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ORIGINAL ARTICLE

Histological evaluation of root canal cleaning by different final irrigation protocols

ABSTRACT

Aim: *This study aimed to evaluate in vitro the ability to remove pulp tissue from the mesial root canals of mandibular molars using three different protocols for the final activation time of irrigation with the Easy Clean (EC) rotary instrument.*

Materials and Methods: *Thirty mandibular molars with vital pulp were instrumented with the ProDesign Logic rotary system and divided into three experimental groups according to the protocol for the final activation time of the irrigants (n=10). Group EC1: the final rinse was performed with 5 mL of 2.5% sodium hypochlorite (NaOCl), followed by 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) and another 5 mL of 2.5% NaOCl solution, with each rinsing agent activated in 3 cycles of 20 seconds. Group EC2 used the same sequence of solutions as group EC1, with each irrigant activated in 6 cycles of 20 seconds. In group EC3, the operation technique and the sequence of solutions were the same as in groups EC1 and EC2, with each rinsing agent being activated in 9 cycles of 20 seconds. At the end, samples from all groups were washed with 20 mL of distilled water using a NavigTip 30-G syringe and needle. The samples were fixed in 10% formaldehyde, cut into micrometers, fixed on histology slides and stained with hematoxylin-eosin (HE). The total area of the canal and remaining tissue was determined using the Image J program to determine the percentage of remaining pulp.*

Results: *In the cervical third, all groups had similar results. In the middle and apical thirds, EC1 and EC2 had similar percentages of pulp remnants and differed from EC3 ($p<0.05$).*

Conclusions: *It can be concluded that the removal of pulp tissue increases with longer contact time of the NaOCl.*

Ana Claudia de Carvalho Cândido[1]
Carlos Eduardo da Silveira Bueno[1]
Ana Grasiela Limoeiro[2*]
Wayne Martins Nascimento[1]
Augusto Shoji Kato[2]
Elesandro José Cândido[3]
Rina Andrea Pelegrine[1]

[1]Department of Endodontics, São Leopoldo Mandic Dental Research Center, Campinas, Brazil.
[2]Department of Dentistry, Endodontics and Dental Materials, Bauru Dental School, University of São Paulo, Bauru, SP, Brazil.
[3]University Center of Caratinga, UNEC, Brazil.

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Corresponding Author
Ana Grasiela Limoeiro | Dep. of Dentistry, Endodontics and Dental Materials, Bauru Dental School, University of São Paulo, Bauru, SP | Brazi
Phone +55 (73)988741956 | Email: grasielalimoeiro@gmail.com

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Introduction

Effective chemical-mechanical preparation in endodontic therapy is crucial for removing both infected or uninfected hard and soft tissues as well as microbial biofilms, with endodontic files and irrigation solutions (1). The advent of rotary or reciprocating endodontic instruments has enhanced the speed of root canal shaping; however, it has not necessarily improved the thoroughness of cleaning (2). The inability of these instruments to touch the entire canal surface (3) resulting in uninstrumented areas and consequently to the failure to remove contaminated or uncontaminated organic tissue (2). Clinically, the thorough removal of pulp tissue is vital, particularly within anatomically complex regions like isthmuses and recessed areas. Isthmuses are narrow connections between two canals, prone to harbor microorganisms and debris. Their challenging accessibility complicates cleansing efforts, potentially compromising endodontic treatment success if efficient disinfection is not achieved (4, 5).

Endodontic irrigation plays a key role in cleaning both the main canal and the isthmus. To this end, it is important that the irrigant penetrates the entire length of the root canal, especially in areas inaccessible to endodontic instruments (6). To achieve better efficacy, irrigants should remain in direct contact with the entire root canal wall for a longer period. In this way, it is possible to improve cleaning efficiency (7).

Factors such as the limited contact area, volume, and renewal of the irrigant solution, individually or in combination, may limit the effectiveness of NaOCl in its tissue-dissolving property (8). NaOCl is most effective up to the first 5 mm, where the canal is wide and allows for solution exchange, whereas in the apical region and in narrower canals, tissue dissolution is less efficient (9). The devices and systems for agitating the irrigant in the canal aim to increase the efficiency of the irrigant by potentiating its action. The Easy-Clean (EC) system (Bassi/Endo, Belo Horizonte, Brazil) is a plastic instrument based on acrylonitrile-butadiene-styrene polymer, with a tip diameter of 25 and a taper of 04, driven by an automatic motor in a reciprocating or rotating motion at speeds between 500 to 15.000 rpm to agitate the irrigant mechanically (10). Histological analysis is a method to measure the removal of the remaining pulp tissue after cleaning and shaping the root canal (11). Due to their anatomical complexity, higher prevalence of isthmuses, mesial roots of mandibular molars were select for this study. The aim of this study was to histologically analyze the capacity of pulp tissue removal from the mesial root canals of mandibular molars using the Easy Clean instrument at three different activation times. The hypothesis was that the different irrigation times would be similar in terms of pulp tissue removal.

