Micro-computed tomographic evaluation of smear layer and accumulated hard tissue debris removal

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Abstract

Background: The isthmus and irregularities present in the root canal system are potential areas for debris accumulation areas and therefore the search for devices and solutions to improve cleaning of these areas is of utmost importance. This study evaluated, by micro–computed tomography, the efficiency of 37% phosphoric acid solution with that of 17% EDTA in the removal of smear layer and accumulated hard tissue debris (AHTD) after canal preparation.

Methods and Materials: Twenty-six mesial roots of mandibular molars were subjected to microtomographic scanning using the SkyScan 1173 X-ray microtomograph at a resolution of 14.97 μm. The canals were prepared using Reciproc R40 instruments, 6% NaOCl and EndoVac System to irrigation/aspiration. After instrumentation, the specimens were divided into two groups: 37% phosphoric acid solution or 17% EDTA.

Results: The results demonstrated that 37% phosphoric acid was significantly more effective in removing smear layer and AHTD than 17% EDTA (Mann-Whitney, p <0.05). It was shown that both 37% phosphoric acid and 17% EDTA are effective agents in the removal of smear layer and debris, but better results were obtained with the use of 37% phosphoric acid.

Conclusion: The results suggest that this solution can be a potential alternative to remove smear layer after root canal preparation.

Keywords: Mandibular molars; micro-computed tomography; phosphoric acid; root canal irrigants; smear layer

INTRODUCTION

The isthmus, anatomical structure with significant prevalence in the mesial root of mandibular molars, can be defined as one or more narrow ribbon-shaped structures, which interconnect two root canals and contain the pulp tissue.[1,2] When these structures do not receive the proper chemical-mechanical treatment, during endodontic therapy, they can either serve as a source of persistent bacteria and infection,[3] or serve as areas for accumulation of smear layer and debris, which may interfere with the penetration of filling materials inside these spaces.[3-5]

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these structures throughout the root canal system (RCS), enabling new techniques to be tested and evaluated for their effectiveness in preventing not only the formation but also the removal of such debris.\(^8\)

The most commonly used substances for smear layer removal are chelator solutions such as ethylenediaminetetraacetic acid (EDTA), especially at a concentration of 17% and with time application ranging from 1 to 5 min.\(^{12,13}\) Studies have indicated a significant number of different chemical agents to remove the smear layer in order to achieve improvements in the physicochemical and biological properties when compared to EDTA.\(^{12,13}\) Phosphoric acid, a chemical agent widely used in the dental practice of conservative dentistry, is a strong acid capable of removing the smear layer from coronal dentin.\(^{12}\) However, only few studies have examined its performance in the root dentin.\(^{13}\)

Therefore, this study aimed to evaluate, by µCT, the efficiency of 37% phosphoric acid solution and 17% EDTA in removing the smear layer and AHTD produced after chemical-mechanical preparation of mesial roots of mandibular molars.

**MATERIALS AND METHODS**

**Specimen selection and initial preparation**

This study was approved by the University Hospital Clementino Fraga Filho (HUCFF/UFRJ) Ethics and Research Committee under the protocol 36865014.0.0000.5257.

Twenty-six human mandibular molars with complete root formation extracted for therapeutic reasons were used. The teeth were stored in 0.1% thymol solution at 5°C until used. Access cavities were performed with round diamond and Endo-Z burs (Maillefer Dentisply, Ballaignes, Switzerland) in a water-cooled, high-speed handpiece. To confirm the existence of two separate and patent root canals in the mesial root, a size 08 K-file (Maillefer Dentisply, Ballaignes, Switzerland) was used passively through the length of the root canal.

**Micro-computed tomography scanning**

A custom-made mold of self-polymerizing resin was created for each tooth to standardize the mounting of the specimen. The teeth were placed in a µCT scanner (SkyScan 1173; Bruker, Kontich, Belgium) on a custom aluminum attachment. This base ensured the standardization of the images obtained initially, after root canal instrumentation and final irrigation, as it allowed precise re-placement of the sample inside the scanner. Scanning was performed through 360° rotation with a rotation step of 0.30 using a 1.0-mm thick aluminum filter, 70-kV energy, 114-mA current, 14.8-mm pixel size, and 21.39-mm resolution.

