

Macro and Microscopic Analysis of the Cleaning of Root Dentin Prepared for Post Space

Cecilia Noemí de Caso ^{1,*}, Claudio Francisco Boiero ¹, and Mario Sezin ²

ABSTRACT

Objective: To analyze *in vitro*, using optical and electron microscopy, the effect of different cleaning methods on the dentin of root canals prepared for prosthetic post placement.

Materials and Methods: Forty incisors with partial endodontic fillings were selected and prepared for prosthetic post placement to a standardized length of 15 mm using size 2 and 3 drills. The samples were divided according to cleaning method (n = 10 per group). Group 1: distilled water-17% EDTA-distilled water-passive irrigation. Group 2: distilled water-Irrisonic tip-ultrasonic activation. Group 3: distilled water-endobrush-type brush-ultrasonic activation. Group 4: 17% EDTA-endobrush-type brush-ultrasonic activation. The longitudinally fractured specimens were examined under a stereomicroscope and scanning electron microscope, and images were analyzed using image-processing software. The Kruskal-Wallis and Pearson's chi-square test were applied.

Results: Under optical microscopy, Group 4 achieved the highest cleanliness scores, followed by Groups 1, 3, and 2, with statistically significant differences (Chi^2 p < 0.01). Regarding canal thirds, the apical third showed the best cleaning performance, while the coronal third exhibited the lowest cleanliness percentages, with significant differences among thirds (Chi^2 p < 0.01). Electron microscopy analysis revealed that Groups 1 and 4 achieved the highest cleanliness values, with significant differences compared with 2 and 3, where all tubules appeared occluded (Chi^2 p < 0.01).

Conclusion: Within the limitations of this study, the chemical action of 17% EDTA, regardless of the irrigation method, produced the highest dentin cleanliness values in root canals prepared for prosthetic post placement.

Keywords: Cleansing agents, root canal irrigants, root canal preparation, smear layer.

Submitted: September 20, 2025

Published: October 30, 2025

 10.24018/ejdent.2025.6.5.411

¹Department of Endodontics, Health Sciences, Dentistry, Catholic University of Cordoba, Argentina.

²Department of Oral Rehabilitation, Faculty of Dentistry, National University of Córdoba, Córdoba, Argentina.

*Corresponding Author:
e-mail: cndecaso@gmail.com

1. INTRODUCTION

Mechanical preparation using manual or rotary instruments produces a granular, amorphous, and irregular smear layer in the root dentin which contains inorganic and organic debris, necrotic debris, odontoblastic processes, pulp tissue residues, and microorganisms with their metabolic products [1]. This layer adheres to the surface of dentin and occludes the dentin tubules, thus preventing irrigating solutions, medications, and root canal sealers from penetrating dentin tubules [2]. A combination of solutions of different chemical nature such as chelating agents, acids or antibacterials should be applied with various techniques or effective delivery and agitation systems to optimize cleaning inside the root canals and the removal

of debris and remaining material remains [3]. Agitation and activation of irrigants using lasers and sonic and ultrasonic devices are additional resources that improve smear layer removal in combination with the solutions used for root canal irrigation [4]. Strong chelating agents such as ethylenediaminetetraacetic acid (EDTA) decalcify root dentin and remove the smear layer, which acts as a barrier preventing irrigants from directly reaching and disinfecting the dentin surface and tubules, as well as affecting the sealing quality of the endodontic filling. The sonic activation of irrigating solutions produces movement in the contact area resulting in improved cleaning compared to conventional irrigation techniques, although it is of lower quality compared to the ultrasonic method [5].



Ultrasonic energy influences fluid dynamics within the root canal system, which can optimize irrigant contact in areas of the root canal that cannot be reached with conventional or current rotary instruments [6]. During the preparation of the space for the placement and fixation of an intraradicular anchor, different rotary instruments are used to produce a layer composed of the endodontic sealer, and gutta-percha remains softened by frictional heat, which in combination with the dentin forms a secondary smear layer that contaminates and obliterates the surface and dentin tubules [7]. For intraradicular anchorage, a surface free of macro- and micro- contaminants is required, as well as the creation of a micro-retentive surface pattern by modifying or removing the layer of debris produced during instrumentation. In addition to the action of the irrigant, the effectiveness of irrigation depends on the possibility of contact with the materials and structures within the endodontic substrate [8]. The smear layer, along with gutta-percha and sealer residues, obliterates the dentin tubules and interferes with bonding procedures. The chemical action of irrigants and fluid dynamics within the root canal are essential for conditioning the dental substrate prior to the final bonding procedure [9]. Therefore, the aim of this study was to analyze in vitro using optical and scanning electron microscopy the action of different cleaning methods on the dentin of the root canal prepared for prosthetic post placement.

