J Oral Res
research in oral sciences

Article

# Removal of the smear layer by passive and continuous ultrasonic irrigation: a scanning electron microscopy study.

## Eliminación de la capa de barro dentinario mediante irrigación ultrasónica pasiva y continua: un estudio de microscopía electrónica de barrido.

**Ricardo Machado.**[1]
**Alexandre Rother.**[2]
**Daniel Comparin.**[3]
**Ajinkya M. Pawar.**[4]
**Felipe de Souza Matos.**[5]
**Thais Christina Cunha.**[5]
**Luiz Rômulo Alberton.**[6]
**Luiz Renato Paranhos.**[7]

**Affiliations:**
[1]Clinical Practice Limited to Endodontics, Navegantes, Santa Catarina, Brazil.
[2]Clinical Practice Limited to Endodontics, Palhoça, Santa Catarina, Brazil.
[3]Department of Endodontics, Paranaense University, Francisco Beltrão, Paraná, Brazil.
[4]Department of Conservative Dentistry and Endodontics, Nair Hospital Dental College, Mumbai, Maharashtra India.
[5]Postgraduate Program in Dentistry, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.
[6]Department of Animal Science, School of Veterinary Medicine, Paranaense University, Umuarama, Paraná, Brazil.
[7]Department of Preventive and Community Dentistry, School of Dentistry, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

Corresponding author: **Ricardo Machado.** Rua Brasília, n. 300, Apto. 503, Centro, Navegantes, Santa Catarina, Brazil. Phone: (55-47) 98409 1561.
E-mail: **ricardo.machado.endo@gmail.com**

Receipt : 03/30/2021 Revised: 07/29/2021
Acceptance: 12/31/2021

**Cite as:** Machado R, Rother A, Comparin D, Pawar AM, Matos FDS, Cunha TC, Alberton LR & Paranhos LR.
Removal of the smear layer by passive and continuous ultrasonic irrigation: a scanning electron microscopy study.
J Oral Res 2021; 10(6):1-8.
**Doi:10.17126/joralres.2021.070**

**Abstract:** Background: Due to the anatomical complexity of the root canal system, irrigation plays an essential role in endodontics. This *in vitro* study was sought to compare the removal of the smear layer (RSL) promoted by conventional irrigation (CI), passive ultrasonic irrigation (PUI) and continuous ultrasonic irrigation (CUI) with 17% EDTA, by using scanning electron microscopy (SEM). **Material and Methods:** Forty single-rooted human mandibular canines were instrumented and randomly assigned to four groups (n=10), according to the irrigation protocol aiming to the RSL: CG (control group) —conventional irrigation with distilled water; CI— conventional irrigation with 17% EDTA; PUI —passive ultrasonic irrigation with 17% EDTA; CUI— continuous ultrasonic irrigation with 17% EDTA. Hemisections from each sample were obtained, and images of each root canal third (cervical, middle and apical) were captured at 1000 X magnification by SEM. Three previously calibrated and blinded evaluators classified the RSL, according to the criteria proposed by Torabinejad *et al.*: small or no smear layer (all dentinal tubules were clean and open); 2 = moderate smear layer (no smear layer on the surface of root canal, but dentinal tubules contained debris); 3 = dense smear layer (covering practically all dentinal tubules entrances). Statistical analysis was performed by Kruskal-Wallis and Bonferroni tests ($p$<0.05). **Results:** Overall, CUI and cervical thirds showed better RSL rates, compared with the other methods and thirds, respectively ($p$<0.05). More specifically, the cervical thirds showed better results in the CG, CI and PUI groups ($p$<0.05), whereas the cervical and middle thirds were not significantly different in the CUI group. **Conclusion:** CUI was the most effective method for the RSL.

