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Original paper

A SEM evaluation of the different techniques effectiveness to remove ozonated oil from root canals

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Keywords

ozone therapy, intracanal medication, endodontics, ozonated sunflower oil, calcium hydroxide, SEM..

Abstract

Ozonated sunflower oil is an alternative to intracanal medication in root canal treatment. Thus, the aim of this study is to evaluate whether ozonated sunflower oil is effectively removed from the root canal with Passive Ultrasonic Irrigation and the XP-Endo Finisher. Materials and methods: Twenty-three human single-root teeth were selected for this experiment. Teeth were divided into 2 groups according intracanal medication, G1: Ultracal calcium hydroxide, and G2: ozonated sunflower oil, and then subdivided according to complementary techniques: G1A (n=5): sunflower ozonated oil + PUI; G1B (n=5): ozonated sunflower oil + XP-Endo Finisher; G2A (n=5): Ultracal calcium hydroxide + PUI; G2B (n=5): Ultracal calcium hydroxide + XP-Endo Finisher; G3 Control (n=3): no intracanal medication. After chemomechanical preparation up to R25 or R40 Reciproc files, root canals were filled with intracanal medication according to experimental groups and remained in a wet chamber for 14 days. The teeth were then treated according to subgroups. Samples were prepared for SEM evaluation and images were analyzed by one evaluator. Data were tabulated and underwent Kruskal-Wallis and Mann-Whitey testing ($p < 0.05$). Results: Image analyses showed all tested protocols were effective in the removal of intracanal medication, but there was no statistical significance among them. Conclusion: Passive ultrasonic irrigation and the XP-Endo Finisher were effective in the removal of ozonated sunflower oil from root canals.
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1.- Introduction

The fact that the chemomechanical stage of root canal treatment has decontamination limitations is a consensus in scientific literature (Roças e Siqueira, 2010; Siqueira *et al*., 2010; Pinheiro *et al*., 2015; Nakamura *et al.*, 2018; Nogales, 2019). Thus, calcium hydroxide-based intracanal dressing has been proposed as an alternative to improve the endodontics protocol. Various studies show a decrease in the amount of bacteria between appointments (Molander *et al*., 2007; Paiva *et al*., 2013; Nakamura *et al*., 2018) to levels lower than those obtained with chemomechanical procedures; however, microorganisms and endotoxins are still detected.

Ozone therapy has been proposed as a therapeutic tool that employs a gaseous mixture composed of oxygen and ozone. In endodontics, research points toward a real potential for its insertion in the endodontics protocol. (Nogales *et al*., 2016; Nogales, 2019). In the case of intracanal medication, ozonated oil is recommended. In an *in vivo* study, Silveira *et al*. (2007) analyzed the histobacteriological, histopathological and radiographic aspects of apical healing. They concluded that ozonated sunflower oil showed the same tissue response as calcium hydroxide. Ferreira (2011), in a randomized clinical trial, used ozonated sunflower oil as an intracanal dressing. The protocols that used ozone as an adjuvant showed the best results in periapical healing and a complete absence of post-operative pain, which is statistically significant in comparison to the standard protocol.

The issue to be raised is the difficulty of removing intracanal dressing. In a prior study, Ferreita *et al*. (2009) evaluated the quality of ozonated oil removal. They concluded that the protocols employed were not effective in completely removing the ozonated oil from the root canal. Several studies have proposed complementary techniques to improve the effectiveness of intracanal medication removal, such as Passive Ultrasonic Irrigation (PUI), as described by van der Sluis *et al*. (2007), Mancini *et al*. (2013) and the XP-Endo Finisher (Carvalho, 2019). The last one shapes the root canal according to the inner anatomy and body temperature and takes on a new shape due to the molecular memory, allowing the instrument to reach and clean difficult areas which the standard traditional endodontic files normally would not reach. Furthermore, the XP-Endo Finisher does not alter the shape of the original root canal (Silva *et al*., 2017).

Thus, the aim of this study is to evaluate the effectiveness of cleaning in root canals after calcium hydroxide- and ozonated oil-based intracanal medication, using PUI and the XP-Endo Finisher.

