Original Article

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Influence of gutta-percha cone disinfection on leakage: comparison of two sealability assessment methodologies

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Abstract

Aim: To clarify the influence of 5.25% sodium hypochlorite disinfection of gutta-percha cones on leakage and to compare the outcomes of two sealability assessment methodologies (fluid filtration and dye penetration methods). **Methods:** Thirty teeth were prepared and filled using the gutta-percha lateral condensation technique. Group I was filled with gutta-percha cones disinfected with sodium hypochlorite 5.25%, while Group II was filled with cones that were not disinfected. Apical leakage was measured using: (a) fluid filtration technique and (b) linear measurement of dye penetration after clearance. **Results:** The Student's t-test showed no significant difference between the groups for both techniques ($p<0.05$). **Conclusions:** Both methodologies employed, even with the inherent limitations, were capable of identifying sealing failures. The disinfection of gutta-percha cones with 5.25% sodium hypochlorite did not alter apical leakage.

Keywords: disinfection, gutta-percha, dental leakage, sodium hypochlorite, endodontics.

Introduction

The hermetic endodontic sealing is considered a major objective for achieving root canal treatment success. On this regard, much emphasis has been placed on the improvement of material sealing ability[1-2] and, therefore, it is of paramount importance to investigate of the quality of root canal filling[2-8].

Several studies using leakage tests have been developed with this purpose[2-8]. On the other hand, the results of leakage studies have been seen as polemical and controversial. The great diversity of methodologies, the lack of studies approaching the comparison among different methods and the difficulty of extrapolating the scientific findings related to the *in vitro* sealing ability to the clinical field have been pointed out[9].

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Another important aspect is that most of the available studies have investigated the sealing capacity of endodontic sealers[1,5-7], although the major portion of endodontic fillings is comprised of a solid material, i.e., the gutta-percha cones. As a matter of fact, chemical disinfection of gutta-percha cones has been suggested[10-11], and its influence on leakage has yet to be properly addressed. Even so, the assumption that surface deformation and physical alterations on this material may influence its sealing ability has been raised[10,12].

Several different substances have been used for cone disinfection, the most common of which are 2% chlorhexidine and sodium hypochlorite, at concentrations that vary from 0.5 to 5.25%[10,13-14]. Chlorhexidine has demonstrated antimicrobial potential for disinfecting gutta-percha cones, but it is ineffective against *Bacillus subtillis* spores even after immersion for 72 h[10,15]. Sodium hypochlorite offers an excellent antimicrobial capacity when in contact with this material, even inhibiting sporogenous forms[10,13]. On the other hand, it is an oxidative solution which breaks down polymer chains, and causes structural deformations to a wide range of materials[12,16-18].

In view of the importance of comparing various sealability assessment methodologies and clarifying the relevance of disinfection on leakage[19-20], the purpose of this study was to investigate the influence of 5.25% sodium hypochlorite cone disinfection on the endodontic sealing and to compare the results obtained with the fluid filtration and dye penetration methods.

Material and methods

This study was approved by the Institutional Review Board (IRB) and Research Ethics Committee of the School of Dentistry of the Federal University of Rio Grande do Sul, Brazil.

Thirty human canines with a straight root canal were selected. Roots with cracks, caries, open apices, or resorption were excluded.

Instrumentation and filling of the canals

The coronal portion of all teeth was removed with diamond disks, so that each specimen was 20-mm long. A #15 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the root canal until the tip was just visible at the apical foramen. Working length was determined by subtracting 1 mm from this length. Apical patency was confirmed by inserting a #15 file through the apical foramen before and after root canal preparation.

The teeth were prepared with hand instrumentation according to a crown-down technique. The last instrument was a # 35 file so that the enlargement of all canals was standardized. Canals were irrigated with 1% sodium hypochlorite (Biodinâmica, Ibiporã, PR, Brazil) using a syringe and a 27-gauge needle, and were dried with paper points.

The prepared roots were randomly divided into 2 groups of 15 roots each. Group I was obturated with 5.25% sodium hypochlorite disinfected gutta-percha cones for 5 min and washed with distilled water, while Group II was obturated with non-disinfected cones. A standardized gutta-percha cone (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil) was fitted with a "tugback" at working length. The canals were obturated using gutta-percha lateral condensation and a zinc oxide and eugenol based sealer (Endofill; Dentsply Ind. e Com. Ltda.).