***Keywords:*** *root canal irrigants; scanning electron microscopy; smear layer; ultrasonics; root canal preparation; endodontics.*

**Resumen:** Antecedentes: Debido a la complejidad anatómica del sistema de conductos radiculares, la irrigación juega un papel fundamental en la endodoncia. Este estudio *in vitro* se buscó comparar la remoción de la capa de barro dentinario (RCBD) promovida por irrigación convencional (IC), irrigación


ISSN Print 0719-2460 - ISSN Online 0719-2479.  

ultrasónica pasiva (IUP) e irrigación ultrasónica continua (IUC) con EDTA al 17%, mediante el uso de microscopía electrónica de barrido (SEM). **Material y Métodos:** Se instrumentaron cuarenta caninos mandibulares humanos de raíz única y se asignaron aleatoriamente a cuatro grupos (n = 10), de acuerdo con el protocolo de riego apuntando a la RCBD: CG (grupo control) —riego convencional con agua destilada; IC— irrigación convencional con 17% de EDTA; IUP: irrigación ultrasónica pasiva con 17% de EDTA; IUC: irrigación ultrasónica continua con 17% de EDTA. Se obtuvieron hemisecciones de cada muestra y se capturaron imágenes de cada tercio del conducto radicular (cervical, medio y apical) con un aumento de 1000X mediante SEM. Tres evaluadores previamente calibrados y ciegos clasificaron la RCBD, según los criterios propuestos por Torabinejad *et al*.: capa de frotis pequeña o nula (todos los túbulos dentinarios estaban limpios y abiertos); 2 = capa de frotis moderada (sin capa de frotis en la superficie del conducto radicular, pero los túbulos dentinarios contenían residuos); 3 = capa de frotis densa (que cubre prácticamente todas las entradas de los túbulos dentinarios). El análisis estadístico se realizó mediante las pruebas de Kruskal-Wallis y Bonferroni ($p$<0,05). **Resultados:** En general, IUC y tercios cervicales mostraron mejores tasas de RCBD, en comparación con los otros métodos y tercios, respectivamente ($p$<0,05). Más específicamente, los tercios cervicales mostraron mejores resultados en los grupos GC, IC y IUP ($p$<0,05), mientras que los tercios cervical y medio no fueron significativamente diferentes en el grupo IUC. **Conclusión:** CUI fue el método más eficaz para la RCBD.

***Palabra Clave:*** *irrigantes del conducto radicular; microscopía electrónica de rastreo; capa de barro dentinario; ultrasonido; preparación del conducto radicular; endodoncia.*

## INTRODUCTION.

Root canal treatment aims primarily at preventing infection/reinfection of the root canal system (RCS), and promoting a significant reduction in the microbial content of infected canals.[1] Mechanical preparation reduces the bacterial load significantly, but complete disinfection cannot be achieved due to the anatomical complexity of the RCS. Therefore, the use of irrigating solutions is essential for the disinfection process, in addition to lubricating the root canal walls, preventing accidents and iatrogenic events.[2]

However, endodontic files used together with irrigating solutions induce the production of a "material", characteristically, a 1-mm to 2-mm-thick amorphous structure composed of inorganic dentin debris, as well as organic substances containing fragments of the odontoblastic process, microorganisms and necrotic pulp tissue. This "material" is called the smear layer.[3]

Removal of the smear layer (RSL) has been recommended after chemomechanical preparation and before applying intracanal dressing or performing root canal filling, because this adherent layer can hinder the spread of antimicrobial agents from intracanal medications to the root dentin,[3] intratubular penetration of the endodontic sealer, and the contact between obturation materials and the root canal walls, thus compromising the sealing and increasing the chances of reinfection.[4]

The most common chelating solutions for the RSL contains ethylenediaminetetraacetic acid (EDTA), which reacts with the calcium ions in dentin to form soluble calcium chelates.[3] However, conventional irrigation (CI) with EDTA, using a syringe and needle without agitation, has been found to be ineffective for the RSL. Thus, the activation of chelating solutions using different methods has been proposed.[5]

