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Original Article

Evaluation of Apical Contamination in Teeth with Prefabricated Posts Fixed with Different Dental Cements

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Abstract

Objective: To assess the sealing capacity of different dental cements regarding apical microleakage on teeth with prefabricated posts. **Material and Methods:** Forty-eight single-rooted teeth were selected and, after having their coronal part removed at 10mm standard, underwent endodontic cleaning and preparation. The space for the post was prepared with # 3 Largo bur at 7 mm standard value. The study included the following groups: Group I, post prefabricated with # 1 intracanal glass fiber cemented with zinc phosphate cement (n = 12); Group II, post prefabricated with # 1 intracanal glass fiber cemented with conventional glass ionomer cement, Vidrion C (n = 12); Group III, post prefabricated with # 1 intracanal glass fiber cemented with chemical action resin cement, Multilink (n = 12). Two groups of six teeth each were also included, one as negative control group and the other as positive control. After cementing, teeth were tested through a dual-chamber experimental model, using Enterococcus Faecalis for apical contamination. **Results:** The control groups behaved as expected: positive control group with total contamination in 11 days and negative control group without any contaminated specimen within observation time of 60 days. Group I showed microleakage in all specimens for up to 29 days (100%). Group II and Group III showed microleakage in 50 and 49 specimens (83.33% and 75%) respectively along the research time of 60 days. Group 1 showed microleakage in all specimens for up to 29 days (100%). Group 2 and Group 3 showed microleakage in 50 and 49 specimens (83.33% and 75%) respectively along the research time of 60 days. The Log-Rank test showed differences between Groups 1 and 2 ($p<0.05$) and between Groups 1 and 3 ($p<0.05$). However, there was no difference between Groups 2 and 3 ($p> 0.05$). The results showed higher delay in bacterial infiltration in Groups 2 and 3 when compared to Group 1. **Conclusion:** No cement completely prevented infiltration during the experimental period. Zinc phosphate cement had the lowest apical sealing capacity while glass ionomer cement and resin cement showed similar results.

Keywords: Dental Cements; Dental Leakage; Post and Core Technique.

Introduction

Coronal microleakage favors the penetration of microorganisms from the oral cavity to the internal part of the tooth, which by means of filling, can reach the periapex, often leading to failure of the endodontic therapy.

Endodontically treated teeth should therefore be restored as soon as possible to preserve the antisepsis of the pulp cavity obtained, since the technical quality of the endodontic treatment and adequate coronal restoration are correlated with high success rates [1-8].

The proper filling of the intraradical space is a preventive factor to infiltration. Several authors report the quality of endodontic treatment as more important compared to restorative treatment, being considered as the main factor associated with the maintenance or development of periapical lesions [5,6,9]. However, the need for removal of part of the filling material is considered a critical factor. The space created for the placement of an intraradical retainer, if not properly filled, allows the infiltration of microorganisms from the oral environment [10,11]. A study has reported that root canals filled and prepared to receive an intraradical retainer have lower sealing ability than those completely filled [11].

The intraradical retainer is designed to retain the core and the crown, promoting occlusal stability, maintaining the aesthetic requirements. Studies in literature have assessed the adhesion of intraradicular posts cemented with different cements [12-15], as well as the fracture resistance of bovine roots restored with different prefabricated posts [16]. Coronal microleakage was assessed by dye ink, comparing posts and cores cemented with permanent and temporary cements, showing that teeth restored with permanent cements had better sealing [17]. Subsequently, using fluid leakage, it was observed that polyethylene fiber and glass fiber posts have less coronal infiltration when compared to stainless steel and zirconia posts [18]. Another material used to verify the sealing capacity of intraradicular posts was methylene blue [19]. After thorough search in databases, scarcity of studies addressing infiltration in teeth with retainers was observed.

Enterococcus faecalis is a microorganism commonly detected in persistent endodontic infections. The prevalence of such infections ranges from 24% to 77%. It presents several survival and virulence factors including its ability to compete with other microorganisms, invade dentinal tubules and resist nutritional deprivation [20]. In addition, it has been widely used in studies evaluating the sealing quality of materials during endodontic treatment [21]. However, few studies have used biomarkers in the sealing capacity of intraradicular posts cemented with different cements.