2. MATERIALS AND METHODS

Forty maxillary central incisors were clinically and radiographically selected, regardless of race, age and sex, and had acceptable endodontic fillings (good adaptation to the walls and homogeneous radiopacity). The study was approved by the Institutional Committee on Health Research Ethics of the Faculty of Dentistry, National University of Córdoba, under number ODO CAI-CIEIS 67, and by Resolution No. 190, dated July 29, 2025. Endodontic fillings were performed using the lateral compaction technique with gutta-percha cones and a zinc oxide-eugenol-based sealer (Farmadental, Buenos Aires, Argentina). The coronary portion was sectioned leaving a 3 mm remnant above the anatomical neck. Preparations for prosthetic anchorage were made up to a length of 15 mm using sequentially Largo # 2 and # 3 drills (Dentsply Maillefer, Tulsa, Oklahoma, USA) and Macro-Lock #2 and #3 finishing drills (RTD, Saint-Egrève, France). Once the prosthetic preparation was complete, they were randomly divided into four groups of 10 pieces each, to carry out different cleaning methods. Group 1: distilled water/17% EDTA/distilled water (Tedequim, Córdoba, Argentina), passive irrigation; Group 2: distilled water/Irrisonic tip (Helse, Santa Rosa de Viterbo, São Paulo, Brazil), ultrasonic activation; Group 3: distilled water/endobrush (Proxabrush Gum Sunstar, Illinois, USA), ultrasonic activation; Group 4: 17%EDTA/endobrush, ultrasonic activation. The total irrigant volume was 5 ml. A P-5 Booster Suprasson ultrasonic device (Satelec-Merignac Cedex, France) was used with a 90° Ultrasonic Endo File Holder adapter (EMS Electro Medical Systems, Nyon, Switzerland). Ultrasonic activation in

groups 2, 3, and 4 was performed in three 5-second cycles at low power, according to the manufacturer's instructions. In groups 3 and 4, the endobrush was designed from a fine cylindrical Proxabrush interdental brush replacement, 0.8 mm in a diameter. After cleaning, the specimens were sectioned longitudinally by fracture. Mesially and distally, grooves were made with diamond discs (Jota, Rüthi, Switzerland), and with a tube cutter tool, tooth fracture was carried out tooth, obtain buccal and palatal halves.

2.1. Optical Microscopy

Each surface ($n=80$) was digitally captured with a 10x stereoscopic magnifier, and analyzed with image processing software (Image Pro Plus version 5). The entire canal area prepared for anchorage was divided into thirds (coronal, medial, and apical), and a score of 1 to 5 was established to quantify cleaning based on the percentage of dentin surface covered by debris. Score 1: 100%/81%; Score 2: 80%/61%; Score 3: 60%/41%; Score 4: 40%/21%; Score 5: 20%/0%.

2.2. Electron Microscopy

The samples ($n = 80$) were digitally observed using a high-resolution scanning electron microscope (FE-SEM Sigma, Munich, Germany) and analyzed using an image processing software (Image Pro Plus version 5). The images were scored according to the following numerical scale: T0, open dentin tubules, free of debris, without smears; T1, visible or partially occluded dentin tubules, moderate presence of smears; T2, occluded dentin tubules, abundant presence of smears.

2.3. Statistical Analysis

A sample size estimation was performed considering a 95% confidence level ($\alpha = 0.05$) and a statistical power of 80% ($\beta = 0.20$). Since the non-parametric distribution of the data was initially unknown, a normal distribution was assumed. Accordingly, the sample size was calculated using the formula for a one-way ANOVA (parametric test), based on preliminary data obtained from a pilot study. The estimated sample size was $n = 36$. After data collection, it was verified that the distribution was not normal; therefore, intergroup comparisons were performed using the non-parametric Kruskal–Wallis test. As there is no simple or universal formula for determining the sample size in non-parametric analyses, specialized statistical software is often used for this purpose. However, in this study, the sample size previously calculated for the one-way ANOVA ($N = 36$) was retained and adjusted according to the relative efficiency of the Kruskal–Wallis test (efficiency factor: 0.955), which represents an acceptable approximation. Thus, $N = 36/0.955$, resulting in an adjusted $N = 37.4$. For equal group allocation, the sample size per group was $n = 10$ (9.4).

