Ex-vivo evaluation of four irrigation protocols for the removal of hard-tissue debris from flattened root canals and isthmus in mandibular incisors

Avaliação ex-vivo de quatro protocolos de irrigação para a remoção de resíduos de tecido duro de canais radiculares achatados e istmo em incisivos inferiores

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ABSTRACT
Introduction This study aimed to compare the effect of ultrasonic activation in comparison to conventional irrigation in the removal of dentin debris from the root canals walls. Methods Sixty single-rooted mandibular incisors with one only straight flattened root canal were selected and allocated across groups (n=15), according to the irrigation protocol: G1: conventional irrigation with 2.5% sodium hypochlorite; G2: conventional irrigation with 2% chlorhexidine gel and saline solution; G3: G1 + ultrasonic activation; G4: G2 + ultrasonic activation. Cross-sectional images were obtained and evaluated before and after root canal cleaning and shaping. Two-way (ANOVA) and t-test ($P < 0.05$) statistical tests were performed. Results G3 irrigation protocol removed significantly more dentin debris (p=0.0065), followed by the G1 protocol (p=0.0165) and by the G2 and G4 techniques, which were comparable (p=0.763). Conclusions Irrigation with sodium hypochlorite followed by ultrasonic activation promoted a significant reduction in dentin debris within the root canal system.

Keywords: irrigation, debris, ultrasonic activation

RESUMO
Introdução Este estudo teve como objetivo comparar o efeito da ativação ultrassônica em comparação à irrigação convencional na remoção de restos de dentina das paredes dos canais radiculares. Métodos Sessenta incisivos inferiores unirradiculares com um único canal radicular reto achatado foram selecionados e alocados nos grupos (n = 15), de acordo com o protocolo de irrigação: G1: irrigação convencional com hipoclorito de sódio 2,5%; G2: irrigação convencional com clorexidina gel 2% e solução salina; G3: G1 + ativação ultrassônica; G4: G2 + ativação ultrassônica. Imagens transversais foram obtidas e avaliadas antes e após a limpeza e modelagem do canal radicular. Foram realizados testes estatísticos two-way (ANOVA) e t-test ($P <0,05$). Resultados O protocolo de irrigação G3 removeu significativamente mais resíduos dentinários (p = 0,0065), seguido pelo protocolo G1 (p = 0,0165) e pelas técnicas G2 e G4, que foram comparáveis (p = 0,763). Conclusões A irrigação com hipoclorito de sódio seguida de ativação ultrassônica promoveu uma redução significativa dos resíduos de dentina no sistema de canais radiculares.

Palavras-chave: irrigação, detritos, ativação ultrassônica

1 INTRODUCTION

The achievement of complete debridement of the root canal system is one of the greatest challenges in endodontics, due to variations in anatomical complexity, containing accessory canals, apical branching, and isthmus (ZHENDER, 2006; VAN DER SLUIS, 2007). It is widely known that great area of the root canal system remains untouched after instrumentation procedures (PETERS, 2001; PETERS, 2004), containing pulp and dentin remnants, biofilms, and byproducts of bacterial metabolism (PETERS, 2004; RICUCCI, 2010). Thus, irrigation is an essential part of endodontic treatment, since it permits cleaning of untouched portions of the root canal (LEONI, 2017).

Several irrigation protocols for root canal cleaning and shaping that enhance the properties of irrigant solutions have been proposed and incorporated into clinical practice (LEONI, 2017; NEELAKANAN, 2016; MEDONÇA, 2015; MARTINS, 2014). Nevertheless, the conventional syringe-and-needle technique remains widely used (DUTNER, 2012). Although the conventional technique provides adequate control of needle penetration (PEREZ, 2016) and volume of irrigant dispensed (BOUTSIOUKIS, 2010), it is not efficient in removing dentin debris and soft-tissue remnants (ADCOK, 2011).