Weller *et al*.,[6] was the first author to describe the passive ultrasonic irrigation (PUI). The technique consists of passively inserting a metal tip/file attached to an ultrasonic device oscillating at a frequency of 30 kHz into the canal filled with a solution (irrigant or chelating).[6] After activation, the instrument is surrounded by acoustic streaming, which agitates the solution to enhance removal of debris and the smear layer.[5]

Continuous ultrasonic irrigation (CUI) does not require the irrigant renewal among ultrasonic file activations. The ultrasonic tip is placed in the canal and the irrigant or chelating solution keeps flowing for the entire activation time. According to Jamleh *et al*.,[7] the effectiveness of CUI in endodontics has not been fully researched. Thus, this *in vitro* study was sought to

compare the RSL promoted by CI, PUI and CUI with 17% EDTA, by using scanning electron microscopy (SEM). The null hypothesis tested was that there would be no significant differences among the protocols researched.

## MATERIALS AND METHODS.

This research was performed considering the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

### Selection and specimen preparation

Forty single-rooted human mandibular canines were selected from donations made to a private dental clinic. Inclusion criteria: teeth with single, straight canals and completely formed root apexes, free of cracks, endodontic treatment, restorations, and curvatures less than 30°, according to a classification system developed by Schneider.[8] Mesiodistal and buccolingual radiographs were performed to confirm these features. Ultrasound (Profi II Ceramic, Dabi Atlante, Ribeirão Preto, SP, Brazil) was used to clean the external root surfaces. After extractions, the specimens were stored in containers of 0.2% thymol disinfection solution, and then rinsed in running water for 24 hours before the initiation of the experiment.

Afterwards, root segments were standardized to a length of 18 mm by sectioning the tooth crowns close to the cementoenamel junction with a double-faced disc (KG Sorensen, Barueri, SP, Brazil). A layer of OpalDam Blue gingival barrier (Ultradent Products, South Jordan, USA) was used to seal the root apex of each root to simulate clinical conditions, always taking care to avoid irrigant extrusion.[9,10] The gingival barrier was kept clear of the canal by inserting a #15 K-file before applying the barrier layer.[9]

### Chemomechanical preparation

Root canal entrance was prepared with a #2 Largo (Dentsply/Maillefer, Ballaigues, Switzerland) and #3082 burs (KG Sorensen). The cervical and middle thirds were instrumented with #4, #3 and #2 Gates-Glidden drills (Dentsply/Maillefer), using the crown-down technique. Afterwards, the working length (WL) was determined by using a #15 K-File (Dentsply/Maillefer) inserted in the canal until its tip was visible at the apical foramen, and then subtracting 1 mm from this measurement.

An experienced operator instrumented all the root canals with the Protaper System up to the F5 file driven with an electric motor (X-Smart Plus, Dentsply-Maillefer) following the manufacturer's instructions. The irrigation was conducted by using a 31-gauge NaviTip Double Sideport needle (Ultradent Products, South Jordan, UT, USA), inserted up to 1 mm from the WL, and a total of 20 mL of 2.5% sodium hypochlorite (NaOCl) per canal.[11]

### Removal of the Smear Layer (RSL)

After chemomechanical preparation, the specimens were randomly divided into 4 groups (n = 10) and the following protocols were applied for the RSL:

**- CG (control group)**

The root canal was filled with 18 mL of distilled water applied for 3 minutes with a 31-gauge NaviTip Double Sideport needle, inserted up to 1 mm from the WL.

**-CI (conventional irrigation group):**

The root canal was filled with 18 mL of 17% EDTA applied for 3 minutes with a 31-gauge NaviTip Double Sideport needle, inserted up to 1 mm from the WL.