Materials and Methods

Study design

This study was conducted as a laboratory-based *in vitro* experiment using extracted human mandibular molars to simulate clinical endodontic conditions. Based on pilot tests, the sample size calculation to achieve a power of 95% and a significance level of 5% (alpha, type I error) was conducted using the G*Power 3.1.9.4 program. The effect size was 0.760, indicating the need for at least 7 mesial roots of mandibular molars per group. Due to the risk of sample loss, an additional 20% was employed, resulting in 10 specimens per group.

Sample selection

This study was approved by the São Leopoldo Mandic research ethics committee (CAAE: 0589119.2.0000.5374). Thirty mandibular molars were used. All teeth were diagnosed as having a vital pulp (positive response to the cold test) and were indicated for exodontia. The teeth were explicitly donated by the patients and immediately placed in distilled water at the time of extraction and stored in a freezer at -18 ˚C for a maximum period of 3 months.

Inclusion criteria

The inclusion criteria were teeth with vital pulp, fully formed roots, mesial canals with distinct and independent pathways, intact

apices, no previous endodontic treatment, curvatures between 10° and 20° according to the method of Schneider (12).

Exclusion criteria
Exclusion criteria were teeth with calcifications, lacerations, root resorption, mesial canals exhibiting confluence, internal or external perforations in the furcation area, root caries and previous endodontic treatment.

Preparation of the teeth
After endodontic access, a #10 K file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the mesiobuccal and mesiolingual canals until its tip was visible in the apical foramen. A silicone stop was placed at the tip of the corresponding cuspid to obtain the first canal measurement. The occlusal surface was ablated with a double-sided diamond disk No. 7020 (KG Sorensen, Barueri, Brazil) attached to a straight handpiece at low speed and under constant water cooling, obtaining a length of 19 mm. The working length (WL) for instrumentation was set 1 mm up to the foramen (18 mm).
The distal root of the specimen was cut under cooling with a double-sided diamond disk # 7020, discarded, and the remaining coronal part was sealed with composite resin Z350 (3M, Sumaré, Brazil).
To simulate a clinical situation, the teeth were inserted into a PVC container filled with condensation silicone (Coltene, Altstaetten, Switzerland) and treated according to the manufacturer's recommendations. This allowed the tooth to be fixed and a closed irrigation and aspiration system to be formed.

Preparation of the root canal
The canals were instrumented by the same operator using the ProDesign Logic system (Bassi/Easy) in conjunction with the Easy Endo SI motor (Bassi/Easy). A hand file type K #10 (Dentsply) was inserted up to the apical foramen, followed by a file 25/01 at 350 rpm and 1 N torque to achieve patency of the foramen. Then, instrument 25/.05 was inserted at 600 rpm and 1.5 N torque with smooth in-and-out movements with a maximum amplitude of 3 mm until WL was reached. Apical shaping was performed with files 30/.01, 35/.01 and 40/.01 (Bassi/Easy) at 350 rpm and 1 N torque.
At each instrument change, the K #10 file was used to confirm patency. The canals were flushed with 5 mL of 2.5% NaOCl (Asfer, São Caetano do Sul, Brazil) at each file change. For this purpose, a NavigTip 30-G syringe and needle (Ultradent Products Inc, South Jordan, UT) was positioned up to WL, with a total volume of approximately 30 mL of irrigation fluid. Final rinsing was performed with 10 mL of distilled water.