The data obtained by optical microscopy were tabulated and statistically analyzed using the Kruskal–Wallis test to assess the differences between groups and subgroups. Electron microscopy micrographs were analyzed using Pearson's chi-squared test, with a significance level of $p < 0.05$ for both studies.

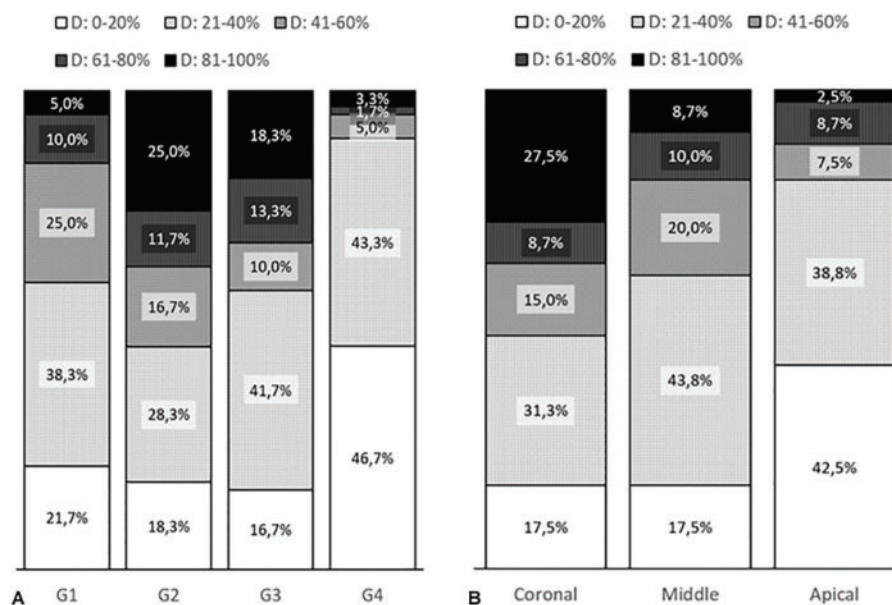


Fig. 1. Percentage of detritus categorized according to score range: (A) by Group and (B) by canal third.