**- PUI (passive ultrasonic irrigation group):**

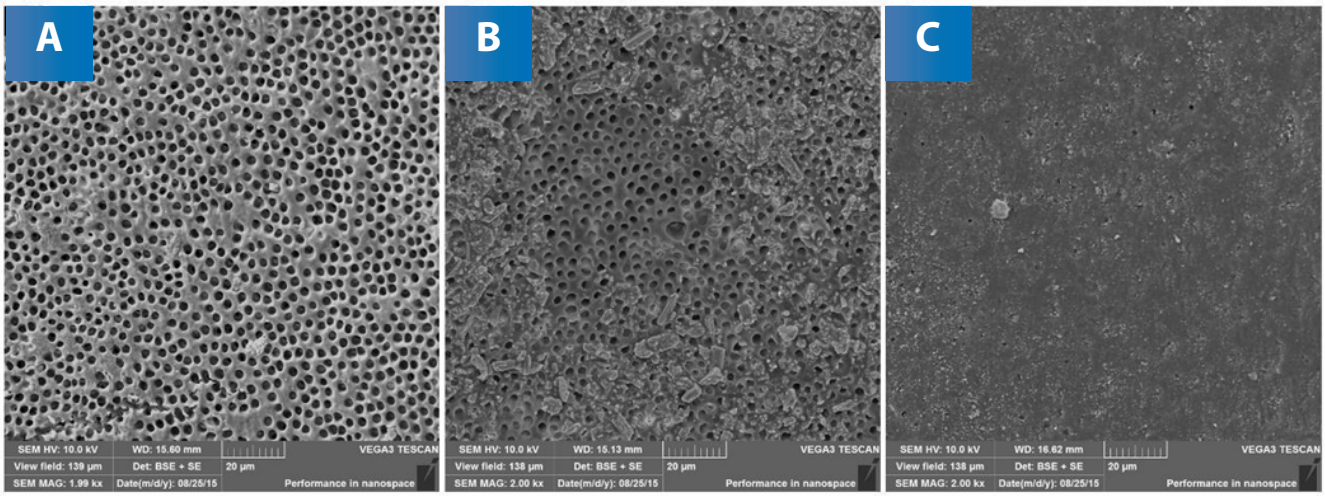
The root canal was filled with 2 ml of 17% EDTA applied with a 31-gauge NaviTip Double Sideport needle, inserted up to 1 mm from the WL. PUI was performed by using the Irrisonic tip (Helse, Santa Rosa de Viterbo, SP, Brazil), calibrated to 1 mm short of the WL, and was ultrasound-activated (Profi Neo – US, Dabi Atlante, Ribeirão Preto, SP, Brazil) at a power of 40%, as indicated by the manufacturer, and taking care to avoid contact with the root canal walls. PUI was performed for 9 cycles of 20 seconds with 2 mL of 17% EDTA replaced before each cycle, totaling 3 minutes and 18 mL of 17% EDTA.

**- CUI (continuous ultrasonic irrigation group):**

The root canal was filled with 2 ml of 17% EDTA by using a 31-gauge NaviTip Double Sideport needle, inserted up to 1 mm from the WL. A 17% EDTA solution was placed in the bottle of the ultrasonic device, which was set to a power of 40%, as indicated by the manufacturer. The ultrasonic device was adjusted to release about 2 mL for 20 seconds of activation, with the tip inserted 1 mm short of the WL, and CUI


**ISSN Print** 0719-2460 - **ISSN Online** 0719-2479.  

**Figure 1.** Representative images of the score system used.




**A.** Small or no smear layer. **B.** Moderate smear layer. **C.** Dense smear layer .

**Table 1.** General analysis of the groups regardless of the thirds.

| Group | Median | Quartile Deviation | *p*-value |
|---|---|---|---|
| CG (Control) | 3.00[a] | 2.00 | 0.000 |
| CI (Conventional Irrigation) | 3.00[a] | 2.00 | |
| PUI (Passive Ultrasonic Irrigation) | 2.00[b] | 2.50 | |
| CUI (Continuous Ultrasonic Irrigation) | 2.00[c] | 1.50 | |

*: Different superscript letters indicate statistically significant differences ($p<0.05$).

