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ORIGINAL ARTICLE

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Coronal bacterial leakage in root canals filled with single cone technique and different endodontic sealers

Infiltração coronária microbiana em canais radiculares obturados pela técnica do cone único com diferentes cimentos endodônticos

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ABSTRACT

Objective: To evaluate coronal bacterial leakage comparing five endodontic sealers (AH Plus, Apexit Plus, Copaifera sp oil, EndoREZ and Polifil), and comparing root canals filled with EndoREZ sealer/ EndoREZ® Points and EndoREZ sealer/conventional gutta-percha points. **Material & Methods:** 84 human teeth were prepared and filled with gutta-percha points using the single cone technique. Roots were randomly divided into 6 groups: Apexit Plus, AH Plus, Copaifera sp oil, Polifil, EndoREZ, and EndoREZ/EndoREZ Points. After setting time, the roots were incorporated in a leakage model, which upper chamber contained a suspension of *Streptococcus mutans*, and lower chamber a broth. Leakage was assessed for turbidity in lower chamber for 60 days. Statistic analysis was performed using the nonparametric Kaplan-Meier method ($p<0.05$). **Results:** All experimental groups presented leakage during the study's period. The medium time of leakage was: Apexit Plus and AH Plus 6.3 days, Polifil 5.1 days, Copaifera 1.2 days, and both EndoREZ groups infiltrated in the first day. **Conclusions:** There was no statistically significant difference between the sealers Apexit Plus, AH Plus and Polifil, but they prevented leakage better than Copaifera sp oil and both EndoREZ groups. However, none of the tested sealers was capable of resisting coronal bacterial leakage for more than 22 days.

KEYWORDS

Dental leakage; Endodontics; Root canal; Root canal filling materials

RESUMO

Objetivo: Avaliar a infiltração coronária microbiana de cinco cimentos endodônticos (AH Plus, Apexit Plus, Copaiba, EndoREZ and Polifil), e comparar canais obturados com cimento EndoREZ/ cones EndoREZ e canais com cimento EndoREZ/ cones de guta-percha. **Material e Métodos:** 84 raízes de dentes humanos uniradiculados tiveram seus canais preparados e obturados pela técnica do cone único. As raízes foram divididas em 6 grupos: Apexit Plus, AH Plus, Copaiba, Polifil, EndoREZ e EndoREZ/ cones EndoREZ. Após endurecimento dos cimentos, as raízes foram adaptadas a um modelo de infiltração, cuja câmara superior continha uma suspensão de *Streptococcus mutans*, e a inferior um meio de cultura, deixando a porção apical da raiz imersa. A infiltração foi verificada diariamente pelo turvamento na câmara inferior, por um período de 60 dias. Os dados foram avaliados pela análise estatística não paramétrica Kaplan-Meier ($p<0,05$). **Resultados:** Todos os grupos experimentais apresentaram infiltração no período do experimento, contudo o tempo máximo foi de 22 dias. O tempo médio de infiltração foi: Apexit Plus 6,3 dias, AH Plus 6,3 dias, Polifil 5,1 dias, Copaiba 1,2 dias, e em ambos os grupos do cimento EndoREZ todos os espécimes infiltraram no primeiro dia. **Conclusão:** Não houve diferença estatisticamente significante entre os cimentos Apexit Plus, AH Plus e Polifil, mas estes apresentaram melhores resultados que Copaifera e ambos os grupos do EndoREZ. Porém, nenhum cimento foi capaz de impedir a infiltração coronária microbiana por mais de 22 dias.

PALAVRAS-CHAVE

Infiltração microbiana; selamento coronário; cimentos endodônticos.

INTRODUCTION

The main purpose of root canal obturation is to provide a complete filling of the canal in all dimensions to create a bacteria-tight seal, in addition to prevent reinfection [1-5]. Although no available filling material and/or technique produce a complete seal of the entire root canal system [1]. When the coronal portion of the canal is exposed to the oral environment, it may allow leakage and it can cause the failure of the root canal treatment [6].

In general, a root filling is composed of two materials: a solid core material and a sealer [4]. There are many materials and the most commonly used are a combination of gutta-percha points and sealer, which is frequently based on resin, calcium hydroxide, or glass ionomer [7,8]. There are also new sealers, as castor oil-based sealers, that should be more studied to determine their properties.