The hypothesis to be investigated is that since manufacturer's recommendations are followed, all materials used for cementing dental posts meet proper sealing requirement of the tooth / apical region complex in restored teeth. Given the above, this study aimed to verify the apical sealing capacity of different dental cements used in clinical practice for fixing intraradicular posts in order to delay the infiltration of microorganisms in the periapical region.

Material and Methods

Selection, preparation and distribution of specimens

This study was approved by the Ethics Research Committee (CEP PUC Minas) under number 43804. Forty-eight single-rooted human teeth from the bank of human teeth of PUC Minas were used in the experiment. By radiographic evaluation, teeth with incompletely formed apex, resorption and extensive caries were excluded.

After disinfection with 2.5% sodium hypochlorite for 12 hours, specimens were stored in distilled water at a proportion of 1000 ml per 10 ml of 2.5% sodium hypochlorite up to the time of preparation of root canals.

Specimens were cut using arborundum discs, removing 2 mm in the apical region and coronal part near the cementoenamel junction, defining the total length of the specimens in 10mm. The root canal patency was achieved by introducing a K # 15 file (Dentsply Maillefer, Ballaigues, Swiss) inside the root canal up to its viewing in the opening of the apical foramen. The measure between the tip of this tool and the occlusal reference was considered the patency length (CPC). The cleaning procedures and formatting of the root canal system were performed in CPC by nickel-titanium rotary instrumentation, ProTaper system (Dentsply Maillefer, Ballaigues, Swiss), using S1, S2, F1, F2 and F3 instruments, followed by manual instrumentation in CPC with K # 15-40 files (Dentsply Maillefer, Ballaigues, Swiss), with preparation being complemented with Gates-Gliden # 3 burs (GG) (Dentsply Maillefer, Ballaigues, Swiss) in the length of 7 mm to intraradical preparation. Throughout instrumentation, irrigation was performed with 2 ml of 2.5% sodium hypochlorite solution at each instrument exchange. The root canal patency was defined and standardized in all specimens with K # 40 file. The prefabricated fiberglass post chosen was Reforpost (Angellus, Londrina, Brazil).

Subsequently, specimens were divided into 3 groups, according to the cement used for cementation: Group 1 (n = 12): intracanal pin cemented with zinc phosphate cement (SS White, Rio de Janeiro, Brazil); Group 2 (n = 12): intracanal pin cemented with conventional glass ionomer cement (Vidrion C, SS White, Rio de Janeiro, Brazil); Group 3 (n = 12): intracanal pin cemented with chemical action resin cement, (Multilink, Ivoclar Vivadent-, Schaan, Liechtenstein). Positive control group (n = 6) showed no sealing and negative control group (n = 6) was completely sealed using cyanoacrylate and epoxy resin.

Cementing of Pins

In Group 1, zinc phosphate cement has been manipulated and applied on pins and inserted in canals for fixation. In Group 2, conditioning with polyacrylic acid for 20 seconds was initially performed. Then, the glass ionomer cement was used according to manufacturer's instructions, being applied on pins and inserted in canals for fixation.

After 60 minutes and after insertion of pins in their respective canals, specimens were kept for 23 hours at 37ºC in incubator, identifying the species and origin of all prepared standards.

For cementation of pins in Group 3, 37% phosphoric acid was initially applied to fiber pins (DFL, Rio de Janeiro, Brazil) for 60 seconds and completely removed with water spray and dried with compressed air. Then, Multilink cement was applied to pins, positioned and maintained under compression. The excess was removed with micro brushes. Specimens of this group were also kept in incubator for 23 hours at 37 ° C.

Preparation of the Test Apparatus

The test apparatus for the manufacture of the dual-chamber experimental model consisted of a structure composed of 10 ml glass vials (Wheaton do Brasil S.A., São Bernardo do Campo, Brazil), rubber stoppers of 20 mm in diameter (Adnaloy Artefatos de Borracha Ltda, São Paulo, Brazil), and 1.5 ml Eppendorf tubes (Cral, Comércio de Artigos para Laboratório, São Paulo, Brazil).