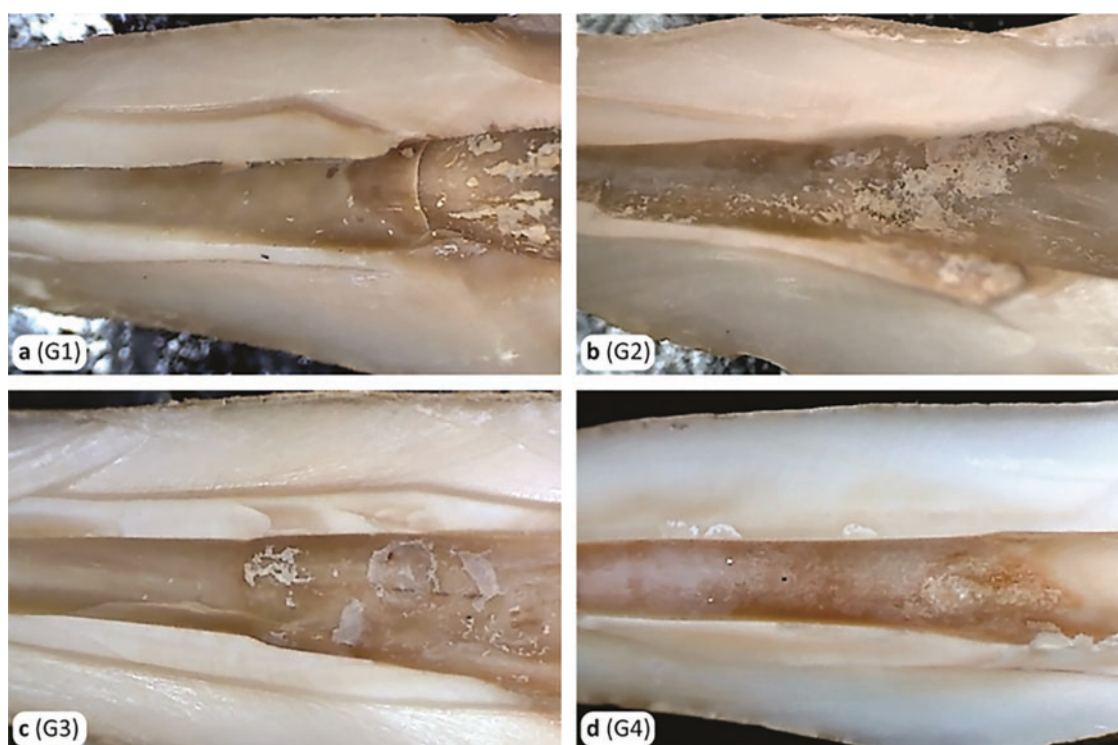


Fig. 2. Images a–d correspond to Groups 1–4, respectively, showing higher cleanliness in Groups 1 and 4 and lower scores in Groups 2 and 3.

3. RESULTS

3.1. Optical Microscopy

Group 4 exhibited high cleanliness scores (4 and 5) in all thirds, followed by Group 1, while Groups 3 and 2 showed low cleanliness percentages. The differences were statistically significant ($\chi^2 p < 0.01$). (Fig. 1A).

In third the analysis, the best cleaning scores were observed in the apical third, followed by the middle third. The lowest cleaning percentages was observed in the coronal third region. The differences between the thirds were statistically significant ($\chi^2 p < 0.01$). (Figs. 1B and 2).

3.2. Electron Microscopy

Group 1 showed the greatest number of open tubules, which was significantly different from that in Group 4 ($\chi^2 p = 0.006$). Groups 2 and 3 exhibited all occluded tubules, with no differences between the two groups, but differences were observed compared to Groups 1 and 4 ($\chi^2 p < 0.01$). (Figs. 3 and 4), respectively.

4. DISCUSSION

This study demonstrated that the chemical action of the irrigating solution was the most effective factor in

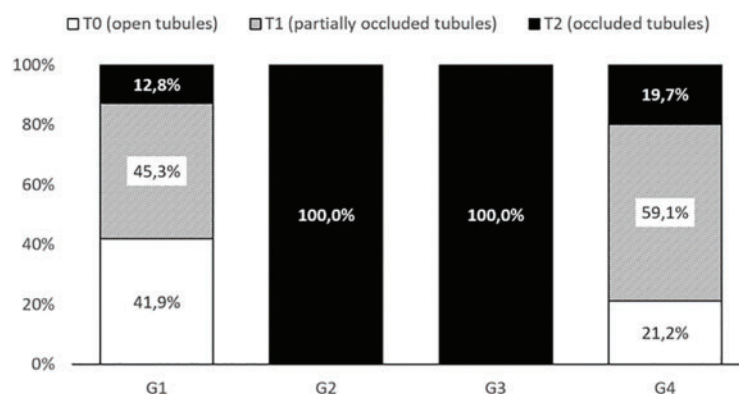


Fig. 3. Cumulative percentages of dentinal tubules, categorized by occlusion level and group.