Experimental protocol for the final irrigation
At the end of the chemical-mechanical preparation, the teeth were randomly divided into three groups according to the experimental time protocol (n=10) (Figure 1).
The Easy Clean instrument was inserted

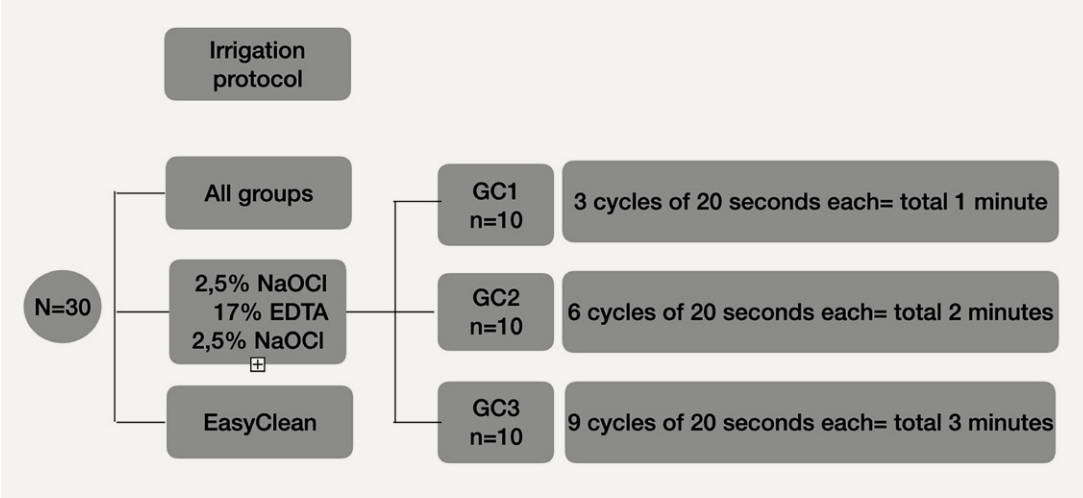


Figure 1
Organizational chart of the experiment.

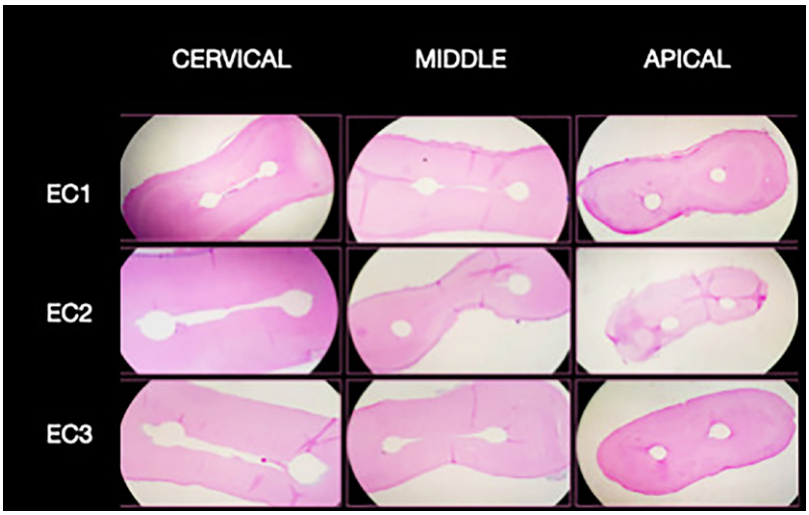


Figure 2
Histological findings of the examined areas with pulp remnants, 4x magnification.

into the WL and rotated with an Easy motor at a speed of 950 rpm. At the end, the canals were rinsed with 20 mL of distilled water using a NavigTip 30-G syringe and needle (Ultradent). The canals were dried with absorbent paper and specimens from all groups were stored in a 10% formaldehyde solution (Anidrol, Diadema, Brazil) without coronal sealing to ensure penetration of the formaldehyde solution throughout the length of the root canal.

Histomorphology analysis

Samples were fixed in 10% buffered formaldehyde for 24 hours and then demineralized in a 20% formic acid solution (Merck, Darmstadt, Germany) for 10 days. After the demineralization step, samples were dehydrated in ascending alcohol chain (70%, 80%, 90%, and absolute), diaphanized in methyl salicylate, and embedded in paraffin. The blocks were mounted on a microtome and sectioned to obtain histological cross sections with a thickness of 4 µm of the cervical, middle, and apical thirds of the root. The obtained sections were placed on glass slides and stained with the hematoxylin-eosin technique to analyze the main histological aspects. The slides with depressions and isthmuses representative of the analyzed areas were selected. The slides were viewed with a Nikon Eclipse Ci-S microscope (Nikon Corporation, Tokyo, Japan) and photographed with a camera attached to this device, at an initial 4x magnification for viewing and overall analysis of the root canal and at a 10x magnification for analysis of the canal alone.

