Effect of three final irrigation protocols on the smear layer removal from the middle third of endodontically treated teeth: a qualitative analysis

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ABSTRACT

Objective: this study evaluated by scanning electron microscopy (SEM) the sealer capacity of dentinal tubules of teeth treated endodontically using different chelating solutions. **Materials and Methods:** The root canals were irrigated with 2.5% sodium hypochlorite (NaOCl), replaced every instrument and filled. The auxiliary solutions used with a passive ultrasonic irrigation (PUI) for 1 minute were: 70% ethanol (control); 10% citric acid; 17% EDTA; and 0,2% chitosan. The roots were split lengthwise into two parts and samples were taken for analysis in a scanning electron microscope to obtain photomicrographs for the qualitative assessment. **Results:** 17% EDTA solution and 10% citric acid removed the smear layer similar to each other. The 0.2% chitosan solution removed the smear layer partially, with lower efficacy than 17% EDTA solution and 10% citric acid. **Conclusion:** There were a greater removal of smear layer with use of 17% EDTA, followed by 10% citric acid, 0,2% chitosan and 70% alcohol.

Keywords: Chitosan. Endodontics. Tooth, nonvital. Chelating agents.

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Introduction

Teeth restorations with major coronary destruction is a dentistry challenge. In several situations, there may be a need for an endodontic treatment and the use of an intrarradicular retainer to perform this rehabilitation. Fiberglass pins are a clinical option for providing retention to coronary restorations. Besides being aesthetic, they have biocompatibility, absence of corrosion and require less clinical time.[1] These pins offer greater resilience, a elasticity modulus similar to dentin and an adhesive cementation.[1] However, dentin/cement interface detachment has been considered the main cause of restorative failure with these retainers.[1] One of the factors considered to be detrimental to the fiberglass pins bond strength is the inefficiency of certain chemical solutions in removing the smear layer from the conduits walls during the chemical-mechanical preparation of the root canals.[2,3] This smear layer may hinder or prevent the penetration of antimicrobial agents through the dentinal tubules, interfering in the adhesion of the endodontic cements and compromising the quality of the root canal obturation.[4,5] Its removal from the root canal system increases the chances of successful endodontic therapy as well as the successful adhesion of retainers to the root canal.[6,7,8]

To date, an ideal irrigant solution, which simultaneously has tissue dissolution capacity, antimicrobial activity, smear layer removal capacity and low toxicity, is not available.[6] The most commonly auxiliary chemical used is sodium hypochlorite (NaOCl) in various concentrations.[1,5,9,10] However, NaOCl alone is not able to perform a complete debridement of the root canals or to eliminate the bacteria from the biofilm.[11] The chelating agents are then used for the final irrigation of the root canals.[11] They react with calcium ions on hydroxyapatite crystals by removing these ions from the dentin.[12]

The 17% ethylenediaminetetraacetic acid (EDTA) is the most commonly used chelating solution in dentistry.[1,13] However, this substance has a strong demineralizing effect, widening the dentinal tubules, softening dentin and denaturing the collagen fibers. These effects may cause difficulty in the adaptation of the obturator material to the wall of the root canal.[12] Another disadvantage is that EDTA is considered a pollutant, since this substance is not originally found in nature.[7] The search for more biocompatible solutions, in order to minimize their harmful effects on the periapical tissues, led researchers to seek alternatives to EDTA.[7,10,14]

That said, another chelator that can be used is citric acid.[15] It reacts rapidly with calcium ions, has relatively low cytotoxicity as well as antimicrobial properties.[14,16] However, chitosan, a natural polysaccharide, has attracted attention due to its biocompatibility, biodegradability, bioadhesion and absence of toxicity.[11,17] It is obtained by the deacetylation of chitin, found in some arthropods and it has become ecologically interesting for various applications due to its abundance in nature and low production costs.[7] Chitosan has high chelating capacity for several metal ions under acidic conditions and it has been widely applied for the removal or recovery of metallic ions in different industrial areas.[2,4,7,14]

Due to this wide range of available chelating materials and to the appearance of chitosan as a less damaging alternative to dental structure, this study aimed to evaluate, by scanning electron microscopy (SEM), the ability of the dentinal tubules to be disembodied from endodontically treated teeth using different chelating solutions.