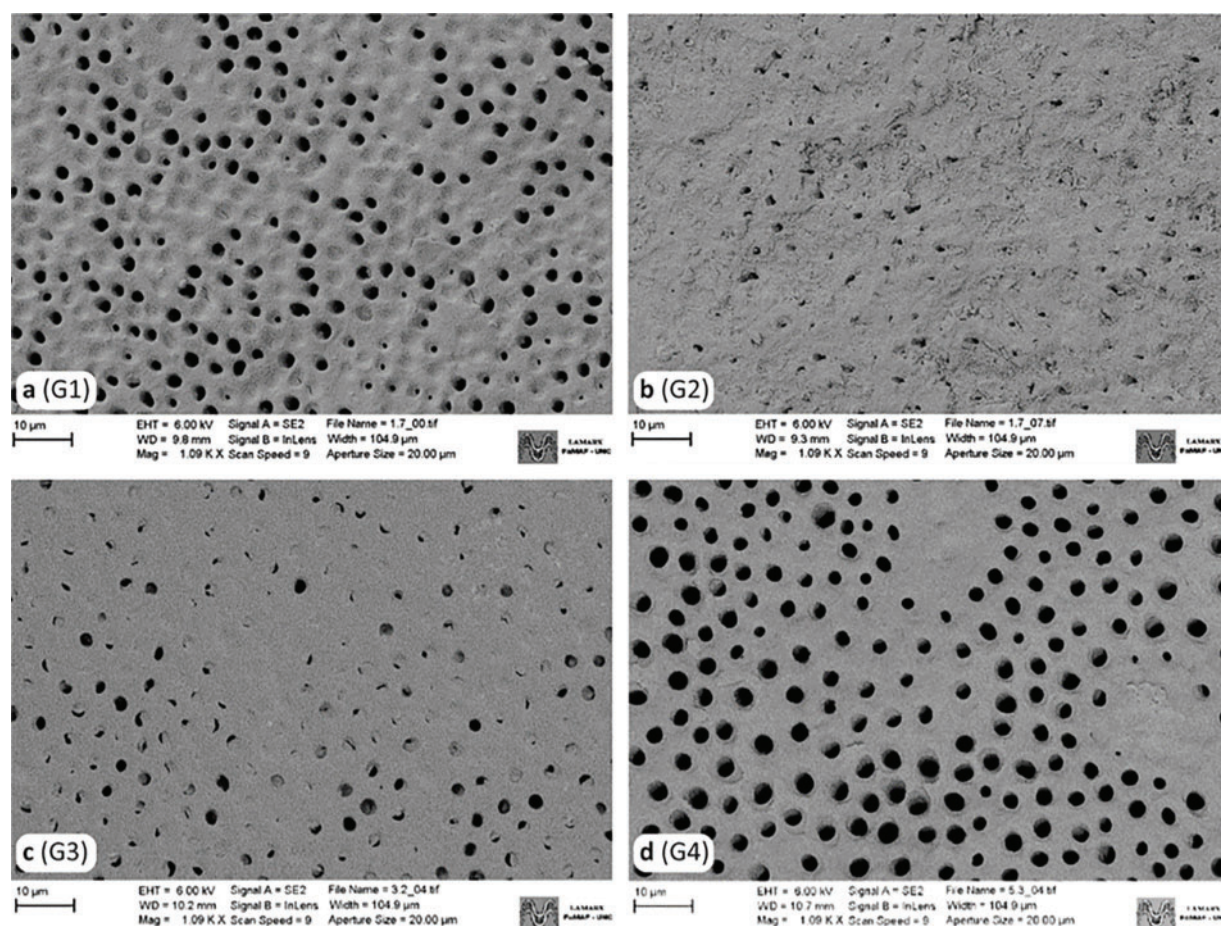


Fig. 4. Representative micrographs a–d correspond to Groups 1–4, respectively, showing different degrees of dentinal tubule occlusion: (a) open and semi-occluded tubules; (b–c) predominantly occluded tubules; (d) mainly open tubules.

cleaning the space prepared for post placement, overcoming ultrasonic activation and the mechanical brushing effect of the endodontic brush. However, together they act synergistically to enhance the cleaning action, since effective smear layer removal is a critical factor for the success of bonding procedures in prosthetic restorations. Therefore, a combination of different irrigation techniques is required, along with the chemical effects of chelating agents [10]. The study has inherent limitations due to its *in vitro* design. Root canal selection was limited to maxillary central incisors to reduce morphological variability, and all canals were treated under standardized conditions. While these measures ensured rigorous experimental control, they cannot fully replicate the *in vivo* clinical

environment. Additionally, 17 % EDTA was selected at controlled volumes because it is a commonly used solution at a concentration considered safe to prevent dentin erosion. Ultrasonic activation was also performed with a controlled activation period to avoid changes in the irrigant temperature. Future prospective clinical studies could validate these results and further assess the efficacy of this irrigation protocol under *in vivo* conditions and typical clinical practice. In clinical situations, it is difficult to evaluate the cleanliness of root dentin therefore, a randomized experimental *in vitro* study was designed to analyze root surfaces using optical microscopy, where the residual filling material was evaluated, and electron microscopy where the cleanliness of the wall and dentin

tubules was assessed, thus complementing the research methods.