**Table 2.** General analysis of the thirds regardless of the groups.

| Third | Median | Quartile Deviation | *p*-value |
|---|---|---|---|
| Cervical | 2.00[a] | 2.50 | 0.000 |
| Middle | 3.00[b] | 2.00 | |
| Apical | 3.00[b] | 2.00 | |

*: Different superscript letters indicate statistically significant differences ($p<0.05$).

**Table 3.** Comparison among thirds, considering the removal of the smear layer scores (median and quartile deviation) in each group.

| Group | Third | | | *p*-value |
|---|---|---|---|---|
| | Cervical | Middle | Apical | |
| CG (Control) | 2.00 ± 2.00[a,A] | 3.00 ± 2.00[b,A] | 3.00 ± 1.50[b,A] | 0.00 |
| CI (Conventional Irrigation) | 2.00 ± 2.50[a,A] | 3.00 ± 2.00[b,A] | 3.00 ± 2.00[b,A] | 0.02 |
| PUI (Passive Ultrasonic Irrigation) | 1.00 ± 1.50[a,B] | 2.00 ± 1.50[b,A] | 3.00 ± 1.50[b,A] | 0.00 |
| CUI (Continuous Ultrasonic Irrigation) | 1.00 ± 1.50[a,C] | 1.00 ± 1.50[a,A] | 2.00 ± 1.00[b,A] | 0.00 |

Different superscript letters indicate statistically significant differences ($p<0.05$). Considering thirds (columns): lowercase letters; considering rows: uppercase letters.

was performed for 9 cycles of 20 seconds, totaling 3 minutes and 18 mL of 17% EDTA.

After completing the irrigation protocol of each group, all the canals were irrigated with 5 mL of distilled water, and then aspirated and dried with absorbent paper points.[10]

### Analysis of the RSL by SEM

First, a F5 gutta-percha cone was introduced into the canal. Then, vertical grooves were made on the mesial and distal external surfaces of each root to facilitate fracturing into halves with double-sided diamond discs (KG Sorensen), operated at low rotary speed, until the gutta-percha cone could be seen, always taking care to avoid accidental contamination and invasion of the canal by sharp debris.[9,12]

The hemisections were then fixed on circular metal stubs to sputter coat the surface with a 30-nm layer of gold (Quorum Q150R ES, Ashford Kent, UK). A scanning electron microscope (VEGA 3, Tescan, Brno, Czech Republic) captured images at 1000X magnification for each hemisection, corresponding to each root third (cervical, middle and apical).

Three different examiners were previously calibrated by analyzing 24 images randomly selected from two specimens of each group, using the score system proposed by Torabinejad *et al.*,[13]:

1= Small or no smear layer (all dentinal tubules were clean and open);

2= Moderate smear layer (no smear layer on the surface of root canal, but dentinal tubules contained debris);

3= Dense smear layer (covering practically all dentinal tubules entrances) (Figure 1).

During this process, communication among examiners was allowed, in order to ultimately establish only one score per image. The same methodology was used in undertaking the definitive process of scoring the images, however, with no communication among examiners. The analysis was blindly performed by examiners.

### Statistical analysis

Initially, the Kappa test was applied to analyze the level of agreement among evaluators, and thus validate the findings. The Kruskal-Wallis test was used to compare the results, based on the obtained scores. Afterwards, the Bonferroni test was applied for pairwise multiple comparisons. Statistical analysis was performed using the SPSS 2.0 software (SPSS Inc., Chicago, IL, USA) ($p<0.05$).

## RESULTS.

Kappa *p*-values of 90.83% were obtained, indicating excellent interexaminer agreement with the scores given. Scanning electron microscopy analysis allowed cleanliness to be evaluated by applying scores to all the samples. Overall, CUI and cervical thirds showed a better RSL rate compared with the other methods and thirds, respectively ($p<0.05$) (Table 1 and Table 2).