Radiographs were taken to evaluate the quality of root filling regarding homogeneity and apical extension. Canal obturation was complemented if voids were detected to obtain well-compacted fillings. Excess material was removed with a heated plugger at coronal level and the remaining filling material was vertically condensed using Schilder pluggers 3 and 4 (Dentsply Maillefer). Thereafter, the specimens were stored at 100% humidity and 37°C.

Fluid Filtration

Two layers of nail polish were applied on the entire extent of the root (except on the apical surface of the root canal).

The apical surfaces of the roots were cemented onto 2x2x0.7-cm pieces of Plexiglass with a cyanoacrylate adhesive (Loctite Henkel Ltda., Itapevi, SP, Brazil). The pieces of Plexiglass had 18-gauge stainless steel tubes placed through their centers, ending flush with the upper surfaces.

The apical openings of the tooth segment were then positioned over the tubes to permit a direct communication between the root canal and the micropipette/microsyringe system. The apical portion of the specimens was filled with water through the 18-gauge needle, taking care to remove all air bubbles.

The roots were mounted in the fluid filtration device designed to measure leakage, assembled in a similar way to that described by Derkson et al.[19], and later adapted for endodontic leakage studies according to Wu et al.[3] and da Silva Neto et al[1].

The leakage of the 2 groups was quantified by following the progress of a tiny air bubble traveling within a 25-ì micropipette (Microcaps, Fisher Scientific, Philadelphia, PA, USA). The entire tubing pipette and the syringe were both filled with distilled water under the pressure of 10 psi.

The fluid filtration volume was measured by observing the air bubble movement. The air bubble displacement was recorded as the fluid filtration result, expressed in ì/min^{-1}.10 psi. Fluid movement measurements were made at 2-min intervals for 8 min and then averaged. After all connection points had been sealed, the system was allowed to equilibrate for 4 min. One sample was excluded during the measurement procedures because a crack was verified.

Dye Penetration

After the fluid filtration measurements, the specimens were kept in India ink for 10 days (John Faber Castel, São Paulo, SP, Brazil) and then washed with tap water to remove the excess of ink. The nail polish layers were then removed with a scalpel and the roots were cleared.

The teeth were demineralized in 5% nitric acid until a milky color was achieved. The solution was renewed every

24 h until the process ended. The specimens were then dehydrated in 80º ethanol for 12 h, followed by 1 h in 90º ethanol and 1 h in 96º ethanol. This last procedure was repeated three times. All ethanol solutions were obtained by the dilution of 100% ethanol in distilled water. The specimens were then allowed to dry naturally for 10 min and were put into glass vials with methyl salicylate ($C_8H_8O_3$). After 2 h, the internal structure of the tooth could be visualized. The ink leakage was measured three dimensionally under stereoscope microscopy (x40) (Meiji Techno Co., Ltd., Tokyo, Japan).

For clearance assessment, the examiner was calibrated using the intra-class correlation coefficient test. The data were subjected to statistical analysis using a Student's t-test. The confidence level was pre-set at 5% for both evaluation methods.

Results

For clearance evaluation, the examiner was calibrated and the ICC test scored 0.939.

There were no statistically significant differences ($p > 0.05$) between the study groups by means of the fluid filtration and clearance test, as shown on Tables 1 and 2.

Table 1- Mean of fluid movements measures and statistical analysis for the groups (μ/min^{-1}.10 psi) ($p < 0.05$).

Group	N	Mean	Sd	T	p
I	15	0.997	0.47	0.499	0.622
II	14	0.910	0.46		

Table 2 - Mean of trace penetration measure in mm after clearance ($p < 0.05$).

Group	N	Min	Max	Mean	Sd	t	p
I	15	0	8	2.33	1.88	1.774	0.087
II	14	0	4	1.29	1.20		

Discussion

The present study has attempted to elucidate two important aspects: the comparison of different sealability assessment methodologies and the influence of cone surface alterations caused by disinfection on leakage.

Several methods have been described aiming at evaluating the sealing quality of root canals fillings. The most common is the dye tracer penetration test[21]. This technique, associated with clearance, makes possible the three-dimensional observation of the dental structure and the identification of the dye penetration extension.