The final success of restoration in endodontically treated teeth with different types of posts depends on several factors and/or conditions, such as the length and type of post, remaining tooth tissue, quality of the hybrid layer and chemical compatibility between the adhesive systems [11]. During the preparation of the space for post placement and intraradicular fixation, different rotary instruments are used to produce a layer composed of the endodontic sealer and gutta-percha remains softened by frictional heat, which in combination with dentin, generate an additional smear layer that contaminates the dentin and obliterates the dentin tubules [12]. Although no unique and exclusive protocol has been established to facilitate cleaning of the root dentin substrate, irrigation and/or dentin conditioning solutions are used to promote adhesion. Furthermore, irrigant solutions activation is used to enhance debris removal and promote dentin tubules opening using sonic or ultrasonic devices. In a literature review, it was concluded that the best dentin conditioning solution without activation was 17% EDTA, which coincides with the results of this study [7]. The 17% EDTA solution can remove the inorganic smear within the dentin tubule generated during root canal instrumentation and preparation of the area for intraradicular fixation. Its demineralizing action on dentin can reach a depth of 20-50 μm . A 17% concentration decalcifies the surface of the root canal wall in less than 1 minute; however, the process is self-limiting because the solution finishes its chelating particles as it acts [13]. This is in agreement with other authors who suggested that the smear removal time should not exceed one minute since EDTA applied for prolonged periods causes excessive peri and intratubular dentin erosion, as corroborated by scanning electron microscopy [14], [15].

There is a vast combination of irrigating substances to treat the dentin surface prior to restorative procedures, but considering the combination of irrigants, better smear removal was found in the middle and apical third of the root canal with the application of 15% EDTA with cetavlon [16]. Other research has confirmed that the effectiveness of smear layer removal increases with the combination of different substances [17]. Analyzing the application of various types of acids, it was found that the most effective solution was 7% maleic acid to eliminate smears in the apical third [18]. Another proposed combination is based on the use of a sodium hypochlorite solution alternating with a chelating agent, such as EDTA [5]. Similarly, the use of etidronic acid with 2.5% sodium hypochlorite was an adequate solution for cleaning the root canal before dentin priming and cementing the fiber post with self-adhesive resin cement [19]. In the present study, the combination of irrigating substances was not used and, although the NaClO solution is widely used in endodontics to disinfect the endodontic space, in this study it was not used either because of its strong oxidizing power that can alter the dentin and significantly reduces the adhesion strength of resin-based materials used in intraradicular restoration [12]. In a review of factors influencing the adhesive cementation of fiberglass posts,

they concluded that irrigation of root canals with chlorhexidine, MTAD, or EDTA (alone or in combination with NaOCl) after post space preparation appears to improve the bond strength. The use of 17% EDTA improved the surface, clearly eliminating the smear from within the dentin tubules [20].

In addition to irrigants, various devices have been incorporated into irrigation procedures to improve their chemical-mechanical effectiveness. The root canal brush, coupled with the ultrasonic system, acts mechanically on the dentin surface, and the friction created between the brush and root canal wall can detach debris and improve cleaning. Although this accessory has not been used for cleaning the space prepared for intraradicular fixation, researchers have found significantly cleaner dentin surfaces with its application in the surgical preparation of the root canal [21]–[25]. However, other studies have observed that it does not significantly increase detritus elimination [26], [27]. Consequently, in view of the results found, it is a resource that can be considered effective and efficient as a complement to cleaning the space for intraradicular anchorage, as long as it is applied with the synergistic effect of ultrasound and an irrigating substance with chelating action.