The correlation between endodontic treatment failure and the persistence of endodontic infection (SIQUEIRA, 2001; ARNOLD, 2013) is directly associated with irrigant solution inability in penetrating the entire root canal system and in reaching the anatomic complexities (SARNO, 2012). Endal et al. (ENDAL, 2011), using micro-CT images, detected large amounts of dentin debris in the isthmus regions of mesial roots of mandibular molars after rotary instrumentation and copious conventional irrigation.

The base of endodontic irrigation is to remove tissue remnants as well as free-floating infectious agents, to disrupt and to eradicate biofilm within the root canal system (VAN DER SLUIS, 2007). Additional objectives include removal of smear layer and dentin debris generated during root canal shaping (NUSSTEIN, 2015). The efficacy of irrigation depends on the mechanisms of action of the irrigant and on the technique employed to deliver it to the elements that need to be cleared from the pulp cavity (MEDONÇA, 2015).

The most widely used endodontic irrigant is sodium hypochlorite (NaOCl), due to its potent antimicrobial action and ability to dissolve organic matter (BASRANI, 2012). However, chlorine, which accounts for the tissue-dissolving and antimicrobial activity of this substance, is unstable and rapidly consumed during the first stage of tissue lysis, which creates the need for continuous renewal (MOORER, 1982).

Chlorhexidine has been widely used in endodontics as an intracanal medicament and as an irrigant (BASRANI, 2012), with excellent outcomes in management of endodontic infection (ZANDI, 2016). Use of chlorhexidine gel as an irrigant was first proposed in 2001 (FERRAZ, 2001), in a study that reported satisfactory results in terms of pulp cavity cleaning and disinfection.

Ultrasound was introduced to endodontic practice in 1957 by Richman (VAN DER SLUIS, 2007), who first proposed its use for endodontic instrumentation with files attached to special adapters or inserts. However, poor control of the ultrasound tip and its displacement while cutting encouraged researches to achieve a different modality whereby the tip would not touch the root canal walls.

Within this context, Weller et al. (WELLER, 1980) introduced the term "passive ultrasonic irrigation" (PUI) to refer to a technique in which the ultrasound tip has no cutting action, instead serving only to vibrate and activate the irrigant solution within the root canals. This promotes cavitation, which increases the penetration capacity of sodium hypochlorite and, by agitating the solution and increasing its temperature, helps maximize its efficacy (HAAPASALO, 2014; CONDE, 2016).

Thus, and given the clinical importance of this topic, this *ex vivo* study was designed to test the null hypothesis that conventional syringe-and-needle irrigation would be as efficient as ultrasonic activation of irrigants in removing dentin debris from flattened root canals and isthmus in mandibular incisors.

2 MATERIAL AND METHODS

2.1 SPECIMEN SELECTION AND PREPARATION

The present study was approved by the Institutional Ethical Review Board (Protocol #1.482.262). The sample comprised 60 extracted mandibular incisors with one only straight flattened root canal. Teeth were screened for eligibility by analysis of buccolingual and mesiodistal radiographs.

The selected teeth were randomly allocated across four groups (n=15) and embedded in acrylic resin (BRAMANTE, 1987; CARVALHO, 2011), as shown in Figure 1 (A and B). Cross-sections were obtained using an IsoMet Low Speed Saw (IsoMet, Buehler, Lake Bluff, IL, USA), with a diamond disc, under constant cooling. Sectioning was performed 3 mm and 6 mm from the root apex.

After sectioning, the samples were immersed in ultrasonic bath with 17% EDTA for 5 minutes, in order to remove debris that might have been deposited in the isthmus or in the root canal. Pre- and post-instrumentation images of each cross-section were obtained with a Sony CyberShot 10.5-megapixel digital camera (Sony, Tokyo, Japan) attached to a microscope (Leica S8AP0, Leica Microsystems GmbH, Germany), at 80X magnification. This was allowed by the flasking procedure, which reunited the tooth sections in alignment, allowing instrumentation and irrigation.

The total root canal area (mm²) and the percentage of dentin debris were calculated using ImageJ software. All analyses were performed in triplicate (before and after instrumentation), by three standardized examiners, and the mean recorded as the final result.

Instrumentation procedures were standardized across all groups. A glide path was created, and apical patency established by scouting a stainless steel #15 K-file (Dentsply-Maillefer) up to the working length, which was established by deducting 1 mm from the root canal length.