Rubber stoppers were perforated at the center, with a steel punch with 11 mm in diameter (Industry and Commerce Graziano, São Paulo, Brazil), and Eppendorf tubes with extremities sectioned in 7 mm with the aid of a carborundum disc mounted in the chuck and driven by micromotor in straight handpiece.

Teeth were placed in the Eppendorf structures after buckling of the sectioned end to obtain better adaptation and adjustment of the cervical third; then, waterproofing of specimens was performed. Therefore, two layers of cyanoacrylate were applied (Super Bonder Henkel Loctite Adesivos Ltda., Itapevi, Brazil) with an interval of one hour between one application to another. Specimens were kept at room temperature to establish drying.

Subsequently, a layer of sealing agent (nail polish) (Colorama Cremoso, Procosa Produtos de Beleza Ltda, São Paulo, Brazil) was applied, varying color according to the group. After drying, the Eppendorf tube-tooth portion was sealed with an epoxy resin layer (Durepóxi, Alba Química Ind. Com. Ltda, Boituva, Brazil) in order to ensure proper sealing in the Eppendorf tube-tooth junction.

After these procedures, a layer of cyanoacrylate was applied to both the surface of the epoxy resin as the sealed root surface, and a new layer of nail polish was used to ensure the best possible sealing of the Eppendorf tube-tooth junction and the sealing of specimens.

Specimens were identified by the different nail polish color according to specimen: Positive Control: Orange; Negative Control: White; Zinc phosphate: Green; Multilink: Blue; Ionomer: Red.

After drying of the sealing agent for a minimum time of 24 hours at room temperature, the entire test apparatus consisting of tooth-eppendorf tube, 10 ml glass vial and rubber stopper properly identified and forming sets are individually numbered and submitted to sterilization in ethylene oxide gas (Curar Centro de Esterilização Ltda, Belo Horizonte, Brazil). The sterilization process was run for a time of 240 minutes of exposure to the agent at set point temperature of 55°C and relative humidity of 60%, followed by aeration process for 180 minutes.

Indicator Microorganism

According to protocol already described in literature [21], the indicator microorganism used was obtained from the American Type Culture Collection (ATCC) - *Enterococcus faecalis* (ATCC 4083).

Distribution and preparation of the Culture Medium

Culture medium Brain Heart Infusion (BHI) broth (BHI Difco Laboratories, Detroit, MI, USA), the container containing the sterile medium, along with the individual packages of test apparatuses sterilized in ethylene oxide gas were opened in Laminar Flow Chapel, where the mounting of the fixation platform was performed and the culture medium was distributed in glass bottles. About 6.5 ml of BHI broth was placed in each bottle and then the rubber stoppers were adapted to these bottles and the introduction of the tooth-eppendorf tube up to the immersion of approximately 3 mm of root in the culture medium.

Contamination Control and Microbial Inoculation of Specimens

Strain maintenance was performed in two weekly subcultures - Mondays and Wednesdays - in petri dishes containing BHI agar (BHI Difco Laboratories, Detroit, MI, USA), from a 24-hour culture in BHI agar.

For inoculums, a microbial suspension was prepared in 5 ml sterile distilled water with turbidity corresponding to the McFarland scale # 1 (3 x 108 cells / ml). About 1 ml was removed from this suspension to prepare a new suspension in 5 ml of BHI broth. About 0.1 ml of the new microbial suspension was used to inoculate the specimens in the upper chamber of the experimental model, i.e., in tooth-eppendorf tubes to be then incubated in bacteriological incubator at 37 ° C under aerobic conditions.

This microbial inoculation was done every two days, Tuesday and Thursday, all with 24-hour culture over a period of 60 days. The viability of the indicator microorganism was verified at each inoculation by inoculating 0.1 ml of the microbial suspension in a test tube with 10 ml of BHI broth.