**Root canal preparation and final irrigation**

The apex of all teeth was sealed with utility wax (Technew, Rio de Janeiro, Brazil). The working length was established 1 mm short of the radiographic apex using a size 08 K-file (Maillefer Dentisply, Ballaignes, Switzerland).

All root canals were instrumented using Reciproc R40 instruments (VDW, Munich, Germany) in reciprocating movement, powered by an electric motor (VDW, Munich, Germany). During the instrumentation, the file moved in the apical direction using an in-and-out pecking motion of about 3 mm amplitude with a slight apical pressure. After three pecking motions, the instrument was removed from the canal and cleaned in a gauze. Next, 1 mL of 6% sodium hypochlorite (NaOCl) (Fórmula e Ação, São Paulo, Brazil) was held within the RCS.

The endodontic irrigation was performed using 6% NaOCl as an irrigant and Endovac System (SybronEndo, Orange, USA) as an irrigation/aspiration system. A modified protocol irrigation described by Nielsen and Craig Baumgartner\(^{14}\) was used in this study.

At the end, the root canals were washed with 1 mL of distilled water using the master delivery tip from EndoVac, and the microcannula was used at working length for aspiration of the agent within the root canal and to remove the excess of distilled water. Finally, the canals were dried with absorbent paper points (Dentisply Maillefer, Ballaignes, Switzerland). After this, the dental elements underwent µCT image acquisition again, as described earlier.

For final irrigation, the samples were allocated into two groups as follows:

- **Group 1** (\(n = 13\)): 3 mL 6% NaOCl + 1 mL distilled water + 3 mL 37% phosphoric acid (1 mL/min) + 1 mL distilled water + 3 mL 6% NaOCl + 1 mL distilled water
- **Group 2** (\(n = 13\)): 3 mL 6% NaOCl + 1 mL distilled water + 3 mL 17% EDTA (1 mL/min) + 1 mL distilled water + 3 mL 6% NaOCl + 1 mL distilled water.

At the end, the canals were dried with absorbent paper points (Dentisply Maillefer, Ballaignes, Switzerland) and the specimens were submitted to a new µCT scan.

**Micro-computed tomographic scanning measurements**

Only the images of the mesial roots of the mandibular molars were reconstructed by NRecon software (Bruker) and analyzed using CTAn software (Bruker, Bruker microCT,
Kontich, Belgium) to provide cross-sections of the internal structure of samples.

DataViewer v. 1.4.4 software (Bruker µCT, Kontich, Belgium) and Seg3D v. 2.1.4 software (National Institutes of Health Center for Integrative Biomedical Computing, University of Utah Scientific Computing and Imaging Institute, Salt Lake City, USA) were used to visualize the presence of the isthmuses. Seg3D v. 2.1.4 software was also used to build 3D models.

The cross-sectional images were segmented, visualized, and quantified to evaluate the changes in root canal volume using CTAn v. 1.14.4 (Bruker µCT, Kontich, Belgium).

**Data analysis**

The cross-sectional images were analyzed by DataViewer v. 1.4.4 software to verify the presence of isthmus in the mesial root.

To analyze the efficiency of the smear layer and AHTD removal, cross-sectional images were evaluated using CTAn software and the following values were obtained: (1) initial volume of the RCS, (2) difference between the volumes after chemical-mechanical preparation and initial volume (Δ1), and (3) difference between the volumes after smear layer removal and after chemical-mechanical preparation (Δ2).

The values for the initial volume of RCS were subjected to normal analysis by the Shapiro-Wilk test, showing a non-normal distribution. Thus, comparisons between groups were evaluated using the Mann-Whitney test. The Chi-square test was used to compare the relative frequencies of isthmuses between the groups. The level of statistical significance was 5% (SPSS v20.0, Chicago, IL, USA).