2.- Materials and methods

This study was approved by the Ethics Committee of the University of Mogi das Cruzes, São Paulo, Brazil, protocol no. 3.348.527, dated 05/27/2019.

2.1 Tooth selection

Twenty-three human single rooted teeth were selected. They were obtained from the Dental Clinic of the University of Mogi das Cruzes and extracted due to periodontal or prosthetic issues, cleaned and kept in saline solution until use. Selection of the teeth was based on size and morphological similarity of the single root, as verified by periapical radiography.

2.2 Sample preparations

Tooth crowns were sectioned to create a 15 mm standard root length. Apical foramen were sealed with 3M composite (3M do Brasil Ltda., Campinas, SP, Brazil) to avoid irrigation overflow. The entire root canals were enlarged to the size of a K-file #25 (Dentsply, Ballaigues, Switzerland). The instrumentation was performed with R25 e R40 Reciproc files (VDW, Munich, Germany) along with 5 mL of 2.5% sodium hypochlorite (Asfer, São Caetano do Sul, SP, Brazil) irrigation at every instrument change. After instrumentation, the root canals were filled with 17% EDTA (Inodon, Porto Alegre, RS, Brazil) for 5 minutes and received a final rinse with 5 mL of 2.5% sodium hypochlorite.

2.3 Experimental groups

Specimens were divided into 2 experimental groups (n=10) according to the intracanal medication used: G1 – Ultracal calcium hydroxide (Ultradent, UT, USA); G2 –ozonated sunflower oil (Philozon, Balneário Camboriú, SC, Brazil), peroxide values (PV) of 500 mEq/kg O_2. Ultrasonic activation (Emisonic 230, MMO, SP, Brazil) was performed for 30 s after root canals were filled with intracanal medication.

Teeth were kept in a wet chamber for 14 days and then randomly subdivided according to the intracanal medication removal method: G1A (n=5): ozonated sunflower oil (PV 500 mEq/kg O_2) + PUI; G1B (n=5): ozonated sunflower oil (PV 500 mEq/kg O_2) + XP-Endo Finisher; G2A (n=5): Ultracal calcium hydroxide + PUI; G2B (n=5): Ultracal calcium hydroxide + XP-Endo Finisher; G3 control (n=3): with no intracanal medication.

2.4 Intracanal medication removal

The root canals were irrigated with 6 mL of 17% EDTA and activated for 30 s every 2 mL according to subgroups: G1A and G2A – (PUI) and G1B and G2B – XP-Endo Finisher. Root canals were dried with paper points and then samples were standardized for SEM.

Images obtained were randomly organized in a Power Point presentation and analyzed by one blinded examiner who has been a specialist for over 10 years. The evaluation was performed qualitatively and scored as described by Ahir *et al*. (2014): score 1 – no smear layer, dentinal tubules clean; score 2 – moderate presence of smear layer, dentinal tubules with debris, and; score 3 – marked presence of smear layer, dentinal tubules closed. Images were then separated by thirds of the root canals: cervical third, medium third and apical third.

Data were tabulated and statistical analyses performed according to the Kruskal-Wallis test and complemented by the Mann Whitney test, with a significance level of 5%.

1. RESULTS

The data were tabulated and submitted to statistical analysis ($p < 0.05$). The data are shown in Table 1.