The presence or absence of turbidity of the culture medium contained in the glass vial was evaluated every day of the experimental period (lasting 60 days), which characterizes the complete microbial infiltration through SCR. Daily observations were recorded in spreadsheets, according to the experimental group and the observation day. For each teeth assessed in each group, the day on which bacterial infiltration or absence of bacterial infiltration occurred at the end of the experimental period was recorded.

Statistical analysis

The results were evaluated by survival analysis. In survival analysis, the results from each group were arranged in survival curves indicating the time (in days) elapsed until the next event: presence of bacterial infiltration. Differences between groups were analyzed using the Log-Rank test

(Mantel-Cox). The significance level was set at 5%. Tests were performed using GraphPad Prism 5:00 software (GraphPad Software, San Diego, California, USA).

Results

Table 1 summarizes the amount of infiltrated samples in each experimental group and control group compared to the infiltration period and shows the percentage of turbid samples in each experimental group.

Table 1. Infiltration Period.

Group	Turbid Samples / Turbidity Day	Number of Turbid Samples	%
Positive	1(1º),1(2º),1(4º),1(5º),1(6º),1(6º),1(7º),1(14º),1(18º), 1(20º),1(29º)	6 in 6	100%
Negative	No Sample	Zero	Zero
Zinc Phosphate	1(1º),1(2º),1(4º),1(5º),1(6º), 1(6º), 1(7º), 1(9º),1(14º),1(18º),1(20º),1(29º)	12 in 12	100%
Glass Ionomer	1(1º),1(4º),2(5º),1(7º),1(9º),1(28º),1(37º),1(47º),1(54º)	10 in 12	83.33%
Multilink	1(7º),1(9º),1(15º),1(16º),1(27º),1(28º),1(30º),1(42º),1(55º)	09 in 12	75%

The Log-Rank test showed the presence of difference between Group 1 (zinc phosphate cement) and Group 2 (glass ionomer cement) ($p < 0.05$). It also demonstrated the existence of differences between Group 1 and Group 3 (resin cement) ($p < 0.05$). However, the Log-Rank test showed no difference between Groups 2 and 3 ($p > 0.05$). The results of this test, associated with the observation of survival curves (Figure 1), allow indicating a higher survival rate (i.e., a delay in bacterial infiltration) in Groups 2 and 3 when compared to Group 1.

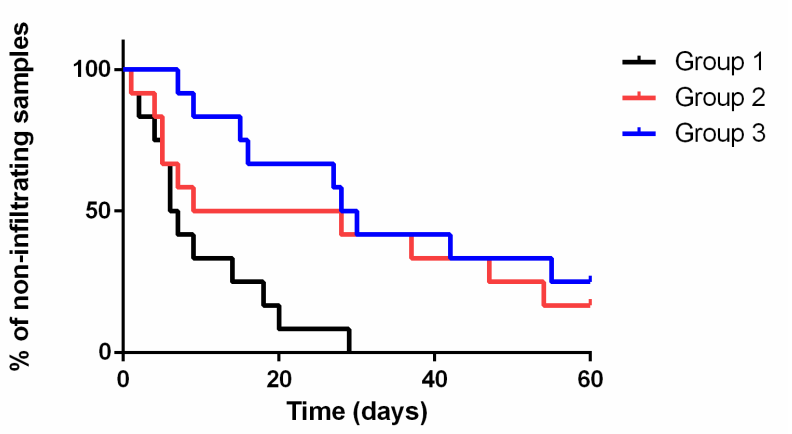

Figure 1. Survival curves indicating the time (in days) elapsed until the next event: presence of bacterial infiltration.

Discussion

Cutting at 02 mm from the apex, followed by preparation with ProTaper system and standardization of the apical foramen of all specimens with #40 file was exclusively aimed at evaluating the sealing ability of cementing materials. In this sense, the absence of filling (condition

verified in a previous study) [22] tried to assess in extreme conditions the efficiency of cementing agents. In conventional clinical situations, even with the presence of filling material, microbial infiltration was observed [21]. Thus, it is essential the presence of an efficient cementing agent to fix the intracanal pin, which will should hinder contamination of the apical segment, a key condition for treatment failure.