In all the experimental groups, the root canals were instrumented with Reciproc® R25 (25/0.08 (VDW GmbH, Munich, Germany) and Mtwo files (30/0.05, 35/0.06, 40/0.06) (MTW, VDW GmbH, Munich, Germany), in a Silver Reciproc motor/Sirona Endo 6:1 contra-angle handpiece (VDW GmbH, Munich, Germany). Instruments were driven according to the preset programs.

Study groups

The samples were distributed according to the irrigation protocol (Table 1).

In all groups, final irrigation was performed for 4 minutes with 3 mL of 17% EDTA, followed by irrigation with NaOCl (G1 and G3) or saline solution (G2 and G4).

In groups where ultrasonic activation was used, irrigants were injected into the root canals and the ultrasound tip (#E1, Irrisonic, Helse Dental Technology, Santa Rosa do Viterbo, Brazil) was passively inserted, 2 mm short of the working length. The activation was performed for 20 seconds, using a Gnatus ultrasound system, on power setting 1 (minimum power) without irrigation, as recommended by the manufacturer.

At the end of instrumentation and irrigation procedures, the specimens were taken apart from the flask system and new cross-sectional images were obtained.

Table 1 Study groups and irrigation protocols

GROUP	IRRIGATION PROTOCOL
G1	The root canals were irrigated with 2.5% NaOCl using 5mL syringe and 19-gauge needle
G2	The root canal was filled with 2% chlorhexidine gel using a 3 mL syringe and a 19G needle before insertion of the instrument into the root canal. After the use of each instrument, 5 mL of distilled water was used as an irrigating solution with a 5-mL syringe and a 30G needle placed 3 mm short of the working length. A chemical auxiliary agent (i.e., CHX) was used with the endodontic instrument to prepare the root canal. Distilled water was used as an irrigating solution to liberate CHX and instrumentation-related debris from the root canal.
G3	As in G1, the root canals were irrigated with 2.5% NaOCl using 5mL syringe and 19-gauge needle, followed by three activations of a passive ultrasound tip (E1, Irrisonic) while canal system was flooded with 2.5% NaOCl after instrumentation.
G4	As in G2, 2% chlorhexidine gel was used as a chemical auxiliary agent during root canal instrumentation, and 5 mL of distilled water was used between instruments as an irrigation

solution. Passive ultrasonic activation was performed in 3 cycles of 20 seconds, using E1 Irrisonic tip. after instrumentation.

2.2 STATISTICAL ANALYSIS

Data regarding the root canal area and the debris-filled area score were tested for normality and compared across groups by two-way analysis of variance (ANOVA) (BioEstat 5.0 software environment (Instituto Marimauá, Manaus, AM, Brazil). Between-group analyses were performed by comparing the middle and apical thirds of each specimen, before and after instrumentation. A paired *t*-test was used for within-group comparisons. The significance level was set at 5%.

3 RESULTS

Result show that, regardless of the irrigation protocol, the middle third was easier to clean ($p<0.05$).

Table 2 –Mean (%) of the root canal area free of debris in the middle (6mm) and apical (3mm) thirds across different study groups.

GROUPS	Middle - 6mm	Apical - 3mm
G1	81.1 ± 22.0^{b}	47.7 ± 20.0^{B}
G2	62.6 ± 21.3^{a}	30.6 ± 21.0^{A}
G3	96.4 ± 22.3^{c}	57.7 ± 21.3^{C}
G4	70.8 ± 18.0^{a}	35.7 ± 15.3^{A}

***Different letters denote significantly different results.**

Comparative analysis by thirds showed that the G3 irrigation protocol removed more dentin debris ($p=0.0065$), followed by G1 ($p=0.0165$) and by G2 and G4 groups, which were similar ($p=0.7630$ (Table 2); This behavior was consistent in both analyzed cross-sections.

It should be mentioned that the results obtained in G1 (conventional irrigation with sodium hypochlorite) were superior to those obtained in G4 (2% chlorhexidine gel and saline solution followed by ultrasonic activation).

