bond strengths of resin adhesives [1, 19, 30]. Schwartz et al. [38] examined the effects of eugenol and non-eugenol sealers on post-retention and found that the sealer type had no effect.

When the eugenol-based sealer or temporary restorative materials were compared to resin-based material or even associated with them, no difference was found on microleakage. Some authors reported that the reduction on resin-dentin bond strength values after pretreatment with eugenol-containing materials is caused by the cement itself rather than eugenol, as remnants of temporary materials are not completely removed before adhesive application [42, 45]. It is well know that eugenol released from zinc oxide and eugenol (ZOE) based materials can penetrate dentin and interact with resin-based restorative materials suppressing the polymerization of polymers; in practice, eugenol in IRM reduces the mechanical properties of composite resin within a limited range, less than 100 mm from the interface [24]. Moreover, the less polymerized composite resin close to the interface generates a graded region and provides a smooth transition from hard resin to a soft ZOE base, which can improve stress distribution [24]. This may also be the reason why no difference was found in microleakage in this study.

CHX gluconate in the form of a salt, i.e., gluconate, acetate or hydrochlorate, has been used since the 1950s at different concentrations as an oral antiseptic in the form of a mouthwash, subgingival irrigant, gel, toothpaste and chewing gum [29]. CHX can be used in endodontics as an irrigant and intracanal medicament due to its biocompatibility, substantivity and wide antimicrobial activity [14, 15]. The antimicrobial effect of chlorhexidine is caused by the cationic molecule binding to negatively charged bacterial cell walls, thereby altering the cell's osmotic equilibrium [22]. It has antimicrobial activity against gram-positive and gram-negative organisms [40]. However, CHX cannot be used as routine medicament as it lacks property of tissue solubility and possesses cytotoxic effect [37].

Comparing the cleaning of the cavity before definitively restoring the tooth, when chlorhexidine was used, the microleakage was reduced [6]. This may have occurred because of its activity on the collagen fibrils, which can protect them from degradation. At low concentrations of CHX, small molecular weight substances will leak out, resulting in a bacteriostatic effect. However, at higher concentrations (2 %), as used in this work, it has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein crosslinking [20], and it presented an excellent diffusion into dentinal tubules. This is due to CHX gel's low contact angle with dentin, which effectively penetrates into the dentinal tubules at a faster rate [17, 28]. Besides this, the gel form was used in this study because the gel formulation contacts well with the canal wall due to its viscosity, and, thus, the time of contact is increased [34].

Two-step etch-and-rinse adhesives, as Single Bond, require the pretreatment of dentin with an acid. This acid, usually 30–40 % phosphoric acid, superficially demineralizes dentin and thereby exposes a 3–5 μm collagen scaffold [7]. If this collagen was less damaged during storage time and protected by the applied chlorhexidine, there is clearly an improvement in the dentin-composite adhesion, leading to a reduced microleakage.

Another CHX property that could reduce microleakage was found in the scanning electron microscopy analysis [17], which showed that, when the root canal walls were treated with 2 % CHX gel, almost all dentinal tubules were opened. The authors [17] believed that 2 % CHX gel was able to effectively clean the root canal walls and their anatomic complexities because of the viscosity of the gel, which promoted a better mechanical cleansing of the root canal and a better removal of dentin debris and the remaining tissue.

Although other properties should be investigated, considering the reduction of microleakage for all groups after one-year storage, it is important to consider the use of chlorhexidine previously to definitive restoration with composite resin. Even in the case of non-use of temporary material, the use of chlorhexidine may also reduce the microleakage. With this reduction, the restoration will probably have a greater longevity.

Conclusions

Eugenol, when associated with endodontic or temporary restorative material, showed no influence on the microleakage of the definitive composite resin restoration; on the other side, chlorhexidine gel positively influenced reducing the microleakage.

Authors' contributions

KRF and RHK confectioned the specimens and performed the microleakage test. SBG conducted the statistical analysis and help in the manuscript written. MBL and AGG delineated the experiment and write the manuscript. All authors read and approved the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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