Polifil is a castor oil-based experimental sealer (based on a polyurethane vegetable resin, Ricinus communis extract), which shows excellent results in relation to physical and biological properties [9-11]. Its promising results in previous researches are encouraging to keep studying its good behavior.

Copaifera sp oil a castor oil polymer-based sealer (polymer extracted from the oil of Ricinus communis). It has been reported that this material presents efficient sealing ability [12], also shows low cytotoxicity and high interaction capacity with human cells [13].

The aim of this study was to evaluate coronal bacterial leakage comparing five endodontic sealers (AH Plus, Apexit Plus, Copaifera sp oil, EndoREZ and Polifil), and comparing root canals filled with EndoREZ sealer/EndoREZ® Points and EndoREZ sealer/ conventional gutta-percha points.

MATERIAL & METHODS

This research has been conducted in accordance with my Institutional Committee on Human Research, process number 009/2011-PH/CEP.

Teeth Preparation

A sample of 84 human teeth with a single root canal and fully developed apices was used for the experiment. Data about age, gender or reason for extraction were not available. Bone, calculus or soft tissues were removed with curettes, with the care not to damage the root surface.

The crowns of teeth were removed at the cement-enamel junction using carborundum disks, and the length of all roots was standardized up to 16 mm. Canal length was determined by passing a size 10 K-file through the apical foramen. Working lengths were established 1 mm short of the apical foramen. Foraminal debridement was made using sizes 15 and 20 K-file.

A crown-down root canal preparation was performed using EndoEZE (Ultradent - South Jordan, Utah, USA) oscillatory system. The canals were enlarged to a diameter of 0.35 mm at the apical stop, using RaCe (FKG Dentaire – La-Chaux-de-Fonds, Switzerland) rotary system. After every change of a file size, the canals were irrigated with 1% NaOCl solution. When preparation was complete, the smear layer was removed using 17% EDTA solution for 5 minutes, then the canals were rinsed with distilled water and dried with papers points.

The roots were sterilized by gamma radiation with the company Embrarad. During obturation and accompaniment of the experiment, all specimens were manipulated into a chamber of laminar flow.

The roots were randomly divided into 6 experimental groups (n = 12) according to the sealer: Apexit Plus (Vivadent – Schaan, Liechtenstein), AH Plus (Dentsply - Konstanz, Germany), Copaifera sp oil (Manaus, Amazonas, Brazil), EndoREZ, EndoREZ with resin coated EndoREZ gutta percha points (EndoREZ Points) (Ultradent - South Jordan, Utah, USA), Polifil

(Poliquil - Araraquara, São Paulo, Brazil). Each group presented one teeth for positive control (n = 6) and one for negative control (n = 6).

Before obturation, samples were reirrigated with distilled water. Canals were dried with sterile paper points, respecting humidity maintenance to the sealer EndoREZ with the use of one paper point.

The sealers were mixed and used according to manufacturer's instructions, and introduced into the root canals with file and gutta percha point. The teeth were filled with gutta-percha points with taper of 4% and D_0 = 0.35 using the single cone technique. And one group of EndoREZ was filled with resin coated gutta percha points. The coronal excess of filling material was removed with sterile scalpel and compacted vertically.

The external surfaces of each root from experimental groups were covered with nail varnish, except the apical 2 mm, due to the possibility of lateral, secondary or accessory canals' presence.

The teeth in positive control group did not receive an external impermeabilization neither endodontic sealers, only the gutta percha point properly locked. And the teeth in negative control group were completely covered with nail varnish and sticky wax, including the apex of the root and coronal access.

All groups were stored in an incubator at 100% humidity and 37 ºC for 14 days to allow the sealers to set completely.

Bacterial leakage test

After setting time, the roots were incorporated in a leakage model, which upper chamber contained a suspension of *Streptococcus mutans*, and lower chamber a broth, leaving the root apical portion immersed. Therefore, leakage test verified the passage of microorganism from the upper to the lower chamber through endodontic filling.

The upper chamber consisted of a 15 mL Falcon plastic tube with its tip cut. Each tooth was inserted into the tube and adapted through the opening until the root apical portion protruded through the tube. The space between the root and the tube was sealed with sticky wax, leaving the root apical portion into the lower chamber.