Figure 2 shows images obtained from specimens after delimitation of the total area of the root canal system and of the area occupied by dentin debris, demonstrating the method used to define these areas. Figure 3 shows representative images of root canals in different groups.

4 DISCUSSION

Flattened root canals are common in mandibular incisors (ESTRELA, 2015) and have been reported as a possible cause of endodontic treatment failure (LIU, 2010) due to the difficulty in removing organic matter and achieving total disinfection (SIQUEIRA, 2014). The present study was conducted on mandibular incisors specifically for this reason (LIU, 2010). The selected teeth were randomly allocated across groups. The inclusion and exclusion criteria, as well as *a priori* definition of the optimal thicknesses for specimen sectioning, helped minimize the risk of anatomy-related error and bias and prevent significant interference with results.

The search for irrigation protocols capable of removing as much dentin debris as possible is based on the principle that these remnants can have a significant negative impact on irrigant flow, thus compromising the neutralizing effect of the irrigating solution on the septic/toxic content of the root canals (PAQUÉ, 2012), and can constitute a physical barrier to proper obturation (FREIRE, 2015).

Several methods have been used to quantify dentin debris within root canal system, including histologic processing, scanning electron microscopy, and root sections (HAAPASALO 2014; WALTERS, 2002). Some recent studies used micro-CT technology for quantification of dentin debris generated during root canal instrumentation (PAQUÉ, 2009; PAQUÉ, 2012; ROBINSON, 2013; DE-DEUS, 2015). Due to its nondestructive nature, this method allows specimens to be reused in further studies (PAQUÉ, 2012; FREIRE, 2015). However, this new technology clearly shows that other methods can also identify the presence of dentin debris effectively, despite their weakness in quantifying debris throughout the root canal system. Although the present study used destructive testing (specimen sectioning), this method is firmly established in the literature (KURUVILLA, 2015) and provides an effective approach. We were able to achieve real-world results that can be used to guide selection of endodontic irrigation protocols. The flask technique (BRAMANTE, 1987), whereby teeth are sectioned after embedding in resin, is also well-established and allows reuse of specimens in further research (PAQUÉ, 2005). The thicknesses chosen for sectioning were based in previous work by Liu et al. (LIU, 2010), who detected root flattening and isthmuses in the apical 6 mm of the examined teeth.

This study detected significant differences in the amount of dentin debris removed from flat root canals and isthmuses in mandibular incisors when passive ultrasonic irrigation was combined with different irrigation protocols (sodium hypochlorite vs. 2%

chlorhexidine gel with saline solution). Therefore, the null hypothesis was rejected. The greater debris buildup observed in isthmus regions was indeed a consequence of the limited cleansing ability of irrigation protocols not potentiated by ultrasound. Application of chlorhexidine gel was associated with a significantly greater buildup of dentin debris, which disagrees from the findings of Ferraz et al. (FERRAZ, 2001).

An important issue is the challenging nature of instrumentation in flat canals and cleaning of isthmuses. In all groups, cross-sections revealed areas of the root canal system that were probably not touched by the instruments, as the canal contour remained unchanged from baseline. This occurred despite attempts to ensure contact of the instruments with all dentin walls. In these cases, canal cleaning was achieved exclusively by irrigation, as demonstrated in several previous studies (ADCOCK, 2011, ROBINSON, 2013). Such failures or limitations in root canal preparation often go unnoticed, as isthmuses and flattened root areas are mostly oriented in buccolingual direction. The fact that endodontic treatment outcomes are assessed on plain radiographs prevents visualization of these areas, because their locations overlap with the radiopaque silhouette of the obturating material.

During root canal irrigation procedures, we attempted to advance the irrigation needle as close as possible to the working length. According to Perez et al. (PEREZ, 2016), placing the needle 1 mm short of the working length provides a 300% improvement in debris removal as compared to irrigation with the needle 5 mm short of the working length. However, bringing the needle closer to the apex may increase the risk of irrigant extrusion (BOUTSIOUKIS, 2013); when using sodium hypochlorite, this may lead to accidents (ZHU, 2013). According to Versiani et al. (VERSIANI, 2016), the use of a negative-pressure irrigation system (Endovac) prevents such clinical accidents and is associated with significant improvement in debris removal from isthmus areas.