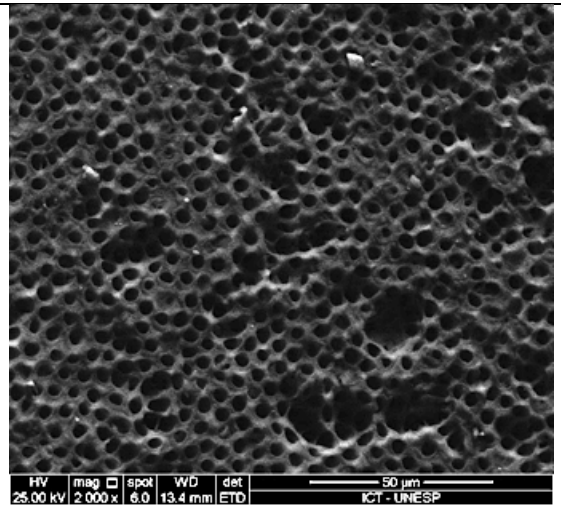
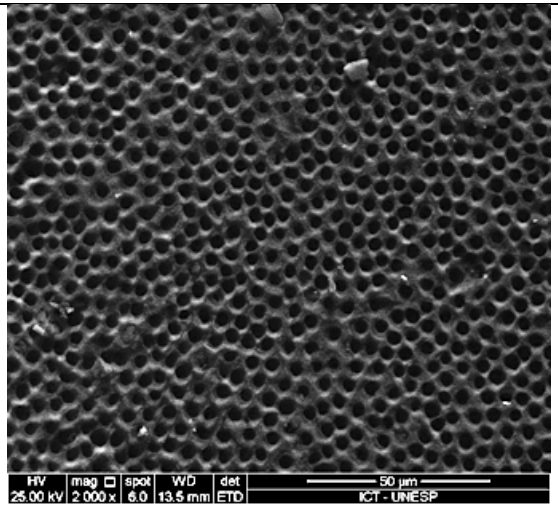
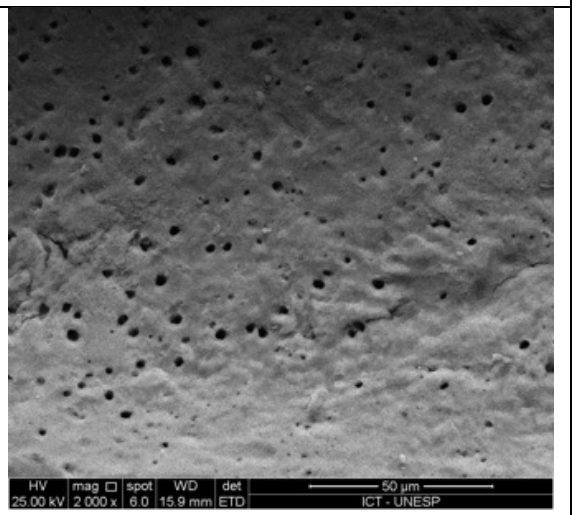
Table 1. Statistical analysis of the ozonated oil remove from root canal