Activation systems employ mechanical, physical, or other forms of energy to agitate and enhance the flow of irrigants within the complexities of the root canal system, to improve the removal of smears, debris and microorganisms [10]. Conventional needle irrigation often fails to effectively deliver and distribute irrigating solutions within the root canal system, particularly in the apical third and isthmus areas. However, in the present study, passive irrigation using a chelator resulted a high percentage of unobstructed dentin tubules similar to those achieved with ultrasound-activated irrigation. In a study using sonic, ultrasonic and 17% EDTA activation, no significant differences were observed between the different protocols applied for smear removal [28]. Ultrasonic energy can influence fluid dynamics within the root canal system, improving the contact of irrigants with regions of the root canal that cannot be reached using conventional endodontic rotary instruments. In our study, ultrasound achieved acceptable levels of cleaning in the apical and middle thirds when used in synergy with EDTA. The application of ultrasonic activation to the final irrigants combined with various chelating agents improves the bond strength of fiberglass posts, regardless of the resin cement used (dual or self-adhesive) [19]. Future multidisciplinary efforts combining the knowledge from basic sciences such as Chemistry, Microbiology and Fluid Dynamics may lead to more effective antimicrobials and improved activation methods to bring them closer to the residual biofilm in the root canal system [29].

In summary, achieving a dentin substrate suitable for adhesion requires the selection of an irrigant that has a chemical effect on the dentin. Based on the results obtained in both studies, it can be inferred that evidence of a surface free of macroscopic debris does not imply the presence of a clean dentin substrate with open dentin tubules.

5. CONCLUSIONS

In both macroscopic and microscopic studies, EDTA irrigation generated the highest levels of cleaning regardless of the irrigation method used. The use of ultrasound and an endodontic brush did not produce significant changes in the cleaning levels. The trend in cleaning levels from the coronal to apical thirds was similar, with the highest levels of cleaning evident in the apical and middle thirds.

The findings of this in vitro study have important clinical implications for developing root canal cleaning strategies and protocols that not only ensure the success of adhesive procedures, but also safeguard the disinfection achieved in endodontic therapy.

ACKNOWLEDGMENT

Lamarx Laboratory, Faculty of Physics, Astronomy and Mathematics, National University of Córdoba. Laboratory of the Oral Biology Area. Faculty of Dentistry, National University of Córdoba.

AUTHORSHIP DECLARATION

All authors contributed significantly to this work and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study.

FUNDING

No funding was received for this study.