This has important implications for clinical practice: even if the clinician chooses to use sodium hypochlorite as irrigant and activate it with ultrasound, saline solution may be used as an irrigant when the solution should be delivered within the canal for physical clearance of debris.

In the present study, the irrigation volume delivered between instruments was standardized at 5 mL across all groups (NaOCl in G1 and G2; normal saline in G3 and G4), for a total volume of 60 mL per tooth. This eliminated the risk of results being biased by differences in irrigant volume (DE-DEUS, 2015; GREGORIO, 2013). Another relevant factor was the set of inclusion criteria. We only selected teeth with no apical curvature, thus ensuring better flow of irrigant solution throughout the root canal (GREGORIO, 2013).

Significant reductions in dentin debris-filled area were observed in G3 and G4, groups in which the irrigant was activated by ultrasonic activation of irrigants. These findings are consistent with those reported by Leoni et al. (LEONI, 2017). This, in fact, is the most important result of the present study, although we did not identify any combination capable of completely eliminating debris from the root canal system. Similar findings were reported by Neelakantan et al. (NEELAKANTAN, 2016) and De Deus et al. (DE-DEUS, 2015).

In our study, ultrasonic activation of irrigants played an essential role in allowing significant removal of the dentin debris generated during root canal instrumentation. The conventional syringe-and-needle irrigation protocols did not provide adequate cleaning, despite delivery of the irrigant as near as possible to the working length and after complete apical preparation. These results are consistent with those of other recent studies (LEONI, 2017; FREIRE, 2015, WALTERS, 2002).

Ex vivo study designs with strictly controlled experimental conditions and specimen selection and distribution can significantly reduce the possibility of biases and confounders, particularly those related to dental anatomy (VERSIANI, 2013). Thus, the results obtained with such methods are more representative of reality and can be used as a foundation for defining future protocols and designing further studies. In the present investigation, measuring the cross-sectional area of the root canal system and the percentage filled by dentin debris – in triplicate and by three standardized examiners – enhanced the robustness of our findings, minimizing accuracy-related errors and mitigating the limitations of our method (NAZARI, 2009).

This study demonstrates the need for combining efficient irrigation protocols (preferably ultrasound-assisted) with instrumentation procedures, to optimize root canal system cleaning and remove as much dentin debris and organic matter as possible. Further research into irrigation protocols with special emphasis on complex root canal anatomies is certainly warranted, and should provide additional evidence on different solutions, volumes, and delivery methods. Although several methods have been reported in the literature, the general consensus is that ultrasound activation provides significant benefit in terms of enhancing dentin debris clearance (van der LUIS, 2007; LEONI, 2017; NUSSTEIN, 2015; DE-DEUS, 2015; KATO, 2016).

5 CONCLUSIONS

Under the conditions of this study, passive ultrasonic irrigation with sodium hypochlorite was associated with a significant quantitative reduction in dentin debris when compared to conventional irrigation protocols, although we were unable to identify any combination capable of eliminating dentin debris completely. Use of 2% chlorhexidine gel allowed greater impaction of dentin debris.

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The authors deny any conflict of interests.