Materials and Methods

The present study was carried out in accordance with the norms and guidelines of #196/96 resolution of the National Health Council, which regulates the research involving human beings, being approved by the Research Ethics Committee of Juiz de Fora Federal University in accordance with opinion #060/2010.

Four unirradicular human canines obtained at the "Teeth Bank" of São Leopoldo Mandic College (protocol nº 1.091.420) were kept immersed in 0.1% thymol until the moment of its use. After external mechanical cleaning of dental surfaces with Ultrasonic Point Pero E (Dabi Atlante, Ribeirão Preto, SP, Brazil), dental crowns were removed using a high rotation drill (Kavo, Joinville, SC, Brazil) and root length obtained was 14mm. The roots were observed through periapical radiographs (parallelism technique) to verify their internal integrity, characterized by the absence of fractures or cracks.

Endodontic treatments were performed according to the regressive step technique (International

Standardization Organization ISO 3630-1, 2008), with a working length of 1 mm of the apex, to the stainless steel file K-File # 45 (Dentsply Maillefer, Switzerland, Switzerland). The root content was irrigated with 2.5% NaOCl (Asfer, São Caetano do Sul, SP, Brazil), substituted for each instrument. At the end of instrumentation, the canals were washed with distilled water and dried with # 40 absorbent paper tips (Dentsply Maillefer, Petrópolis, RJ, Brazil). The obturation was performed with gutta percha (Odous de Deus Indústria e Comércio Importação e Exportação Ltda., Belo Horizonte, MG, Brazil) and epoxy resin paste (AH Plus Dentsply, Konstanz, Germany).

After 14 days, the conduits were unclogged with #1, #2 and #3 drills, and filled with a selected chelator solution, under passive ultrasonic irrigation (PUI) for 60 seconds. The manipulated substances used (10mL) were: alcohol 70% (CO), control group; 10% citric acid (AC); EDTA 17% (ED) and chitosan 0.2% (QU). The last three chelating substances were used in the experimental groups.

Then, the canals were cleaned with deionized water (Asfer, São Caetano do Sul, SP, Brazil), and lightly dried with #40 absorbent paper tips (Dentsply Maillefer, Petrópolis, RJ, Brazil), avoiding complete drying. After this, the samples were fixed to a metal surface with sticky wax and positioned to a precision metallographic (IsoMet® 1000 Precision Saw, Buehler, Lake Buff-IL, USA) and with the use of a diamond disk (Extec Corp, São Paulo / SP, Brazil), longitudinal sections were performed in the cervico-apical direction in order to obtain two sections (buccal and lingual).

After this process, the samples were washed in an ultrasonic bath (Ultrasonic Washer Cristófoli, Campo Mourão, Paraná, Brazil) for 5 min, dried in an oven (Olidef, Ribeirão Preto, São Paulo, Brazil), metallized and taken for analysis in an electron microscope (Inspect S50 scanning, FEI, Czech Republic) with a kilovoltage of 20kv to obtain photomicrographs with 4000X magnification and conduct a qualitative evaluation of the surfaces of the tooth middle thirds.

Results

The visual analysis of the middle third images of the control group (CO) (Fig 1) indicated that the dentinal tubules remained obliterated by the smear layer. The tubules were not visible in the images obtained. In addition to this layer, it was possible to visualize a small amount of residues from root canal filling.

In the tooth irrigated with 10% citric acid (AC), a large disobliteration of the dentinal tubules was observed, with complete smear layer removal and just little residue from the root canal filling (Fig 1).

In the images corresponding to the middle third of the tooth irrigated with 17% EDTA (ED), a large disobliteration of the dentinal tubules, which appear open, with complete removal of the smear layer, and no residue from the root canal obturation were observed (Fig 1).