Since apical contamination by oral fluids occurs in a short period of time [23], it is prudent to restore teeth in a minimum possible time using prefabricated posts instead of extensive sessions using temporary and fused nuclei [24]. Immediate restoration is considered a positive factor to prevent apical recontamination, since the use of definitive cement has better result in relation to apical contamination, compared to temporary cementing [1]. Moreover, teeth prepared to receive pins are at higher risk for apical contamination [11,17].

Some authors consider the quality of endodontic treatment as the main factor associated with maintenance or development of periapical lesions [5,6,9]. However, the need for removal of the filling material is considered a critical factor. The space created for the placement of an intraradical retainer, if not properly filled, can allow infiltration of microorganisms from the oral environment [10,11]. Root canals filled and prepared to receive an intraradicular retainer show risk regarding the sealing ability compared to those completely filled [11]; however there was no difference in infiltration when fused pins and cores were compared [25].

Various in vitro study methods have been used to evaluate the sealing quality, which are usually based on an evaluation of the penetration of a tracer along the filled canal. The most commonly used tracers are dyes, radioisotopes, bacteria and their by-products. Isotopes and dyes such as methylene blue are much smaller than bacteria and their by-products; therefore, although they can be a good means to compare relative infiltration, they do not simulate the types of microbial infiltration can occur in clinical practice [23,26]. The penetration of saliva was significantly slower compared to dye infiltration [27]. Most experimental models designed to study microleakage using biological indicators (bacteria or saliva) use dual-chamber system, in which the microbial indicator associated with a culture medium is inoculated into the upper chamber, and contamination is evaluated by observing the turbidity or color change of the existing culture medium in the lower chamber [21,26]. The absence of turbidity in the culture medium of the negative control group and its presence in all specimens of the positive control group showed that the system used was reliable and provided important reproducible information. Enterococcus faecalis is one of the most resistant species of the oral cavity, often involved in persistent endodontic infections, being therefore responsible for most treatment failures 21. Furthermore, it was demonstrated that this bacterial species, when coming from contaminated food, can infiltratep through restorative materials [28].

After thorough search in databases, scarcity of studies addressing infiltration in teeth with retainers was observed. Works using dyes, although showing limitations, have still been used to verify the capacity of cementing agents [29,30].

The literature shows that the cementing of pins with zinc phosphate cement has higher apical contamination rates [31,32], which was confirmed in this study during the daily control, since all the specimens were infected within 29 days. The results of this study showed no significant difference in relation to bacterial infiltration, when glass ionomer and resin cements were compared. Additionally, it was demonstrated that these cements exhibited a delayed bacterial infiltration, when compared to zinc phosphate cement. The use of resinous cement, possibly due to adhesion, has been demonstrated to have a slight superiority compared to other types of cements [12-15,31,33], being indicated in all cases and cementing with correct cavity cleaning, aiming to eliminate eugenol from endodontic cements, which could alter the adhesive properties of resin cements [34]. In this study, the results obtained with resin cement were similar to glass ionomer cement.

Endodontically treated teeth should therefore be restored as soon as possible, preserving the antisepsis of the pulp cavity, considering that in addition to the technical quality of the endodontic treatment, adequate coronal restoration is correlated to high periapical success rates [1-8,23]. So, it seems reasonable to think that the combination of good endodontic treatment followed by efficient cementing of the pin / restoration set may delay or hinder the access of contaminants from the oral cavity to the periapical region, possibly increasing the success rate of the dental treatment.

Finally, the statistical method used in this study should be highlighted. Survival analyses are used to evaluate a phenomenon in relation to the period of time elapsed until the occurrence of a specific final event. Therefore, this statistical technique does not only measure the final event "death" (as suggested by the term "survival"), but also other events with dichotomous outcomes [35]. In this study, the final event assessed was the occurrence of bacterial infiltration.

Conclusions

Among the results found in this study, it was concluded that:

a) Zinc phosphate cement showed the smallest capacity of apical sealing when compared to glass ionomer cement and resin cement;

b) Glass ionomer cement and resin cement showed similar results with no significant difference between them;

c) No cement completely prevented infiltration during the experimental period.

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