REFERENCES

1. Zhender M. Root canal irrigants. J Endod. 2006;32:389-98.
2. van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007;40:415-26.
3. Peters OA, Schonenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J. 2001;34:221-30.
4. Peters OA. Current challenges and concepts in the preparation of root canal systems. A review. J Endod. 2004;30:559-606.
5. Ricucci D, Siqueira Jr JF. Biofilms and Apical Periodontitis: Study of Prevalence and Association with Clinical and Histopathologic Findings. J Endod. 2010;36:1277-88.
6. Leoni GB, Versiani MA, Silva-Sousa YT, et al. Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. Int Endod J. 2017;50:398-406.
7. Martins Justo A, Abreu da Rosa R, Santini MF, et al. Effectiveness of final irrigant protocols for debris removal from simulated canal irregularities. J Endod. 2014;40:2009-14.
8. Mendonça DH, Colucci V, Rached-Junior FJ, et al. Effects of various irrigation/aspiration protocols on cleaning of flattened root canals. Braz Oral Res. 2015;29:1-9.
9. Nusstein JM. Sonic and ultrasonic irrigation. In: Bettina B, ed. Endodontic irrigation: chemical disinfection of the root canal system. Switzerland: Springer; 2015:173–98.
10. Neelakantan P, Devaraj S, Jagannathan N. Histologic assessment of debridement of the root canal isthmus of mandibular molars by irrigant activation techniques ex vivo. J Endod. 2016;42:1268-72.
11. Dutner J, Mines P, Anderson A. Irrigation trends among American Association of Endodontists members: a web-based survey. J Endod. 2012;38:37-40.
12. Perez R, Neves AA, Belladonna FG, et al. Impact of the needle insertion depth on the removal of hard-tissue debris. Int Endod J. 2016 Apr 9. doi: 10.1111/iej.12648.
13. Boutsioukis C, Lambrianidis T, Verhaagen B, et al. The effect of needle-insertion depth on the irrigant flow in the root canal: evaluation using an unsteady computational fluid dynamics model. J Endod. 2010;36:1664-8.
14. Adcock JM, Sidow SJ, Looney SW, et al. Histologic evaluation of canal and isthmus debridement efficacies of two different irrigant delivery techniques in a closed system. J Endod. 2011; 37: 544-8.
15. Siqueira Jr JF. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J. 2001;34:1-10.
16. Arnold M, Ricucci D, Siqueira JR JF. Infection in a complex network of apical ramifications as the cause of persistent apical periodontitis: a case report. J Endod. 2013;39:1179-84.
17. Sarno MU, Sidow SJ, Looney SW, et al. Canal and isthmus debridement efficacy of the VPro EndoSafe negative-pressure irrigation technique. J Endod. 2012;38:1631-4.
18. Endal U, Endal U, Shen Y, et al. High-resolution computed tomographic study of changes in root canal isthmus area by instrumentation and root filling. J Endod. 2011;37:223-7.
19. Basrani B Haapasalo M. Update on endodontic irrigating solutions. Endodontic Topics. 2012;27:74-102.

20. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. Int Endod J. 1982;15:187-96.
21. Zandi H, Rodrigues RC, Kristoffersen AK, et al. Antibacterial effectiveness of 2 root canal irrigants in root-filled teeth with infection: a randomized clinical trial. J Endod. 2016;42:1307-13.
22. Ferraz CC, Gomes BP, Zaia AA, et al. In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod. 2001;27:452-5.
23. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod. 1980;6:740-3.
24. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Brit Dent J. 2014;216:299–303.
25. Conde AJ, Estevez R, Loroño G, et al. Effect of sonic and ultrasonic activation on organic tissue dissolution from simulated grooves in root canals using sodium hypochlorite and EDTA. Int Endod J. 2016 Nov 3. doi: 10.1111/iej.12717.
26. Bramante CM, Berbert A, Borges RP. A methodology for evaluation of root canal instrumentation. J Endod. 1987;13:243-245.
27. Carvalho-Sousa B, Costa-Filho JR, Almeida-Gomes F, et al. Evaluation of the dentin remaining after flaring using Gates Glidden drills and Protaper rotary files. RSBO. 2011;8:194-9.
28. Estrela C, Rabelo LE, de Souza JB, et al. Frequency of root canal isthmi in human permanent teeth determined by cone-beam computed tomography. J Endod. 2015;41:1535-9.
29. Liu CX, Li W, Zheng QH, et al. Anatomic study of canal isthmuses of mandibular permanent incisors by micro-CT. Hua Xi Kou Qiang Yi Xue Za Zhi. 2010;28:177-80.
30. Siqueira JF Jr, Rôças IN, Ricucci D, Hulsmann M. Causes and management of post-treatment apical periodontitis. Br Dent J. 2014;216:305–12.
31. Paqué 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.
32. Freire LG, Iglecias EF, Cunha RS, et al. 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.
33. Walters MJ, Baumgartner JC, Marshall JG. Efficacy of irrigation with rotary instrumentation. J Endod. 2002;28:837-9.
34. Robinson JP, Lumley PJ, Cooper PR, et al. 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.
35. De-Deus G, Marins J, Silva EJ, et al. Accumulated hard-tissue debris produced during reciprocating and rotary nickel-titanium canal preparation. J Endod. 2015;41:676–81.
36. Paqué F , Laib A, Gautschi H, Zehnder M. Hard-tissue debris accumulation analysis by high-resolution computed tomography scans. J Endod. 2009;35:1044–7.
37. Kuruvilla A, Jaganath BM, Krishnegowda SC, et al. A comparative evaluation of smear layer removal by using edta, etidronic acid, and maleic acid as root canal irrigants: An in vitro scanning electron microscopic study. J Conserv Dent. 2015;18:247-51.
38. Paqué F, Musch U, Hülsmann M. Comparison of root canal preparation using RaCe and ProTaper rotary Ni-Ti instruments. Int Endod J. 2005;38:8-16.