Fluid filtration is based on the principle that no fluid movement will be detected if the root canal system is completely sealed. Measurements of fluid transportation are carried out by observing an air bubble and its movement along a capillary. Further, the fluid filtration technique quantifies microleakage and allows repeated measurements because it is nondestructive[9,20,22-23]. For this reason, in the present study, it was possible to evaluate the same sample using two different *in vitro* methodologies.

Since so far there is no leakage method able to effectively assess clinically relevant aspects, comparison between different materials and techniques used in endodontic practice must still be carried out based on preliminary tests such as those described in this study.

The efforts to reduce artifacts and improve methodologies have not generated conflicting results for sealing capacity, since both methods employed in the present study have not shown significant differences between the groups. However, the statistical analysis shows some variations that should be further explored. Fluid filtration presented a p value of 0.662, and die penetration tests a p value of 0.087. These values show a smaller difference between the groups when fluid infiltration is used, which may indicate that dye penetration tests produce some confounding factors that might be increasing the variability of measurements. On the other hand, despite of the technique used, leakage could be detected.

Although it has been currently documented that dye penetration measurements yield some level of variation, in the present study, ICC data showed that this fact is not related to the variability in the operator's experience.

The variation in the analysis of the dye penetration is probably influenced by some factors: (a) gaps between the root filling and the canal wall may contain air and/or liquid, which would prevent the penetration of the tracers and influence the results; (b) the potential of the tracer to react or affect the filling material itself might make the results from some of these tests unreliable (c) because of dentinal tubule permeability, different leakage tracers could penetrate through root dentin and not through the canal[4].

Another problem for extrapolating the findings related to the dye penetration tests to the clinical field is that there is no equivalence between the ink particles and the bacteria and their toxin sizes. Also, this technique does not provide any information about the volume of tracer that penetrates the dental structure[2]. Due to these aspects, dye penetration tests have their relevance related to the identification of visible gaps in the sealing.

Generally, *in vivo* microleakage is expected to be not as severe as the ones observed in *in vitro* methodologies because it is accepted that a root canal filling should be bacteria tight. On the other hand, the importance of bacterial toxins should not be neglected, and further investigations are necessary to determine the clinically relevant amount of leakage that can be considered as pathological[2].

Considering the influence of disinfection on sealing, this procedure did not cause alterations on leakage. One can infer that the possible changes in the structure and surface of gutta-percha cones do not jeopardize the seal of root canal system.

According to Pang et al.[11], chemical disinfection promotes alterations on the tensile strength and elongation rate of the gutta-percha cones. Valois et al.[12] observed significant surface alterations in gutta-percha cones after disinfection in sodium hypochlorite solutions, particularly at elevated concentrations, using atomic force microscopy. Furthermore, some studies have confirmed the sodium hypochlorite capacity to cause alterations during the process of disinfecting gutta-percha cones[11]. The aim of the present research did not encompass the analysis of structural and surface alterations. Even though, it is presumed that these occurred in the disinfected gutta-percha cones used in this study.

Short et al.[17] proved that crystals are formed on the surface of gutta-percha cones after the immersion of them in sodium hypochlorite. However, washing the material in alcohol, saline or distilled water efficiently removes these crystals[10,17]. In the present study, after disinfection, the cones were washed with distilled water, thus avoiding the influence of the presence of crystals in analysis of the results.

Microbiological studies have confirmed the efficiency of disinfection of gutta-percha cones using 5.25% sodium hypochlorite for 1 mine[10,14], but in the present study the immersion time chosen was 5 min. A longer period was used in order to increase the contact time of the cones with the solution. It is important to note that, even exposing gutta-percha cones for 5 min, disinfection did not lead to higher infiltration levels, when compared to non-disinfected cones. The influence of the disinfection of gutta-percha cones in endodontic sealing is one of the most important aspects in the determination of protocols. The results of the present study do not indicate negative effects, in this sense.

Further investigations are necessary to determine whether material alterations caused by disinfection could impair long-term outcomes of endodontic sealing, causing, for instance, a faster onset of components degradation. According to Maniglia-Ferreira et al.[24], the oxidation reaction of gutta-percha components is a slow process, but significant degradation can be accelerated by some factors, such as the presence of periapical lesions.

In conclusion, both methodologies employed in this study, even with the inherent limitations, were capable of identifying sealing failures in the root canal system. The disinfection of gutta-percha cones with 5.25% sodium hypochlorite did not cause changes in apical leakage.

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