Images were captured, stored on a USB flash drive, and opened in Image J software (Bethesda, Maryland, USA). The cursor was used to delineate and determine the total area of the canal. Then, using the same procedure, the area of the remaining tissue was delineated and determined. In the presence of fragmented areas, all were delineated and added at the end to obtain the final total area of remaining tissue. The percentage

Graph 1
Comparison between the means obtained from each of the studied measurements among the groups.

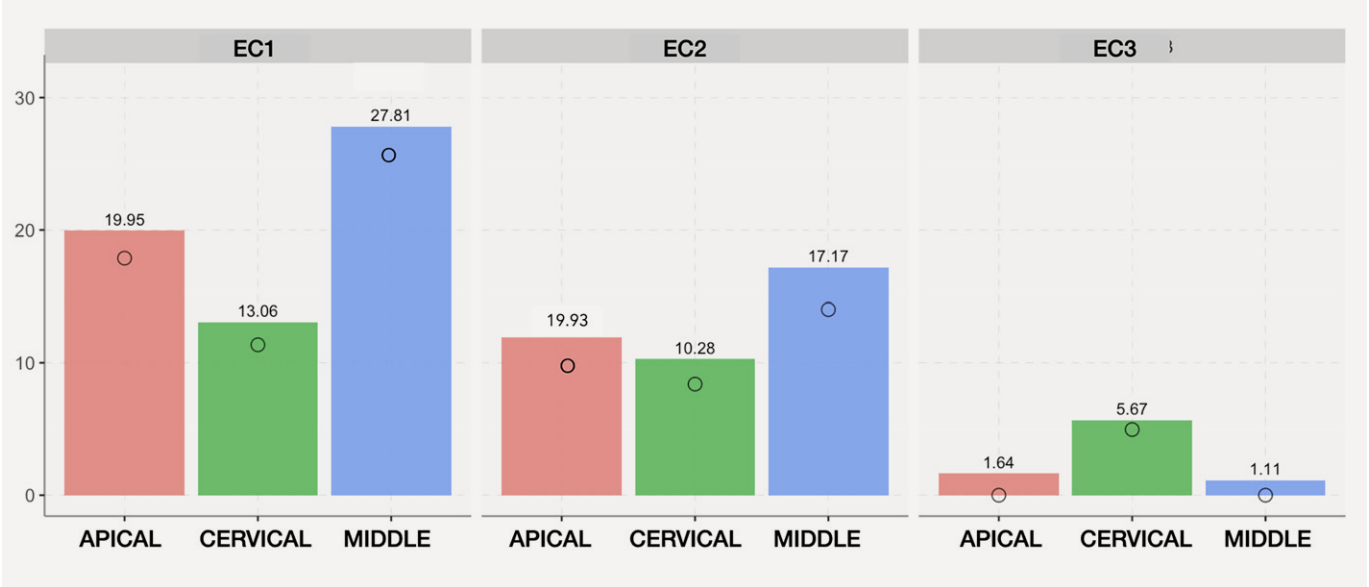


Table 1

Mean and standard deviation of the percentage of pulp remnants related to the analyzed group and the respective third (Wilcoxon Mann-Whitney U test for p<0.05

Group	EC1	EC2	EC3
Cervical	13.06^{aA} (8.46)	10.28^{aA} (5.21)	5.67^{aA} (4.65)
Middle	27.81^{aA} (12.94)	17.17^{aA} (10.27)	1.11^{bA} (1.58)
Apical	19.95^{aA} (9.54)	11.93^{aA} (7.42)	1.64^{bA} (2.36)

Lowercase letters indicate the statistical difference between the thirds of each experimental group (within a line).

Capital letters indicate the statistical difference between the thirds within each experimental group (within a column).

of remaining tissue was calculated using the following formula:

$$X\% \text{ (percentage of remaining tissue)} = \frac{\text{area of remaining tissue x100}}{\text{Total area of the canal}}$$

These data were tabulated and statistically analyzed in an Excel spreadsheet (Microsoft Corporation, 2016 Redmond, USA). All evaluations were performed by the same calibrated investigator.

Graph 2
Comparisons of tissue remnants across the cervical, middle, and apical thirds.

Statistical analysis

Two comparative analyzes were performed: Between experimental groups (EC1, EC2, and EC3) in each third (cervical, middle, and apical) and within each group in relation to the analyzed third (cervical, middle, and apical). The data obtained were subjected to the nonparametric Wilcoxon Mann-Whitney U test. The analysis was performed using R. software (formerly AT &T, now Lucent Technologies). The assumed significance level was 5% (p<0.05).

