Evaluation of the influence of smear layer removal on the apical leakage: in vitro study

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Abstract

Aim: The aim of this study was to evaluate the influence of different irrigant agents (sodium hypochlorite only, sodium hypochlorite + EDTA and sodium hypochlorite + BioPure MTAD) for smear layer removal, on apical leakage.

Materials and Methods: Three groups of premolars (n=49) were instrumented to size 45/.04 ProFile, according to the irrigant agent and filled with gutta-percha and AH Plus sealer. Four premolars formed the control group. Leakage was measured, and was determined as μL/min-1.10 psi, by means of the fluid filtration method after the specimens had been stored at 37℃ and 100 % humidity for 2 weeks. The data were submited to Tukey HSD test.

Results: BioPure MTAD showed the highest leakage means, differing statistically from the other groups. The sodium hypochlorite group showed the lowest leakage values.

Conclusion: Considering the experimental conditions and results observed, it was concluded that the removal of the smear layer did not contribute to a reduction in apical leakage.

Clinical significance: The work confirms that sodium hypochlorite remains the irrigating substance of choice in endodontics. We can speculate that the use of solutions to remove the smear layer without sonic or ultrasonic physical agitation is insufficient.

Key Words: Fluid filtration, BioPure MTAD, Leakage

Introduction

One of the objectives of endodontic treatment is the hermetic filling of the root canal system, which is achieved by complete, tridimensional sealing of the root canal system, helping in preventing microorganisms and their products invasion, through the coronal and apical pathways [1,2].

Continuous research on filling materials is based on the concept which is the apical migration of bacteria and their byproducts into insufficiently filled obturations, allowing leakage and becoming the main reason for the failure of root canal treatment [3].

Chemical-mechanical preparations usually produce debris-free root canals that can be safely filled. Nevertheless, complete cleaning of the root canal system may not be obtained as a result of the deposition of a layer of material on the root canal walls, referred to as the smear layer, which is produced by the cutting action of endodontic instruments during chemical-mechanical preparation [4].

Although the proportions of the smear layer components have not yet been clearly determined, scanning electronic microscopy exams have shown that it consists of an organic portion (coagulated proteins, necrotic and non-necrotic pulp tissue, odontoblastic processes, saliva, blood cells and microorganisms), and an inorganic portion (mineral components of the dentinal structure) [5]. Because of these two portions, smear layer removal requires a combination of irrigant agents, as usage of a single solution would be unlikely to be capable of dissolving both the organic and inorganic components [6].

Whether to allow the smear layer to remain before endodontic filling or not continues to be a controversial subject. Many authors consider it favorable to keep it as it fills the dentinal tubule entrances, thus reducing dentinal permeability [7]. Others foresee difficulties if it remains because it prevents medications from penetrating into the root canal walls and dentinal tubules [8] moreover it acts as a barrier between the filling materials and root canal walls, interfering in the formation of appropriate sealing [9]. Furthermore, any factors that may influence the adaptation of the filling material to the root canal wall are of great importance in determining the depth and extent of leakage, and finally, the prognosis of endodontic therapy.

Sodium hypochlorite at concentrations ranging from 1 % to 5.25 %, have been widely used as endodontic irrigants, as they are efficient for dissolving organic matter and eliminating microorganisms. Nevertheless, it is incapable of completely removing the smear layer from dentinal walls. In contrast, ethylenodiaminotetracetic acid (EDTA) used as an irrigant agent in endodontics, has an efficient chelating action, and dissolves mineralized tissues [10]. The use of EDTA at 17 % followed by 5.25 % sodium hypochlorite produces excellent results in smear layer removal [11].

Torabinejad et al. [12] investigated the effect of an endodontic irrigant denominated MTAD. The product is composed of an isomer of tetracycline (doxycycline), citric acid and a detergent, with the purpose of simultaneously removing the smear layer and promoting disinfection of the instrumented root canal walls. They demonstrated that BioPure MTAD in association with the use of sodium hypochlorite was effective as a final irrigant for smear layer removal, producing minimal erosive alterations on dentinal surfaces.

The aim of this study was to analyze the influence of smear layer removal on apical leakage in root canals treated with sodium hypochlorite alone, sodium hypochlorite + EDTA and sodium hypochlorite + BioPure MTAD, filled with gutta percha and AH Plus resinous cement. The null hypothesis was

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that there is no difference in the mean leakage values among the tested groups.

Materials and Methods

After obtaining approval from the Research Ethics Committee (CEP-PUCPR), forty-nine extracted human mandibular premolar teeth, with uniradicular, straight roots, obtained from the PUCPR tooth bank, were selected. The teeth were immediately stored in 10 % buffered formol and washed in physiological solution at the time of use. Roots with fissures, extensive caries, open apexes or resorptions were discarded.