Finally, in the images corresponding to the middle third of the tooth irrigated with 0.2% chitosan (QU), only partial disobliteration of the dentinal tubules was observed, with removal of part of smear layer and remaining of some residues coming from the instrumentation (Fig 1).

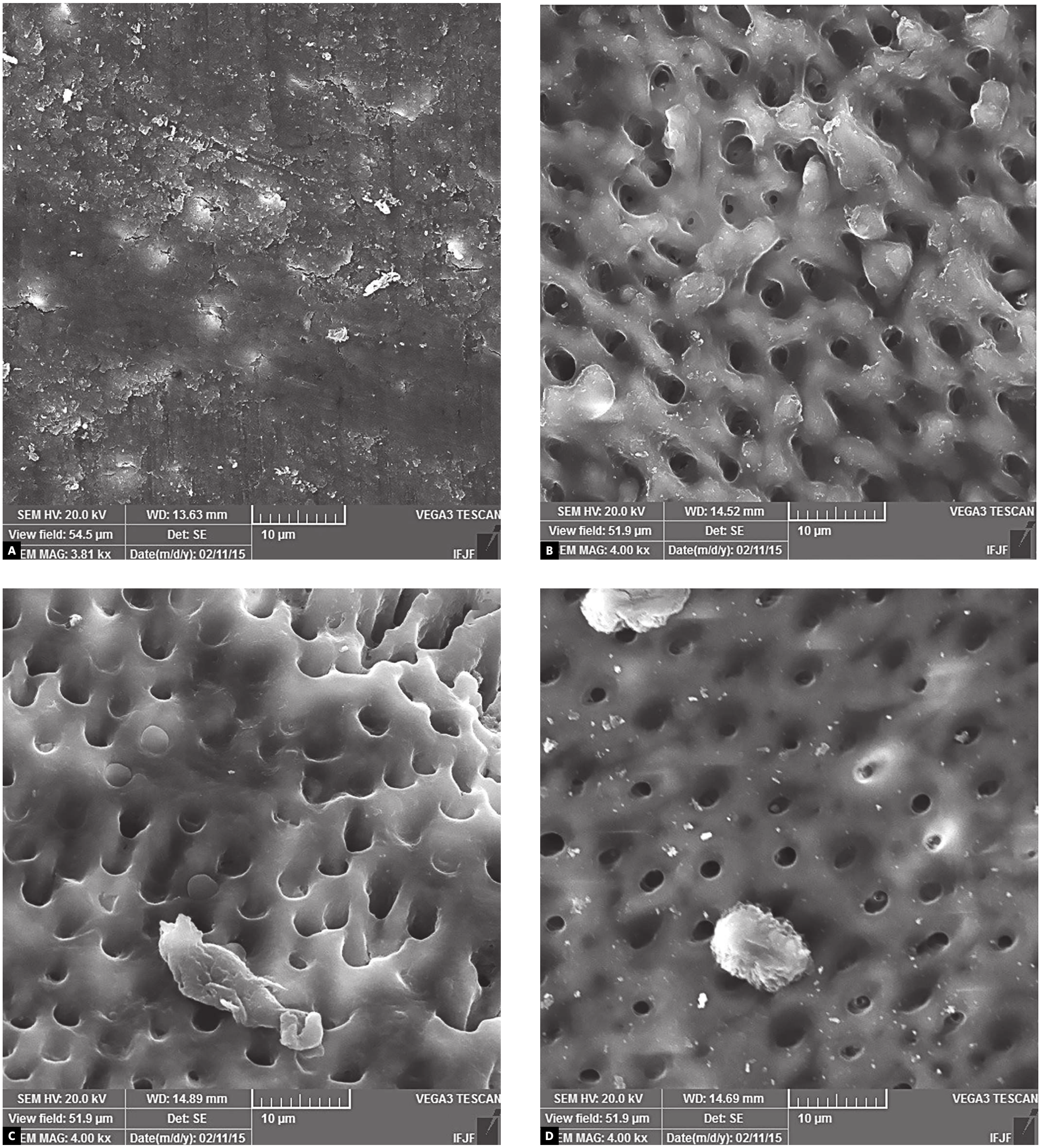

Figure 1. Middle third of the root canals of the CO (**A**), AC (**B**), EG (**C**) and QU (**D**) samples (4000x magnification).