**RESULTS**

The distribution of the teeth with isthmus within group 1 (37% phosphoric acid) and group 2 (17% EDTA) is shown in Table 1. There was no statistical difference between the groups in the total number of isthmuses (P > 0.05). Isthmus were present in 15 of the 26 teeth (57.7%) analyzed, 7 (53.9%) in group 1, and 8 (61%) in group 2.

Table 1: Number and relative frequency distribution of isthmuses in mesial roots of mandibular molars according to the analyzed group

| Isthmus location (root third) | 37% phosphoric acid (n=13) | 17% EDTA (n=13) |
|-------------------------------|---------------------------|-----------------|
| Cervical, middle, and apical    | 3 (23.1)\(^a\)           | 2 (15.4)\(^a\)  |
| Cervical and middle            | 0 (0)\(^a\)              | 1 (7.7)\(^a\)   |
| Middle                        | 1 (7.7)\(^a\)           | 0 (0)\(^a\)     |
| Middle and apical             | 2 (15.4)\(^a\)           | 4 (30.8)\(^a\)  |
| Apical                        | 1 (7.7)\(^a\)           | 1 (7.7)\(^a\)   |
| Total                         | 7 (53.9)\(^a\)          | 8 (61.6)\(^a\)  |

Different subscript letters in the same line indicate statistical difference (Chi-square test, P<0.05). EDTA: Ethylenediaminetetraacetic acid

There was no statistically significant difference (P = 0.130) between the groups in the initial root canal volume and Δ1 (P = 0.489). The results of Δ2 showed statistically significant differences between the two groups (P = 0.000015), indicating that 37% phosphoric acid was more effective than 17% EDTA in removing smear layer and debris [Table 2].

Representative reconstructed images for the groups are shown in Figures 1 and 2. The images confirm the Δ2 value that matches the amount of debris removed by the demineralization agents.

**DISCUSSION**

This study aimed at evaluating the efficiency of 37% phosphoric acid solution and 17% EDTA in removing the smear layer and AHTD produced by chemical-mechanical preparation. There was no difference in the initial volume of the RCS and the total number of isthmuses present between the groups, which verifies the equivalence of the groups’ anatomy and also that the distribution of teeth with/without isthmuses occurred similarly between the two groups. Moreover, the difference between the RCS volume after chemical-mechanical preparation and initial volume (Δ1) showed no difference between groups. This analysis was performed to confirm that the instrumentation process did not influence the amount of smear layer and AHTD formed; since both groups received exactly the same instrumentation protocol. Finally, the difference between RCS volume after removal of smear layer and volume after chemical-mechanical preparation (Δ2) was calculated to examine the smear layer and AHTD removal during the final irrigation. It was found that the 37% phosphoric acid solution was more effective in removing the smear layer and AHTD than 17% EDTA.

An isthmus prevalence of 57.7% was found, of which 23.1% (6 teeth) were located in the middle and apical thirds and 7.7% (2 teeth) in the apical third only, regions most critical to the access of instruments, irrigation, and
intracanal dressing. These results are partly according to those described by other studies, which also found a high prevalence of isthmus in the root canal.

In the three-dimensional images, it was possible to visualize the presence of debris accumulated [Figures 1 and 2]. However, the debris formed inside the main canal only were visualized after its removal process by chemical agents because the analysis program does not differentiate between dentin present in the initial image and the debris and smear layer impacted after instrumentation. The accumulation of debris in those areas, although not quantified in this study, was visualized in the images and is displayed according to other studies.

The isthmus and irregularities present inside the root canal are potential areas of debris accumulation and therefore some authors suggest the use of continuous rotation instead of reciprocating systems because they tend to accumulate more debris in these regions. However, De-Deus, et al. using reciprocating systems and a continuous rotation system observed that the type of movement does not influence the quantity of accumulated debris. In the present study, Reciproc R40 was used in order to reach a final apical diameter compatible with the use of the EndoVac microcannula.