Microbiological test

Streptococcus mutans, strain ATCC 35688, was grown on BHI agar plates (Brain Heart Infusion – BHI, Difco, Detroit, EUA) for 48 hours at 37 ºC in 5% of CO_2. Then, tubes containing BHI broth (Brain Heart Infusion – BHI, Difco, Detroit, EUA) were inoculated with the microorganism for 18 hours at 37 ºC in 5% of CO_2. The microbial growth was centrifuged at 2000 rpm for 10 minutes, the supernatant was despised and the sediment was suspended in 10 mL of BHI broth. This procedure was repeated and the count of cells in the suspension was made spectrophotometrically (B582, Micronal, São Paulo, Brasil) at 398 nm and optical density of 0.620, to a turbidity of 106 cells/mL.

A volume of 2 mL of S. mutans suspension was placed in the upper chamber, in contact with the coronal filling material. The lower chamber was filled with 8 mL of BHI broth plus neutralizer 1% sodium tiosulfate. The upper chamber was inserted into lower chamber, so that the root apical portion was kept immersed in the broth. The sealing between the chambers was also made with sticky wax.

Samples were transferred to an incubator of CO_2 at 37º C and left for 60 days. The specimens were verified daily as turbidity of broth in the lower chamber, indicating bacterial growth. Each 48 h, 1.9 mL of the suspension in the upper chamber was removed and replaced by new BHI broth to the maintenance of S. mutans; and aliquots of this culture of 6 tubes (1 of each group) were randomly chosen to be seeded in BHI agar to verify the viability of the bacterial culture.

Data analysis

Data was submitted to the non-parametric Kaplan-Meier statistical analysis, to a 5% significance level ($p < 0.05$), estimating the mean time of leakage in days for all groups.

RESULTS

All positive control teeth exhibited bacterial leakage in the first day, and there was formation of apical biofilm in the 22º day, other indicative of bacterial growth. The lower chamber of the negative control teeth remained uncontaminated throughout the experiment, proving the efficacy of the impermeabilization.

All experimental groups presented leakage, as seen in Figure 1, in a period lower than 60 days. The elapsed time to turbidity of groups is shown in Figure 2. The mean time of leakage was: Apexit Plus 6.3 days, AH Plus 6.3 days, Polifil 5.1 days and Copaifera sp oil 1.2 days, but in both EndoREZ groups all specimens infiltrated in the first day.

There was no statistically significant difference between the sealers Apexit Plus, AH Plus and Polifil, but they prevented leakage better than Copaifera sp oil and both EndoREZ groups. The sealer EndoREZ filled with conventional gutta percha points and with resin coated gutta percha points showed the same results, and they were statistically similar to Copaifera sp oil.

Figure 1 - Specimens A) without and B) with leakage

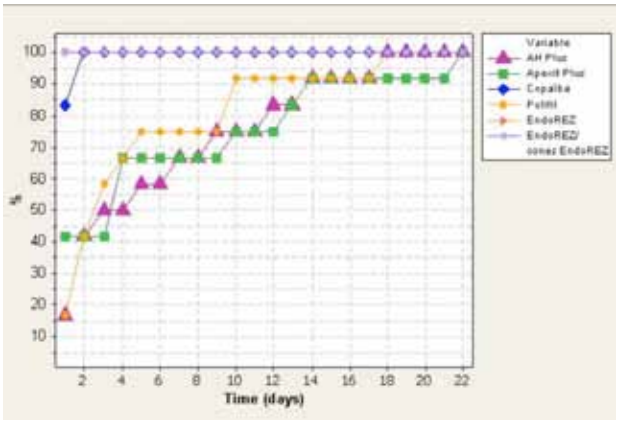

Figure 2 - Curves with cumulative percentages of leaking specimens during time in days.

DISCUSSION

This study demonstrated that leakage occurred after loss of coronal seal in filled root canals, as had previously been shown by other studies [1,9,14-16]. None of the tested filling materials fully prevented apical contamination after coronal exposure to microorganisms.

Removal of smear layer may improve the sealing ability by allowing adhesion and penetration of sealers into the dentinal tubules [4,5]. This procedure was followed in the present study, using EDTA after instrumentation.

There are several root canal filling materials and different techniques. It is generally agreed that the use of gutta percha points with sealer is one of the most reliable methods for filling the root canal [4,7,8].