More specifically, the cervical thirds showed better results in the CG, CI and PUI groups ($p<0.05$), whereas the cervical and middle thirds were not significantly different in the CUI group (Table 3).

## DISCUSSION.

Chemomechanical preparation produces amorphous and irregular surface sediments formed by inorganic and organic components, such as necrotic pulp tissue, remains of odontoblastic processes and microorganisms, which accumulate on the root canal walls. This "material" is called the smear layer.[14,15] Considering the success of endodontic treatment mainly depends on the cleaning of the RCS,[16] the RSL has been recommended. This in vitro study was sought to evaluate the efficiency of the RSL promoted by CI, PUI and CUI, according to SEM. The null hypothesis was rejected because CUI showed better results.

EDTA was used in the current study because it is a chelating agent capable of removing calcium ions from the dentin, and of acting on the inorganic portion of the smear layer to open the dentinal tubules.[17,18] In addition, recent research has shown that ultrasonic activation renders EDTA effective in reducing endotoxin levels in infected root canals.[18-20]

The volume of irrigant is an important factor for the RSL, since it mediates the flow rate, which influences the delivery, renewal and mechanical effect of the solution on the root canal walls. The reflux created by irrigation and aspiration promotes more effective debris elimination.[21,22] For this reason, it was deemed important to standardize the volume of irrigant used in the 3 irrigation techniques evaluated in this study (CI, PUI and CUI). The tested irrigation protocols


**ISSN Print** 0719-2460 - **ISSN Online** 0719-2479.  

basically involve the delivery of irrigation solution with or without activation. Irrigation activation devices have been developed to minimize the apical vapor lock effect that prevents the solution from reaching the final millimeters of the root canal.[23,24]

Many studies have shown that ultrasonic irrigation can improve the dispersion of the irrigating solution along the entire length of the root canal,[25,26] and increase the contact of the liquid with the canal walls.[27,28] It uses a process that transmits acoustic energy from an oscillating tip to the irrigant, ultimately resulting in acoustic microextraction and cavitation, and improving cleaning efficacy through effective fluid flow dynamics.[28,29]

The comparison among the CI, PUI and CUI systems was conducted only in human mandibular canines to avoid sampling bias, and the RSL rate was assessed by SEM. Despite the limitations regarding subjective, quantitative and non-reproducible evaluation,[30] SEM is still the most common method available and used for this type of research.[30,31]

The current results showed the RSL was best by CUI. This outcome was similar to those reported by Jamleh *et al*.,[7] and Layton *et al*.,[32] CUI allows the irrigant to penetrate the RCS dynamically and effectively, in addition to being renewed uninterruptedly. It induces an increase in the flow in the apical portion,[28,33,34] and maintains the canal constantly filled with the irrigating solution.[32] Overall, the efficiency of the 3 irrigation techniques for the RSL was greater in the coronal thirds, as previously reported by other studies.[35,36] This may be attributed to the number and diameter of dentinal tubules that decrease from the cervical to the apical third,[37] and to the greater difficulty for the irrigant to be delivered to the final millimeters of the root canal.

Our study used a clinical configuration of extracted human teeth with a closed system similar to that of previous studies.[38-40] However, since an in vitro environment was used, it is difficult to predict whether the resistance exerted by the root, created by the closed system, is greater than, less than or equal to that exerted by the periradicular tissues. Hence, more research should be conducted to ascertain the reliability of the method used.

## CONCLUSION.

According to the results of the present *in vitro* study, it can be concluded that CUI is the most effective method for the RSL, especially in the cervical and middle thirds of the root canal.

Conflict of interests: The authors declare no conflicts of interest.

Ethics approval: This research was performed considering the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

Funding: The present study was funded by the authors.

Authors' contributions: All authors contributed at different stages of the project, all the authors read and approved the final manuscript.

Acknowledgements: None.