REFERENCES

- [1] Shahi S, Yavari HR, Rahimi S, Reyhani MF, Kamarroosta Z, Abdolrahimi M. A comparative scanning electron microscopic study of the effect of three different rotary instruments on smear layer formation. *J Oral Sci.* 2009;51(1):55–60.
- [2] Kokkas AB, Boutsoukis AC, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. *J Endod.* 2004;30(2):100–2.
- [3] Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. *Br Dent J.* 2014;216(6):299–303.
- [4] Susila A, Minu J. Activated irrigation vs. conventional non-activated irrigation in endodontics: a systematic review. *Eur Endod J.* 2019;4(3):96.
- [5] Dioguardi M, Di Gioia G, Illuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: different methods to improve efficacy and related problems. *Eur J Dent.* 2018;12(3):459–66. doi: 10.4103/ejd.ejd_56_18.
- [6] George R. Evaluation of the evidence of effectiveness of ultrasonic activated irrigation for root canal treatment. *Evid Based Dent.* 2019;20(3):83–4.
- [7] Moreno-Sánchez PL, Ramírez-Álvarez M, Ayala-Ham ADR, Silva-Benitez EDL, Casillas-Santana MÁ, Leyva del Rio D, et al. Dental surface conditioning techniques to increase the micromechanical retention to fiberglass posts: a literature review. *Appl Sci.* 2023;13(14):8083.
- [8] Schwartz RS. Adhesive dentistry and endodontics. Part 2: bonding in the root canal system—the promise and the problems: a review. *J Endod.* 2006;32(12):1125–34.
- [9] Van Meerbeek B, Yoshihara K, Van Landuyt K, Yoshida Y, Peumans M. From Buonocor's pioneering acid-etch technique to self-adhering restoratives. A status perspective of rapidly advancing dental adhesive technology. *J Adhes Dent.* 2020;22(1):7–34.
- [10] Gomes BPFA, Aveiro E, Kishen A. Irrigants and irrigation activation systems in endodontics. *Braz Dent J.* 2023;34(4):1–33. doi: 10.1590/0103-6440202305577.
- [11] Perdigão J. Dentin bonding—variables related to the clinical situation and the substrate treatment. *Dent Mater.* 2010;26(2):e24–31.
- [12] Breschi L, Mazzoni A, De Stefano Dorigo E, Ferrari M. Adhesion to intraradicular dentin: a review. *J Adhes Sci Technol.* 2009;23(7–8):1053–83.
- [13] Basrani B, Haapasalo M. Update on endodontic irrigating solutions. *Endod Top.* 2012;27(1):74–102. doi: 10.1111/etp.12031.
- [14] Darda S, Madria K, Jamenis R, Heda A, Khanna A, Sardar L. An in-vitro evaluation of effect of EDTAC on root dentin with respect to time. *J Int Oral Health.* 2014;6(2):22.
- [15] Kazeminejad E, Niazi M, Geraili E, Rajabi A, Mohammadi M. Effect of endodontic irrigation on mineral content of root canal dentine: a systematic review and meta-analysis. *Eur Endod J.* 2023;8(2):114.
- [16] Singh S, Arora V, Majithia I, Dhiman RK, Kumar D, Ather A. Comparative evaluation of 15% ethylenediamine tetra-acetic acid plus cetavlon and 5% chlorine dioxide in removal of smear layer: a scanning electron microscope study. *Contemp Clin Dent.* 2013;4(1):67–70.
- [17] Mankeliya S, Singhal RK, Gupta A, Pathak VK, Kushwah A. A comparative evaluation of smear layer removal by using four different irrigation solutions like root canal irrigants: an in vitro SEM study. *J Contemp Dent Pract.* 2021;22(5):527–31.
- [18] Kaushal R, Bansal R, Malhan S. A comparative evaluation of smear layer removal by using ethylenediamine tetraacetic acid, citric acid, and maleic acid as root canal irrigants: an in vitro scanning electron microscopic study. *J Conserv Dent.* 2020;23(1):71–8.
- [19] Vrebac M, Mohenski D, Sevar EK, Janković B, Bergman L, Ivanišević A. The impact of different irrigation protocols on the bond strength of self-adhesive resin cement to radicular dentin. *Acta Stomatol Croat.* 2024;58(3):233.
- [20] Tzolomitis P, Diamantopoulou S, Papazoglou E. Contemporary concepts of adhesive cementation of glass-fiber posts: a narrative review. *J Clin Med.* 2024;13(12):3479.
- [21] Al-Hadlaq SM, Al-Turaiki SA, Al-Sulami U, Saad AY. Efficacy of a new brush-covered irrigation needle in removing root canal debris: a scanning electron microscopic study. *J Endod.* 2006;32(12):1181–4.
- [22] Salman MI, Baumann MA, Hellmich M, Roggendorf MJ, Termaat S. SEM evaluation of root canal debridement with Sonicare CanalBrush irrigation. *Int Endod J.* 2010;43(5):363–9.
- [23] Narmatha VJ, Thakur S. Evaluation of manual dynamic activation, passive ultrasonic irrigation and canalbrush on smear layer removal: a scanning electron microscopic study. *Int J Adv Res.* 2015;3(3):390–400.
- [24] Marella KM, Sampathi NR, Pavani LN, Manduru CS, Moosani GK. An in vitro evaluation of smear layer removal with non-activated self-adjusting file, XP-Endo Finisher and rotary canal brush: a scanning electron microscopic study. *J Dr YSR Univ Health Sci.* 2022;11(1):17–22.
- [25] El-Halaby OI, Diab A, Morsy DA. The efficacy of brush file used for irrigation activation versus passive ultrasonic irrigation in terms of smear layer removal and canal cleanliness in single rooted teeth: a comparative in-vitro study. *Adv Dent J.* 2024;6(4):675–90.
- [26] Protogerou E, Arvaniti I, Vlachos I, Khabbaz MG. Effectiveness of a canal brush on removing smear layer: a scanning electron microscopic study. *Braz Dent J.* 2013;24:580–4.
- [27] da Costa Lima GA, Aguiar CM, Câmara AC, Alves LC, Dos Santos FAB, 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.
- [28] Tekinarslan D, Erkal D, Koç S, Er H, Er K. Effects of irrigation activation techniques on root canal walls after post-space preparation: a scanning electron microscopy study. *Rev Port Estomatol Med Dent Cir Maxilofac.* 2024;65:121–8.
- [29] Boutsoukis C, Arias-Moliz MT. Present status and future directions - irrigants and irrigation methods. *Int Endod J.* 2022 May;55(3):588–612. doi: 10.1111/iej.13739.