39. Boutsioukis C, Psimma Z, van der Sluis LW. Factors affecting irrigant extrusion during root canal irrigation: a systematic review. Int Endod J. 2013;46:599-618.
40. Zhu WC, Gyamfi J, Niu LN, et al. Anatomy of sodium hypochlorite accidents involving facial ecchymosis - a review. J Dent. 2013;41:935-48.
41. Versiani MA, Alves FR, Andrade-Junior CV, 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.
42. Gregorio C, Arias A, Navarrete N, et al. Effect of apical size and taper on volume of irrigant delivered at working length with apicalnegative pressure at different root curvatures. J Endod. 2013;39:119-24.
43. Versiani MA, Pecora JD, Sousa-Neto MD. Microcomputed tomography analysis of the root canal morphology of single-rooted mandibular canines. Int Endod J. 2013;46:800-7.
44. Nazari Moghaddam K, Mehran M, Farajian Zadeh H. Root canal cleaning efficacy of rotary and hand files instrumentation in primary molars. Iran Endod J. 2009;4:53-7.
45. Kato AS, Cunha RS, da Silveira Bueno CE, et al. Investigation of the efficacy of passive ultrasonic irrigation versus irrigation with reciprocating activation: an environmental scanning electron microscopic study. J Endod. 2016;42:659-63.

Figure 1: The selected teeth were randomly allocated across four groups and embedded in acrylic resin.

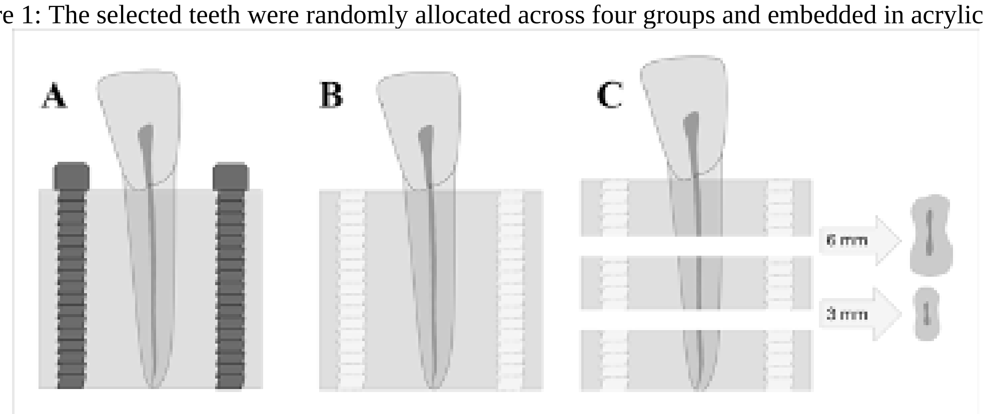

Figure 2: Images obtained from specimens after delimitation of the total area of the root canal system and of the area occupied by dentin debris

Figure 3: Representative images of root canals in different groups.