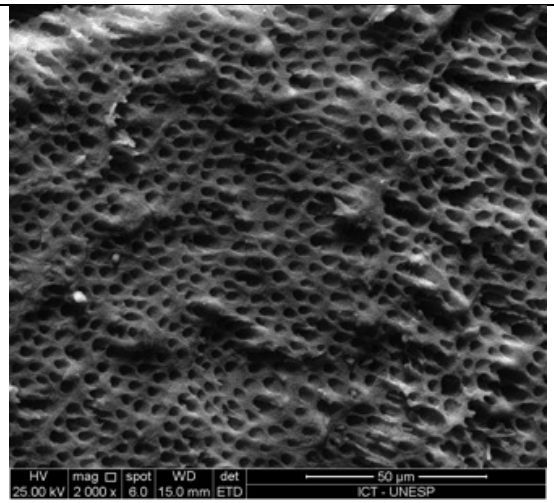
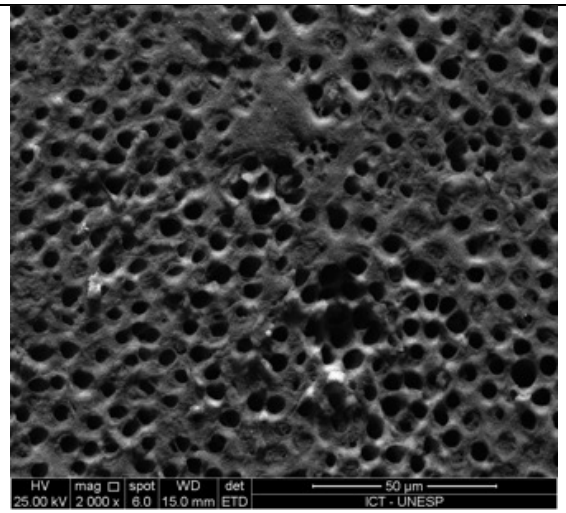
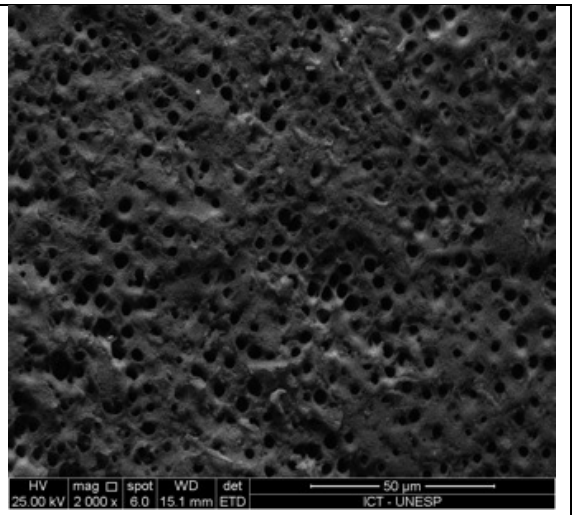
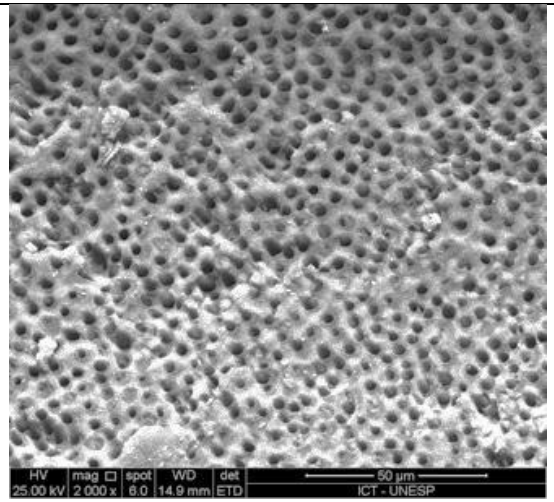
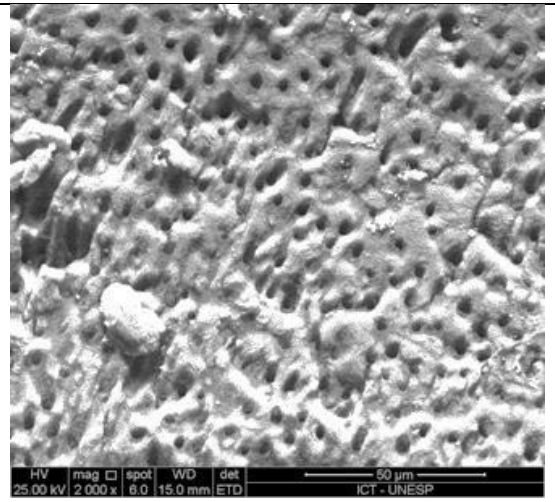
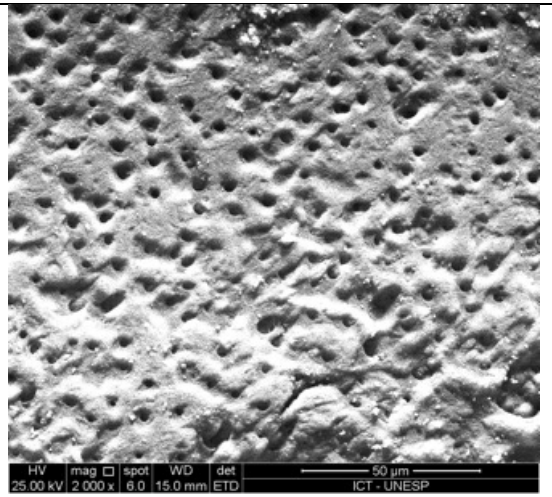
Group	N	Median	Middle Rank	Z-value
Group 1A	5	2ª	10,7	0,09
Group 1B	5	2ª	10,7	0,09
Group 2A	5	2ª	10,7	0,09
Group 2B	5	1ª	9,9	-0,26
Global	20		10,5	