The irrigation system EndoVac was selected herein due to its excellent performance in terms of cleaning. Versiani, et al. (2016) assessed by µCT, AHTD removal from mesial roots using either a 30G Navitip needle or the EndoVac system. The results showed that the irrigation with EndoVac was more efficient in removing AHTD than that of 30G Navitip needle.

Phosphoric acid is a chemical widely used in the dental practice of conservative dentistry, and few studies using this substance as a root dentin smear layer removal agent have shown promising results. It shows cytotoxic effects comparable to those observed by 5.25% NaOCl and 2% chlorhexidine and although it is capable of causing dentinal erosion, this effect can also be observed with EDTA.

In the present study, 37% phosphoric acid solution was more effective in removing the smear layer and AHTD than 17% EDTA. This result is in agreement with the study by Prado et al. who compared, by SEM, the effectiveness of 17% EDTA, 37% phosphoric acid gel, 37% phosphoric acid solution, and 10% citric acid in removing smear layer. The authors observed that in 3 min, same time employed in this

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**Figure 1:** Representative cross-sectional micro-computed tomography images of 37% phosphoric acid group (a-c) and 17% ethylenediaminetetraacetic acid group (d-f). Initial images prior to instrumentation (a and d); images after instrumentation (b and e) and after final irrigation (c and f). Root canal preparation (red), the accumulated debris (blue) and the removed debris (brown).

**Figure 2:** Representative three-dimensional reconstruction images of 37% phosphoric acid group (a-c) and 17% ethylenediaminetetraacetic acid group (d-f). Initial images prior to instrumentation (a and d); images after instrumentation (b and e) and after final irrigation (c and f). Root canal anatomy prior to instrumentation (green), root canal preparation (red), the accumulated debris (blue) and the removed debris (brown).
study, 37% phosphoric acid solution was the most effective chemical agent. However, the results found here are discrepant to those observed in the study of Garberoglio and Becce, where 3% EDTA, 17% EDTA and a mixture formed by 24% phosphoric acid and 10% citric acid were able to effectively remove the smear layer of the RCS, with no significant difference between the agents. Moreover, Takeda et al. found that irrigation with 17% EDTA, 6% phosphoric acid, or 6% citric acid were not able to remove any smear layer of the RCS.

Recent studies by micro-CT have shown that instruments, such as XP-Endo® finisher, Self-adjusting file, EasyClean, and ultrasonic irrigation when used with different irrigants as final irrigation protocols, are effective in the removal of AHTD. However, none of these techniques, agents, or instruments used were able to complete remove AHTD in areas of anatomic complexity, such as the isthmus, present in the teeth used in our study.