Results

Histological Findings

Analysis of Remaining Tissue in Different Root Thirds

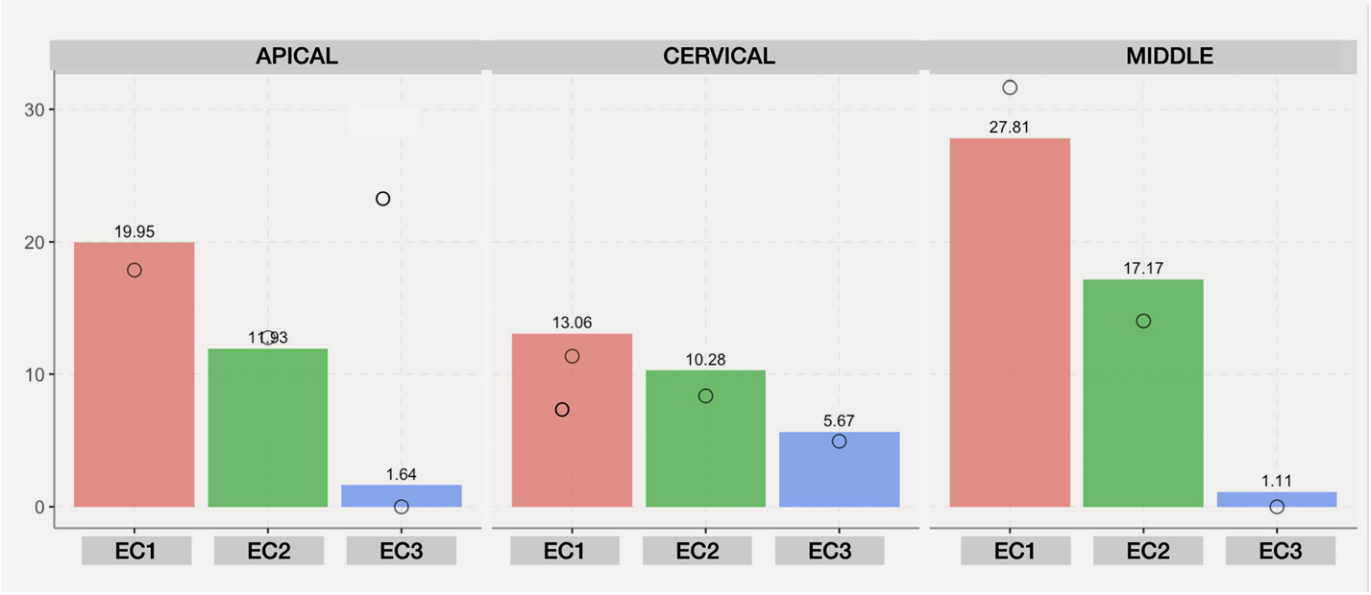
Regarding the analysis between groups in the cervical third, all showed similar results. In the middle and apical thirds, groups EC1 and EC2 had similar percentages of pulp remnants and differed from group EC3, in which the percentage of tissue remnants was lower (p<0.05) (Figure 2 and Graph 1).

Comparative Analysis Between Groups

In all groups, there was no difference between the thirds of the same group (p>0.05) (table 1 and Graph 2).

Discussion

In the present study, the cleaning ability of root canals in mesial roots of mandibular molars was evaluated after three different agitation times of NaOCl 2.5% and EDTA 17%. The null hypothesis tested was rejected