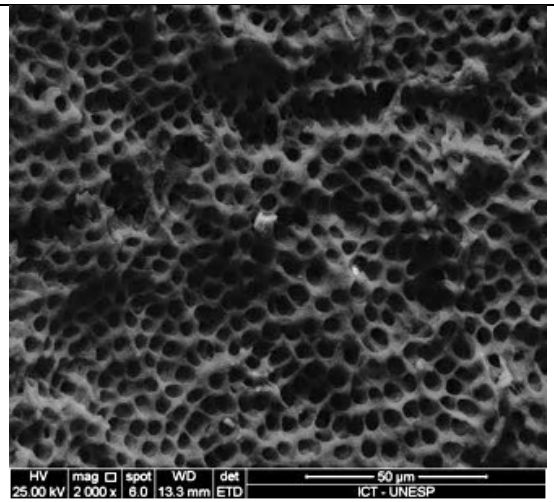
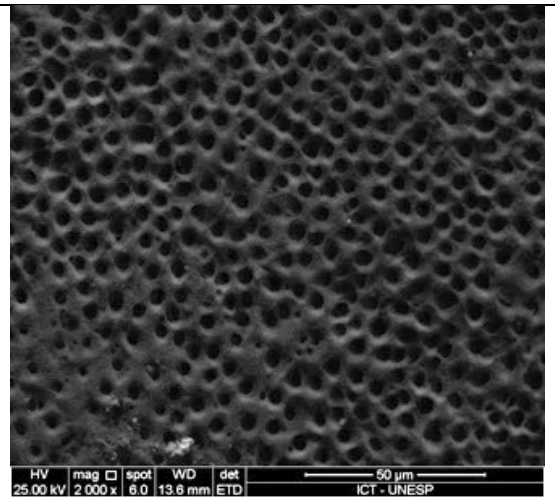
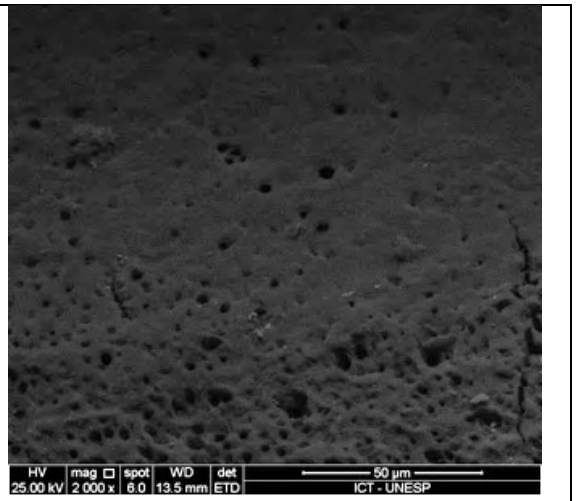
After the Kruskal-Wallis statistical test was applied, complemented by the Mann-Whitney test, the results revealed that the protocols applied were effective in removing the tested intracanal medication, however there was no significant statistical difference among the groups (p=0.933).

SEM images are displayed in Figure 1. The presentation shows a representative sampling, due to the large number of images.

Figure 1 – Distribution of the SEM images with 2000x magnification, showing the dentinal tubules of the experimental groups in respect to the region of the root canal.

	Cervical third	Middle third	Apical third
G1A			

G1B			
G2A			

G2B	 HV 25.00 kV mag 2 000 x spot 6.0 WD 13.3 mm det ETD 50 µm ICT - UNESP	 HV 25.00 kV mag 2 000 x spot 6.0 WD 13.6 mm det ETD 50 µm ICT - UNESP	 HV 25.00 kV mag 2 000 x spot 6.0 WD 13.5 mm det ETD 50 µm ICT - UNESP

2. DISCUSSION

The main goal of this study was to answer the question of whether ozonated sunflower oil as an intracanal medication would be effectively removed from the root canals by improving the cleaning ability of the irrigating solution with complementary techniques, like PUI and the XP-Endo Finisher. Since ozonated oil is not a water-soluble solution, it may possibly present debris adhering to the root canal walls interfering with the adhesion of the endodontic cement.