## REFERENCES.

1. Siqueira JF Jr, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-1301.
2. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Brit Dent J. 2014;216(6):299-303.
3. Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J. 2010;43(1):2-15.
4. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod. 2007;33(2):96-105.
5. Mozo S, Llena C, Forner L. Review of ultrasonic irrigation in endodontics: increasing action of irrigating solutions. Med Oral Patol Oral Cir Bucal. 2012;17(3):e512-6.
6. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod. 1980;6(9):740-3.
7. Jamleh A, Suda H, Adorno CG. Irrigation effectiveness of continuous ultrasonic irrigation system: An ex vivo study. Den Mat J. 2018;37(1):1-5.
8. Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 1971;32(2):271-5.
9. Bueno CRE, Cury MTS, Vasques AMV, et al. Cleaning effectiveness of a nickel-titanium ultrasonic tip in ultrasonically activated irrigation: a SEM study. Braz Oral Res. 2019;33:e017.
10. Mancini M, Cerroni L, Iorio L, Armellin E, Conte G, Cianconi L. Removal of the smear layer and canal cleanliness using different irrigation systems (EndoActivator, EndoVac, and passive ultrasonic irrigation): field emission scanning electron microscopic evaluation in an in vitro study. J Endod. 2013;39(11):1456-60.
11. Silva EJNL, Carvalho CR, Belladonna FG, Prado MC, Lopes RT, De-Deus G, Moreira EJL. 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(2):681-7.
12. Schmidt TF, Teixeira CS, Felippe MC, Felippe WT, Pashley DH, Bortoluzzi EA. Effect of Ultrasonic Activation of Irrigants on Removal of the smear layer. J Endod. 2015;41(8):1359-63.
13. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. J Endod. 2003;29(3):170-5.
14. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod. 1984;10(10):477-83.
15. Peeters HH, Suardita K. Efficacy of removal of the smear layer at the root tip by using ethylenediaminetetraacetic acid and erbium, chromium: yttrium, scandium, gallium garnet laser. J Endod. 2011;37(11):1585-9.
16. Salzgeber RM, Brilliant JD. An in vivo evaluation of the penetration of an irrigating solution in root canals. J Endod. 1977;3(10):394-8.
17. Machado R, Garcia L, da Silva Neto UX, Cruz Filho AMD, Silva RG, Vansan LP. Evaluation of 17% EDTA and 10% citric acid in removal of the smear layer and tubular dentin sealer penetration. Microsc Res Tech. 2018;81(3):275-82.
18. Herrera DR, Martinho FC, de-Jesus-Soares A, et al. Clinical efficacy of EDTA ultrasonic activation in the reduction of endotoxins and cultivable bacteria. Int Endod J. 2017;50(10):933-40.
19. Rodrigues RCV, Zandi H, Kristoffersen AK, et al. Influence of the Apical Preparation Size and the Irrigant Type on Bacterial Reduction in Root Canal-treated Teeth with Apical Periodontitis. J Endod. 2017;43(7):1058-63.
20. Matos FS, Khoury RD, Carvalho CAT, Martinho FC, Bresciani E, Valera MC. Effect of EDTA and QMIX Ultrasonic Activation on the Reduction of Microorganisms and Endotoxins in Ex Vivo Human Root Canals. Braz Dent J. 2019;30(3):220-6.
21. Darcey J, Jawad S, Taylor C, Roudsari RV, Hunter M. Modern Endodontic Principles Part 4: Irrigation. Dent Update. 2016;43(1):20-33.
22. Marques AC, Aguiar BA, Frota LM, Guimarães BM, Vivacqua-Gomes N, Vivan RR, Duarte MA, de Vasconcelos BC. Evaluation of Influence of Widening Apical Preparation of Root Canals on Efficiency of Ethylenediaminetetraacetic Acid Agitation Protocols: Study by Scanning Electron Microscopy. J Contemp Dent Pract. 2018 ;19(9):1087-94.
23. Agarwal A, Deore RB, Rudagi K, Nanda Z, Baig MO, Fareez MA. Evaluation of Apical Vapor Lock Formation and comparative Evaluation of its Elimination using Three different Techniques: An in vitro Study. J Contemp Dent Pract. 2017;18(9):790-4.
24. Chan R, Versiani MA, Friedman S, Malkhassian G, Sousa-Neto MD, Leoni GB, Silva-Sousa YTC, Basrani B. Efficacy of 3 Supplementary Irrigation Protocols in the Removal of Hard Tissue Debris from the Mesial Root Canal System of Mandibular Molars. J Endod. 2019;45(7):923-9.
25. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. Int Endod J. 1982;15(4):187-96.
26. da Costa Lima GA, Aguiar CM, Camara AC, Alves LC, Dos Santos FA, do Nascimento AE. Comparison of smear layer removal using the Nd:YAG laser, ultrasound, ProTaper Universal system, and CanalBrush methods: an in vitro study. J Endod. 2015;41(3):400-4.
27. Walmsley AD. Ultrasound and root canal treatment: the need for scientific evaluation. Int Endod J. 1987;20(3):105-11.
28. Jiang LM, Lak B, Eijsvogels LM, Wesselink P, van der Sluis LW. Comparison of the cleaning efficacy of different final irrigation techniques. J Endod. 2012;38(6):838-41.
29. Jiang LM, Verhaagen B, Versluis M, van der Sluis LW. Evaluation of a sonic device designed to activate irrigant in the root canal. J Endod. 2010;36(1):143-6.
30. De-Deus G, Reis C, Paciornik S. Critical appraisal of published smear layer-removal studies: methodological issues. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112(4):531-43.
31. Machado R, Comparin D, Back E, Garcia L, Alberton LR. Residual smear layer after root canal instrumentation by using Niti, M-Wire and CM-Wire instruments: A scanning electron microscopy analysis. Eur J Dent. 2018;12(3):403-9.
32. Layton G, Wu WI, Selvaganapathy PR, Friedman S, Kishen A. Fluid Dynamics and Biofilm Removal Generated by Syringe-delivered and 2 Ultrasonic-assisted Irrigation Methods: A Novel Experimental Approach. J Endod. 2015;41(6):884-9.
33. Huang TY, Gulabivala K, Ng YL. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. Int Endod J. 2008;41(1):60-71.