as a statistically significant difference was found between the experimental groups.
One major clinical implication is that personalized irrigation protocols can enhance pulp tissue removal, particularly in complex canal anatomies where residual tissue is common (13). Clinicians might consider adapting their strategies based on the specific characteristics of each case, considering the anatomical challenges presented by isthmuses and recessions. By optimizing agitation times, practitioners can potentially improve treatment outcomes and reduce the risk of endodontic failures caused by inadequate cleaning (14).
To ensure the reliability of the results, several methodologies were employed to minimize bias in the histological analysis. The study focused on standardizing variables, allowing the inclusion of teeth with similar internal anatomy, size, and curvature. This careful selection process mitigated variability that could confound results. Additionally, teeth were all preserved under controlled conditions (frozen) to maintain pulp structure integrity, further enhancing the reliability of histological evaluations. Different methodologies have been proposed to evaluate the outcome of flushing agent activation in relation to canal cleaning: bacteriological culture methods (2), scanning electron microscopic analysis (15), optical microscopy (16), histological sections (17), micro-CT (18) and diaphanization (19). In the present work, the methodology of histological analysis was used to evaluate the capacity of pulp tissue removal by the irrigant in mesial canals of mandibular molars. The methodology used aimed to standardize variables that could in any way affect the results obtained. Therefore, teeth with similar internal anatomy, size and degree of curvature were selected.
All teeth were diagnosed as having a vital pulp (positive response to the cold test) and were stored in the freezer for a maximum period of 3 months. This procedure aimed to keep the pulp structure intact through the freezing method, in which microbial activity is paralyzed and the rate of chemical reactions is significantly reduced, which is an effective method for tissue preservation (20).
In this study, a single chemical agitation system called Easy Clean was used, mechanically driven in a rotary motion at 950 rpm (10,15). The Easy Clean tips were positioned in the WL (21) and acted directly on the last apical millimeters.
The hypothesis and focus of the study were whether the agitation time of the 2.5% NaOCl would result in more pulp tissue removal, especially in the uninstrumented areas of the canal, such as the recessions and isthmus. The cervical, middle, and apical thirds were sectioned with a thickness of 4 µm, and those showing these anatomical features in each third of the canal were selected from each specimen. Based on the results obtained, we can conclude that the pulp tissue dissolution in the middle and apical thirds of the canal was lower than in the 3-minute excitation group by the time of 2-minute excitation. This result can be explained by the fact that the strength of tissue dissolution is related to other factors, such as the dentin structure, the surface of the contact area, and the exposure time of the irrigant to the remaining pulp tissue (9). The dissolution time of the tissue is directly related to the contact time, and EDTA have a negative effect on the action of NaOCl (22). Incorporating EDTA into irrigation protocols is crucial for removing the smear layer and ensuring thorough cleaning. However, its effects on dentin, particularly concerning demineralization and collagen modifications, must be taken into account (23). All irrigation protocols have been shown to significantly decrease dentin microhardness, highlighting the important relationship between these protocols and dentin properties. Effective root canal cleaning must balance these factors while preserving dentin integrity. Additionally, research indicates that certain irrigation techniques, along with the use of calcium hydroxide, can notably impact the microhardness of root canal dentin (24). Dentin has a high concentration of carbonates, which promote neutralization of the acid-base effect that occurs during pulp tissue saponification (25). Thus, neutralization of the NaOCl may occur, rendering the substance ineffective in terms of its dissolution properties. Therefore, longer renewal cycles and more time are required to achieve the desired effect. Regarding the percentage of tissue in each third within the group, similar results were observed, showing that the effect

of the substance is uniform inside the canal. An important clinical observation related to the results obtained was the presence of remaining pulp tissue in group EC1. A total of 30 mL of NaOCl 2.5% was used throughout the mechanical preparation process, supplemented by stirring for 1 minute with the Easy Clean System. This study suggests that the removal of pulp tissue from the root canal, particularly in the isthmus and recession areas, is dependent on the irrigant and requires further study and the development of more appropriate protocols.

The results of this study suggest that the activation time and the durability of the rinsing substance influence the process of organic tissue removal from inside the root canal, especially in non-instrumented areas such as isthmuses and recessions.

Despite the positive implications of the study, it is important to acknowledge some limitations. The sample size and the specific inclusion criteria may limit the generalizability of the findings. The study assessed only mesial roots of mandibular molars; therefore, results might differ in other tooth types or in cases with more complex canal systems. Furthermore, histological analysis primarily captures the immediate effects of the irrigants on tissue removal, without necessarily reflecting long-term outcomes related to canal disinfection and healing.

Future research should explore various irrigants and agitation techniques, as well as the optimal concentrations and volumes required for effective cleaning in diverse anatomical scenarios. Long-term studies evaluating the healing outcomes of treated canals and the impact of irrigation protocols on the success rate of endodontic treatments would further advance the field. Additionally, exploring combined approaches that integrate chemical and mechanical cleaning may yield enhanced results in tissue removal and canal disinfection.

Conclusion

In conclusion, this study highlights that longer irrigant activation times enhance the removal of pulp tissue from root canals, indicating a need for endodontic practitioners to refine their irrigation protocols to ensure optimal cleaning, especially in challenging anatomical areas.

Clinical Relevance

The findings emphasize the significance of irrigation technique in endodontics and provide practical insights for improving the procedural outcomes in root canal therapy.

Conflict of Interest

None.

Acknowledgments

None.

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