Intracanal medication in endodontics is still a controversial issue. There is no consensus about the recommendation in medical literature, as described by Manfredi *et al*. (2016). Intracanal medication is classified as a late complementary procedure that aims to supplement the deficiencies of the decontamination achieved in the root canal system by chemomechanical procedures (Nogales 2019).

Calcium hydroxide is the elected intracanal medication in the treatment of apical periodontitis. However, the main concern lies in its removal (Ferreira *et al*., 2009), which is one of the most controversial issues and indicative of the need for complementary procedures to enhance the quality of the irrigation to remove the intracanal medication. The results presented in this study confirmed the importance of PUI and the XP-Endo Finisher to supplement calcium hydroxide removal from the root canal.

Passive ultrasonic irrigation has been the most studied complementary procedure nowadays (van der Sluis *et al*., 2007; Carvalho, 2019; Nogales, 2019). Results from this study are in accordance with several studies which proved its effectiveness (Molander *et al*., 2007; Paiva *et al*., 2013; Nakamura *et al*., 2018; Nogales 2019). Mozo *et al*. (2012) clarified that during PUI, energy is transmitted from the file or ultrasonic insert to the irrigator through ultrasonic waves to induce the acoustic wave and cavitation. Cavitation is characterized by the production of expanding steam microbubbles, contraction and implosion of pre-existing bubbles in a liquid. In turn, the acoustic wave is a rapid movement of the fluid in a circular or swirling manner around the oscillating instrument (van der Sluis *et al*., 2007). Chen *et al*. (2013) observed an increase in the speed of the intracanal irrigator, as well as an increase in contact with the canal walls by the irrigator, as described by Macedo *et al*. (2014).

Another possibility for activation of the chemical substance which has shown promising results is the XP-Endo Finisher, as concluded by Pereira *et al*. (2017), Carvalho (2019) and corroborated by this study. The XP-Endo Finisher proved to be efficient in removing intracanal medication because, in addition to aiding agitation of the irrigating solution, it also allows for mechanical cleaning of areas of the canal that were previously inaccessible, as concluded by Carvalho (2019). This fact can be justified by the instrument's small diameter and high flexibility, which can increase its reach by up to 6 mm in diameter when inserted into the canal. It is exposed to body temperature and changes shape due to molecular memory and is resistant to cyclic fatigue, thus being able to reach areas of high complexity in the root canals (Pereira *et al*., 2017).

Technically, the action of PUI and the XP-Endo Finisher acts directly to promote a more effective removal of intracanal medication, thus providing for cleaner dentinal tubules, as evidenced by the images acquired with SEM in this study.

The analysis was performed by 1 specialist in endodontics with over 10 years of experience; this same methodology was used by Ferreira *et al.* (2009). The results of the present study show that the majority of the specimens in the cervical and middle thirds had a minimum amount of intracanal medication and remaining or almost absent smear layer in the dentinal tubules in relation to the apical third, which presented the greatest amount of intracanal medication and remaining smear layer, when compared with the cervical and middle thirds in both experimental groups.

Regarding intracanal chemical activity, ozone therapy has shown great potential for application in endodontic treatment, thanks to the oxidizing power and reactivity of ozone. This study, like Silveira *et al*. (2007), Ferreira *et al*. (2009) and Ferreira (2011), used the form of ozone associated with sunflower oil. The antimicrobial action of ozonated oil has been proven over time, as described by, Bocci (2002), Ferreira (2011), and Farac *et al*. (2013).

In an initial study, Ferreira *et al.* (2009) concluded that conventional irrigation with syringe and EDTA was not effective in removing ozonated oil from the root canal. The present study has proved the effectiveness of removal, as long as it is associated with the complementary procedures employed. There was no statistically significant difference between PUI and the XP-Endo Finisher in terms of quality in removal of intracanal medication, and both were effective in providing clean dentinal tubules. Thus, it is of the utmost importance to stress the use of complementary techniques.

3.- Conclusion

In light of the results obtained and the methodological limitations of this study, it is possible to conclude that the removal of intracanal medication by means of calcium hydroxide and ozonated sunflower oil is effective when PUI and the XP-Endo Finisher are used. However, further studies are needed to prove the effects of ozonated oil as an alternative in intracanal medication.

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