To the adequate sealing of root canal system, the largest area has to be filled by gutta-percha and sealers fill empty areas where gutta-percha points were unable to fill [17]. The purpose of this study was to evaluate the sealing ability of endodontic sealers, and considering that it was an in vitro study, a larger amount of sealer could be used. That way, the single cone technique was chosen. This technique also allows a comparison of all materials under relatively standardized conditions [14].

The sealing of root canal system can be tested by many models, as: microbial leakage [1,6,15,16,18,19], saliva leakage [5], dye

penetration [2], glucose model [20], fluid filtration [8,11,17]. All methods present considerable results, however bacterial leakage copies well in vitro the in vivo conditions.

Streptococcus species are often found in endodontic infections, and S. mutans is convenient and practical to use for the purpose [1,19]. The number of microorganisms that caused turbidity in the lower chamber was not measured as the purpose was only to test if S. mutans was capable of penetrating through the filled root canal.

In this study, there was no significant difference between the sealers Apexit Plus, AH Plus and Polifil, but they prevented leakage better than Copaifera sp oil and EndoREZ.

AH Plus is an epoxy-based sealer with satisfactory physico-chemical properties, good adhesion [1], low solubility and disintegration [21], good biological properties and antimicrobial activity [22]. In this study, the mean leakage of AH Plus was 6.3 days. This result is consistent with the findings of other studies. AH Plus presented mean leakage of 5.3 days in Salz et al. [19] study, and 4 days in Eldeniz and Ørstavik [1] study. Also provided lower apical leakage rates when compared to other materials in Oliveira et al. [3] study.

All specimens of Apexit Plus presented leakage with mean time of 6.3 days. This result is contrasting with other studies. Many specimens of Apexit group (66.67%) resisted bacterial penetration up to 40 days in the study of Eldeniz and Ørstavik [1]. Apexit showed the worst sealing ability compared to other sealers, including AH Plus, in Miletic et al. [8] study. Also presented significantly higher leakage than AH Plus in the study of Timpawat et al. [15]. Although, these studies used Apexit, not the new generation Apexit Plus.

Methacrylates present high polymerization contraction, which can exceed adhesion strength to dentin, leading to microleakage and lack of clinical success [23]. That can help understanding the result presented by EndoREZ in this study. Also, EndoREZ is very unstable and sensitive to the presence of oxygen, hardening in its absence, which is a complicated condition to achieve experimentally. The study of Neto et al. [17] also showed more leakage in EndoREZ compared to AH Plus. But Karapinar-Kazandag et al. [20] showed no significant difference between AH Plus with conventional gutta percha points, and EndoREZ sealer with EndoREZ Points.

In this study, Polifil showed similar results when compared to AH Plus and Apexit Plus, with 5.1 days of mean leakage. Souza et al. [10] also found comparable results for the sealer Polifil, AH Plus and EndoREZ. But this experimental sealer showed the least leakage, compared to other sealers as AH Plus, in the study of Pinheiro et al. [18]. Also presented less leakage compared to AH Plus in Souza et al. [11] study. The favorable result exhibited by Polifil can be attributed to its high polymerization expansion [9] and antibacterial activities.

The other experimental sealer, Copaifera sp oil, presented shorter sealing ability in this study. There are few studies in the literature testing this material as endodontic sealer. But it has been reported that this material presents efficient sealing ability, compared to MTA and GIC [12], and has quite good biological response [13]. Camargo et al. [13] found that Copaifera sp oil was the least cytotoxic sealer when compared to AH Plus and other endodontic sealers.

Therefore, new research should be performed to study the properties of these materials, as there is little information regarding it in the literature. So that a better clinical result can be achieved, once mean leakage around 6 days is insufficient in case of exposure of endodontic filling material to the oral environment, which can lead to the failure of endodontic treatment.

According to the methodology and analysis used in this study, it follows that the sealers EndoREZ and Copaifera sp oil presented shorter capacity of impermeabilization, without differences among EndoREZ groups, with conventional gutta percha points and resin coated gutta percha points. While the sealers Apexit Plus, AH Plus and Polifil were

similar ($p < 0.05$). However, none of the tested sealers was capable of resisting coronal bacterial leakage for more than 22 days.

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