Discussion

This research consisted in a qualitative evaluation of the smear layer removal capacity and consequent dentin tubules disobliteration, using different chelating solutions for the particles dissolution that make up this layer. The control group, which one used alcohol as solution, presented a lower result than all the experimental materials.

In this study, EDTA was effective to remove the smear layer from the uncured dentine surface with wide drills. This efficacy, observed in SEM images, has already been extensively documented in the literature,[4,16,18,19,20,21] being this substance considered the most effective chelating agent with prominent lubricating properties and with wide use in endodontic therapy.[12] This is because EDTA reacts with calcium ions in the dentin and forms soluble calcium chelates.[11] However, this chelator can alter the dentin structural characteristics, resulting in compromised mechanical integrity and increased potential for bacterial adhesion to collagen.[12] Due to these harmful effects on dental tissue, several papers seek alternatives to their use.[22,23,24]

Some studies[23,24] reported that citric acid solutions could be used as an alternative to EDTA and claim to have a similar effect. Citric acid is organic and weak, capable of reacting rapidly with calcium ions, in addition to relatively low cytotoxicity.[4,14] The SEM images analysis obtained in this study also showed a similar pattern of smear layer removal and disobliteration of the dentinal tubules with 10% citric acid and 17% EDTA. However, the use of these substances caused a tubular disobliteration markedly higher than 0.2% chitosan.

Chitosan was used because of its stability, low toxicity and simple preparation. This substance is a natural, biocompatible and biodegradable polysaccharide abundant in nature, which makes its use very interesting.[4,14] Its chelating capacity was demonstrated pioneering in Silva[4] research, acting satisfactorily in cleaning root canal, but, as in this work, still inferior to EDTA and citric acid. However, in another recent study,[7] the same author stated that 15% EDTA, 0.2% chitosan and 10% citric acid had similar patterns of smear layer removal. It is worth mentioning that the 0.2% chitosan solution, even at low concentration, was able to remove the smear layer with results that are statistically similar to those with highest concentrations.[7] If the solutions had a similar chelating effect, therefore, the less concentrated and more abundant solution in nature should be preferred, even due to environmental and economic issues, since the chitin polysaccharide, precursor of chitosan, is the most abundant substance in nature after cellulose, and the cost of production of this substance is considerably low, making its use environmentally attractive.[14]

The chitosan chelating behavior demonstrated in the present study indicated that this solution acted on the inorganic part of the smear layer, favoring its removal. Two models were reported in the literature as possible mechanisms of action. One, known as the "bridge template," it is based on the theory that there are two or more amino groups of a chitosan-binding chain for the same metal ion. The other model supports the theory that only one amino group of the structure is involved in the bond, the metal ion being "anchored" in that amino group.[11,14]

One fact that may justify a lower efficacy of chitin at 0.2% in our study is the use time of the chelating substance. It is known that the efficiency of a chelating agent depends on several factors (application time, pH, concentration and amount of solution). The working time of 60s used here was based on a tendency to try to promote less aggression to the tissues, not causing an excessive erosion of the inter and peritubular dentin[12,14] besides optimizing clinical time. However, in other works, the use of chitosan was effective only after completing 180s of application. Despite the divergences, the fact that the chitosan solution is concentrated at only 0.2% is already impressive for its proven chelating ability.

Conclusion

Within the limitations of this study, based on the methodology used and the images analysis, it can be concluded that the solution of 17% EDTA and 10% citric acid removed the smear layer similarly to each other and higher than 0,2% chitosan.

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