**ISSN Print** 0719-2460 - **ISSN Online** 0719-2479.  

34. McGill S, Gulabivala K, Mordan N, Ng YL. The efficacy of dynamic irrigation using a commercially available system (RinsEndo) determined by removal of a collagen 'bio-molecular film' from an ex vivo model. Int Endod J. 2008;41(7):602-8.
35. Nogo-Zivanovic D, Kanjevac T, Bjelovic L, Ristic V, Tanaskovic I. The effect of final irrigation with MTAD, QMix, and EDTA on removal of the smear layer and mineral content of root canal dentin. Microsc Res Tech. 2019;82(6):923-30.
36. Matos FS, da Silva FR, Paranhos LR, Moura CCG, Bresciani E, Valera MC. The effect of 17% EDTA and QMiX ultrasonic activation on smear layer removal and sealer penetration: ex vivo study. Sci Rep. 2020;10:10311.
37. Carrigan PJ, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. J Endod. 1984;10(8):359-63.
38. Iriboz E, Bayraktar K, Turkaydin D, Tarcin B. Comparison of apical extrusion of sodium hypochlorite using 4 different root canal irrigation techniques. J Endod. 2015;41(3):380-4.
39. Fukumoto Y, Kikuchi I, Yoshioka T, Kobayashi C, Suda H. An ex vivo evaluation of a new root canal irrigation technique with intracanal aspiration. Int Endod J. 2006;39(2):93-9.
40. Mitchell RP, Yang SE, Baumgartner JC. Comparison of apical extrusion of NaOCl using the EndoVac or needle irrigation of root canals. J Endod. 2010;36(2):338-41.