CONCLUSION

The continuous search for devices and solutions to improve the cleaning of RCS, especially teeth with complex anatomy, is necessary. Our study has shown that both 37% phosphoric acid and 17% EDTA are effective agents in the removal of smear layer and debris, but better results were obtained with the use of 37% phosphoric acid solution, which points out to a potential alternative chelate agent to be used after root canal preparation.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Weller RN, Niemczyk SP, Kim S. Incidence and position of the canal isthmus. Part 1. Mesiodibuccal root of the maxillary first molar. J Endod 1995;21:380-3.
2. Mannocci F, Peru M, Sherriff M, Cook R, Pitt Ford TR. The isthmuses of the mesial root of mandibular molars: A micro-computed tomographic study. Int Endod J 2005;38:558-63.
3. Teixeira FB, Sano CL, Gomes BR, Zaia AA, Ferraz CC, Souza-Filho FJ. A preliminary in vitro study of the incidence and position of the root canal isthmus in maxillary and mandibular first molars. Int Endod J 2003;36:276-80.
4. Endal U, Shen Y, Knut A, Gao Y, Haapasalo M. A high-resolution computed tomographic study of changes in root canal isthmus area by instrumentation and root filling. J Endod 2011;37:229-7.
5. Freire LG, Iglescas EF, Cunha RS, Dos Santos M, Gavini G. Micro-computed tomographic evaluation of hard tissue debris removal after different irrigation methods and its influence on the filling of curved canals. J Endod 2015;41:1660-6.
6. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: Mode of action and indications for their use. Int Endod J 2003;36:810-30.
7. Violoich DR, Chandler NP. The smear layer in endodontics-A review. Int Endod J 2010;43:2-15.
8. Paque F, Boessler C, Zehnder M. Accumulated hard tissue debris levels in mesial roots of mandibular molars after sequential irrigation steps. Int Endod J 2011;44:148-53.
9. Versiani MA, Pécora JD, Sousa-Neto MD. The anatomy of two-rooted mandibular canines determined using micro-computed tomography. Int Endod J 2011;44:682-7.
10. Marceliano-Alves MF, de Lima CO, Augusto CM, Almeida Barbosa AF, Vieira Bruno AM, Rosa AM, et al. The internal root canal morphology of single-rooted mandibular canines revealed by micro-computed tomography. J Conserv Dent 2018;21:589-91.
11. Paque F, Laib A, Gautsch H, Zehnder M. Hard-tissue debris accumulation analysis by high-resolution computed tomography scans. J Endod 2009;35:1044-7.
12. Pérez-Heredia M, Ferrer-Luque CM, González-Rodríguez MP, Martín-Peinado FJ, González-López S. Decalciying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite on root canal dentine. J Endod 2008;41:418-23.
13. Prado M, Gusman H, Gomes BP, Simão RA. Scanning electron microscopic investigation of the effectiveness of phosphoric acid in smear layer removal when compared with EDTA and citric acid. J Endod 2011;37:255-8.
14. Nielsen BA, Craig Baumgartner J. Comparison of the EndoVac system to needle irrigation of root canals. J Endod 2007;33:611-5.
15. Usman N, Baumgartner JC, Marshall JG. Influence of instrument size on root canal debridement. J Endod 2004;30:110-2.
16. Srivastava S, Airogaibah NA, Aljartbou G. Cone-beam computed tomographic analysis of middle mesial canals and isthms in mesial roots of mandibular first molars-prevalence and related factors. J Conserv Dent 2018;21:526-30.
17. Paquè F, Al-Jadaa A, Kfir A. Hard-tissue debris accumulation created by conventional rotary versus self-adjusting file instrumentation in mesial root canal systems of mandibular molars. Int Endod J 2012;45:413-8.
18. Robinson JP, Lumley PJ, Cooper PR, Grover LM, Walsmey AD. Reciprocating root canal technique induces greater debris accumulation than a continuous rotary technique as assessed by 3-dimensional micro-computed tomography. J Endod 2013;39:1067-70.
19. De-Deus G, Marins J, Silva EJ, Souza E, Belladonna FG, Reis C, et al. Accumulated hard tissue debris produced during reciprocating and rotary nickel-titanium canal preparation. J Endod 2015;41:676-81.
20. Versiani MA, Alves FR, Andrade-Junior CV, Marceliano-Alves MF, Provenzano JC, Rôças IN, et al. Micro-CT evaluation of the efficacy of hard-tissue removal from the root canal and isthmus area by positive and negative pressure irrigation systems. Int Endod J 2016;49:1079-87.
21. Prado M, Silva EJ, Duque TM, Zaia AA, Ferraz CC, Almeida JF, et al. Antimicrobial and cytotoxic effects of phosphoric acid solution compared to other root canal irrigants. J Appl Oral Sci 2015;23:158-63.
22. Garberoglio R, Becce C. Smear layer removal by root canal irrigants. A comparative scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1994;78:359-67.
23. Takeda FH, Harashima T, Kimura Y, Matsumoto K. A comparative study of XP-endo finisher and passive ultrasonic irrigation as final irrigation protocols on the removal of accumulated hard-tissue debris from oval shaped canals. Clin Oral Investig 2019;23:3087-9.
24. Silva EJ, Carvalho CR, Belladonna FG, Prado MC, Lopes RT, De-Deus G, et al. Micro-CT evaluation of different final irrigation protocols on the removal of hard-tissue debris from isthmus-containing mesial root of mandibular molars. Clin Oral Investig 2019;23:681-7.