Canal Instrumentation and Filling

The coronal portions of all the teeth were removed with a double faced diamond disk, to standardize the root length of each specimen to 15 mm.

Diamond tips (KG Sorensen, Rio de Janeiro, Brazil) were used to obtain direct access to the root canals. Instruments of the 15 K-Flexofile type (Dentsply Maillefer, Ballaigues, Switzerland) were used to locate and explore the canals, and were afterwards introduced up to the apical foramen. Then the working length was determined by subtracting 1 mm from this measurement. Apical patency was confirmed by the insertion of a 25 K-Flexofile instrument through the apical foramen before and after root canal preparation.

Root canal instrumentation was performed by the crown-apex technique, using ProFile instruments (Dentsply Maillefer, Ballaigues, Switzerland) with a 0.04 taper to 1 mm short of the real length of the roots. All the specimens were shaped up to instrument ProFile 45.04.

The specimens were randomly divided into 3 experimental groups of 15 teeth in each, according to the irrigant agent used during instrumentation. Four specimens formed the control group.

- Irrigation was performed with a Luer-lock syringe and gauge 27 needle, as follows:
- G1 (15): 5mL of 2.5% NaOCl at each change of instrument + 5mL of 17% liquid trisodium EDTA for 3 minutes, at the end of instrumentation;
- G2 (15): 5mL of 2.5% NaOCl at each change of instrument + 5mL of Biopure MTAD for 5 minutes, at the end of instrumentation;
- G3 (15): 5mL of 2.5% NaOCl at each change of instrument.

In all groups, final irrigation was performed with 5 ml of distilled water to remove debris and remaining irrigant agents. The canals were dried with absorbent paper tips (Dentsply Latin America, Petrópolis, Brazil) and the roots externally sealed with two layers of nail varnish, except for 1mm around the apical foramen.

The control group consisted of 4 specimens; 2 being used as positive control, which were filled with gutta percha cones, without filling cement. The other 2 were used as negative control, being completely sealed by the application of nail varnish, including the apical foramen.

After selecting the main cone, according to the surgical diameter determined by instrumentation, the filling maneuvers were performed. The root canals were filled by the lateral condensation technique, using standardized gutta-percha cones (Dentsply Latin America, Petrópolis, Brazil) and AH Plus endodontic cement (Dentsply De Trey, Konstanz, Germany). The cement was manipulated according to the manufacturer's instructions and previously introduced into the canals with the aid of No.4 Lentulo spirals. The main cones No. 45.04 were introduced immediately afterwards to the working length.

The lateral condensation technique was performed with the aid of a digital C spacer, used for laterally compacting the accessory cones that were introduced. The process was repeated until no more accessory cones could be inserted into the canal.

Heated shims were used for removing gutta percha from the canal entrances, preserving 13 mm of filling material in the apical region.

Afterwards, the specimens were stored in an oven at 37 ℃ and 100 % humidity for 15 days to guarantee that the filling cement had set completely.

Leakage Test

The fluid filtration method was used to determine leakage [1,2]. The root apex was connected to a metal needle of the Luer type by means of a plastic tube [2].

The leakage allowed by the tested groups was quantified according to the movement of a small air bubble inside a 25 µL micropipette 65 mm long (Microcaps, Fisher Scientific, Philadelphia, PA). The inside of the pipette and the entire system were filled with distilled water and submitted to a pressure of 10 psi. After ensuring that there was no leakage at the connections, the system was activated and balanced for 4 minutes.

The volume of fluid was calculated by observing the displacement of the air bubble, and expressed in µL/min-1.10 psi. The measurements were performed at intervals of 2 minutes in periods of 8 minutes for each specimen. The data obtained were submitted to the analysis of variance (Levene's Test) and multiple comparisons, using the Tukey-HSD test. The level of confidence used was 95% ($p < 0.05$).

Results

In the fluid filtration test, no movement of the air bubble was detected in the negative control group, whereas in the positive control group, the air bubble was displaced rapidly and uninterruptedly.

The overall values (Mean ± sd) expressed in µL/min-1.10 psi of leakage allowed by the three tested groups, are described in *Table 1*.

The group irrigated with BioPure MTAD presented the highest mean leakage, differing from the other agents which, in turn, differed between them, since the 2.5% sodium hypochlorite used alone presented the lowest mean leakage.

Table 1. Mean leakage values in µL/min-1.10 psi, produced by the different irrigant agents

Group	n	Mean ± sd
Sodium hypochlorite + EDTA	15	0.603 ± 0.295
Sodium hypochlorite + BioPure MTAD	15	1.256 ± 0.143
Sodium hypochlorite only	15	0.356 ± 0.232
Source: Research data.		

Discussion

The null hypothesis was rejected, because there was difference in the mean leakage values among the different groups tested.

Leakage in root canals is defined as the passage of bacteria, fluids and chemical substances between the tooth and the root canal filling material, which is the result of the presence of spaces filled by fluid at the interface of the filling material with the root canal wall. These spaces may result from deficient adaptation of the filling material to the root canal walls, solubility of the cements as well as their expansion or shrinkage. There are two possibilities of leakage: at the interface between the gutta-percha and the cement, and between the cement and root canal walls [13].

In this study, a fluid filtration model [1, 2, 14] was used to evaluate the influence of different irrigant agents for smear layer removal on endodontic leakage. The fluid filtration method has several advantages over the other methods used for evaluating leakage [15], as the models are not destroyed, allowing measurements over the course of time [16] and no marker is required, preventing problems related to molecular size, affinity for dentin, or pH [15, 17]. No specific material is required, as in the models of bacterial penetration or with radioactive markers [17, 18.] Nevertheless, WU et al. [15] indicated that the length and anatomy of samples, as well as patency and the diameter of the foramen after instrumentation should be standardized, in order to reduce the variables of this methodology.

When root canal walls are mechanically instrumented, a layer of residues is formed on the surface and extends into the dentinal tubules. This layer, known as the smear layer [8], is not completely removed by irrigation with sodium hypochlorite [19].

The effect of this layer on the prognosis of root canal treatment is unknown [20], but the thing which is known is it can be degraded by bacterial toxins and acids [21], which allows a pathway to form, through which leakage could occur [22].

Recently, a large number of researches have focused on the properties of BioPure MTAD [23]. The ability of this product to remove the smear layer quickly and in a self-limiting manner has been reported. It has been observed that in comparison with EDTA and citric acid, BioPure MTAD produced minimal erosion in intraradicular dentin [24]. Although the real consequences of destruction of the dentinal matrix remain undefined, Park et al. [25] affirmed that the increased leakage in samples treated with EDTA could be caused by its erosive properties. The study of De Deus et al. [26], conducted by means of glucose leakage is not in agreement with these findings, since no statistically significant difference in leakage was found in the specimens treated with EDTA, BioPure MTAD or 5.25% sodium hypochlorite. Whereas, the present study obtained results demonstrating that sodium hypochlorite + BioPure MTAD allowed greater endodontic leakage, followed by sodium hypochlorite + EDTA and sodium hypochlorite used alone, these results showing significant difference.

At present there has been great interest in investigating the possible effect of smear layer removal on endodontic leakage. Nevertheless, as yet there is no consensus about its real influence on effective sealing of the root canal system [2]. Some researchers have affirmed that the presence or absence of the smear layer has no significant effect on the sealing of root canal fillings [25-29], whereas others, such as Kont Çobankara et al. [2], Gençoglu et al. [30], Taylor et al. [31], Economides et al. [32], Kokkas et al [33] affirmed that its removal produces a positive effect on sealing, as it enables the cement to penetrate into the dentinal tubules, promoting better adaptation of the filling materials to the root canal walls [1]. Moreover, there are authors that have reported that allowing the smear layer to remain, could reduce leakage [13, 34], thus corroborating the results of the present study, because they considered that the smear layer could diminish dentinal permeability, preventing the bacterial infiltration into the dentin. Michelich et al. [35] and Drake et al. [34], demonstrated that smear layer removal favored the access of bacteria to the dentinal tubules, suggesting that allowing it to remain has the potential to block bacterial penetration into the tubules by reducing dentinal permeability.

Shahravan et al. [36], in a systematic and metanalysis study of the effects of the smear layer on root canal leakage, reported that 7 of the 65 comparisons used the fluid filtration method. Nevertheless, 4 of the 7 studies that used the fluid filtration method and 2 of 6 studies that used the bacterial penetration method indicated significant difference in favor of smear layer removal, while only 1 study that used the fluid filtration method showed significant difference in favor of keeping it. As a result of the variations among the methodologies, it was not possible to combine the results of all the studies; hence the results of studies conducted with color infiltration tests were only combined.

These conflicting results are not new in the literature about endodontic leakage, and could be attributed to the differences among the types of cements, filling techniques, cement layer thickness, manner in which the smear layer was produced, use of different irrigant agents for smear layer removal and the different methodologies used in the various published studies, which creates difficulties in making comparisons among the researches and defining a final conclusion.

Under the conditions of the present in vitro study, the associations tested (2.5% sodium hypochlorite + EDTA and 2.5% sodium hypochlorite + BioPure MTAD) had a negative influence on apical leakage. Therefore, it could be concluded that removal of the smear layer does not appear to contribute to a reduction in apical leakage.

Conclusion

Considering the experimental conditions and results observed, it was concluded that the removal of the smear layer did not contribute to a reduction in apical leakage.

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