



# ***In vitro* experimental study comparing continuous and intermittent irrigation protocols: influence of sodium hypochlorite volume and contact time on tissue dissolution**

Alfredo Iandolo<sup>1,2</sup> , Dina Abdellatif<sup>1,2</sup> , Davide Mancino<sup>3</sup> , Gwenael Rolin<sup>1,4</sup> , Camille Coussens<sup>5</sup> , Aurelian Louvrier<sup>2,5,6</sup> , Felipe G Belladonna<sup>7</sup> , Edouard Euvrard<sup>1,2</sup> , Emmanuel João Nogueira Leal da Silva<sup>7,8,\*</sup>

<sup>1</sup>Faculty of Dental Surgery, University of Franche-Comte, CHU Besançon, Besançon, France

<sup>2</sup>Laboratoire Sinergies EA 4662, University of Franche-Comté, Besançon, France

<sup>3</sup>Faculty of Dental Surgery, Federation of Medicine Translational of Strasbourg and Federation of Materials and Nanoscience of Alsace, University of Strasbourg, Strasbourg, France

<sup>4</sup>INSERM CIC-1431, CHU Besançon, Besançon, France

<sup>5</sup>Plateforme I3DM (Impression 3D Médicale), CHU Besançon, Besançon, France

<sup>6</sup>Chirurgie maxillo-faciale et stomatologie, CHU Besançon, Besançon, France

<sup>7</sup>Department of Endodontics, School of Dentistry, Fluminense Federal University, Rio de Janeiro, Brazil

<sup>8</sup>Department of Endodontics, School of Dentistry, Grande Rio University, Rio de Janeiro, Brazil

## **ABSTRACT**

**Objectives:** This study aimed to evaluate whether continuous irrigation with larger volumes or allowing sodium hypochlorite (NaOCl) resting time is more critical for pulp tissue dissolution using a controlled artificial root canal system.

**Methods:** A three-dimensional printed artificial root canal with a lateral canal in the apical third was fabricated. Standardized bovine pulp tissue specimens were inserted, and three irrigation protocols were tested: group A (continuous NaOCl irrigation at 1 mL/min via syringe pump), group B (intermittent NaOCl irrigation with 0.1 mL and a 3-minute resting period), and group C (control, saline irrigation). The time for complete dissolution and the total NaOCl volume were recorded.

**Results:** Complete dissolution occurred in groups A and B, with significant differences in NaOCl volume and time ( $p < 0.05$ ). In group A, complete dissolution was consistently observed after the 6th irrigation cycle, corresponding to a total NaOCl volume of  $6.0 \pm 0.66$  mL per test. The average time required for complete dissolution in this group was  $6 \pm 0.66$  minutes. In group B, complete dissolution occurred after the 4th cycle, with a total NaOCl volume of  $0.4 \pm 0.06$  mL per test and a mean dissolution time of  $12.6 \pm 1.8$  minutes.

**Conclusions:** NaOCl volume and exposure time significantly influence pulp tissue dissolution.

**Keywords:** Endodontics; Sodium hypochlorite; Tissue dissolution

**Received:** April 17, 2025 **Revised:** July 15, 2025 **Accepted:** August 5, 2025

## **Citation**

Iandolo A, Abdellatif D, Mancino D, Rolin G, Coussens C, Louvrier A, Belladonna FG, Euvrard E, Silva EJNL. *In vitro* experimental study comparing continuous and intermittent irrigation protocols: influence of NaOCl volume and contact time on tissue dissolution. Restor Dent Endod 2025;50(4):e36.

## **\*Correspondence to**

Emmanuel João Nogueira Leal da Silva, DDS, PhD

Department of Endodontics, School of Dentistry, Grande Rio University, Rua Heróides de Oliveira, 61/902, Icaraí, Niterói, RJ 24230-230, Brazil  
Email: [nogueiraemmanuel@hotmail.com](mailto:nogueiraemmanuel@hotmail.com)

© 2025 The Korean Academy of Conservative Dentistry

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## INTRODUCTION

Sodium hypochlorite (NaOCl) is widely recognized as an effective irrigant in endodontic treatment due to its potent tissue-dissolving and antimicrobial properties [1]. However, its efficacy depends on several interrelated factors, including concentration, temperature, flow dynamics, canal anatomy, volume, and contact time with the root canal system [2]. While higher concentrations and temperatures enhance its tissue-dissolving ability [3], they also increase cytotoxicity and the risk of extrusion beyond the apex [4]. Similarly, irrigation volume and duration play critical roles in tissue dissolution and debris removal—greater volumes improve flushing action, while contact sustains chemical efficacy [5,6]. As advancements in instrumentation have made canal preparation faster and more efficient, optimizing these variables is essential for safe and effective root canal disinfection.

The evolution of endodontic instrumentation, particularly with rotary and reciprocating systems, has significantly reduced the time required for canal shaping [7]. With fewer instruments and more efficient techniques, clinicians can now achieve effective canal preparation in a fraction of the time previously needed. This shift towards faster procedures has amplified the importance of the chemical phase of root canal treatment, emphasizing irrigation's role in cleaning areas beyond the reach of mechanical instrumentation [1]. The concept of 'shaping for cleaning' highlights the reliance on irrigating solutions, particularly NaOCl, to dissolve residual pulp tissue and biofilms [8]. As a result, optimizing the irrigation protocol—balancing time and volume—has become a key focus in recent years [1]. Understanding how these parameters influence NaOCl's efficacy is crucial to achieving thorough disinfection while maintaining efficiency and patient safety.

Despite advancements in endodontic instrumentation and irrigation techniques, the practical implications of balancing irrigation time and volume remain poorly understood. While the effects of NaOCl concentration and temperature have been extensively studied, fewer investigations have explored the interplay between these two factors [9,10]. This study addresses this gap by using an artificial root canal model designed to evaluate pulp tis-

sue dissolution under controlled irrigation conditions. By isolating and analyzing these parameters, it aims to provide evidence-based insights into their impact on tissue dissolution. Therefore, the aim of the current study was to evaluate whether continuous irrigation with larger volumes or allowing NaOCl resting time is more critical for pulp tissue dissolution. The null hypothesis tested was that there would be no difference in the tissue dissolution effectiveness between continuous and intermittent irrigation protocols using 3% NaOCl.

## METHODS

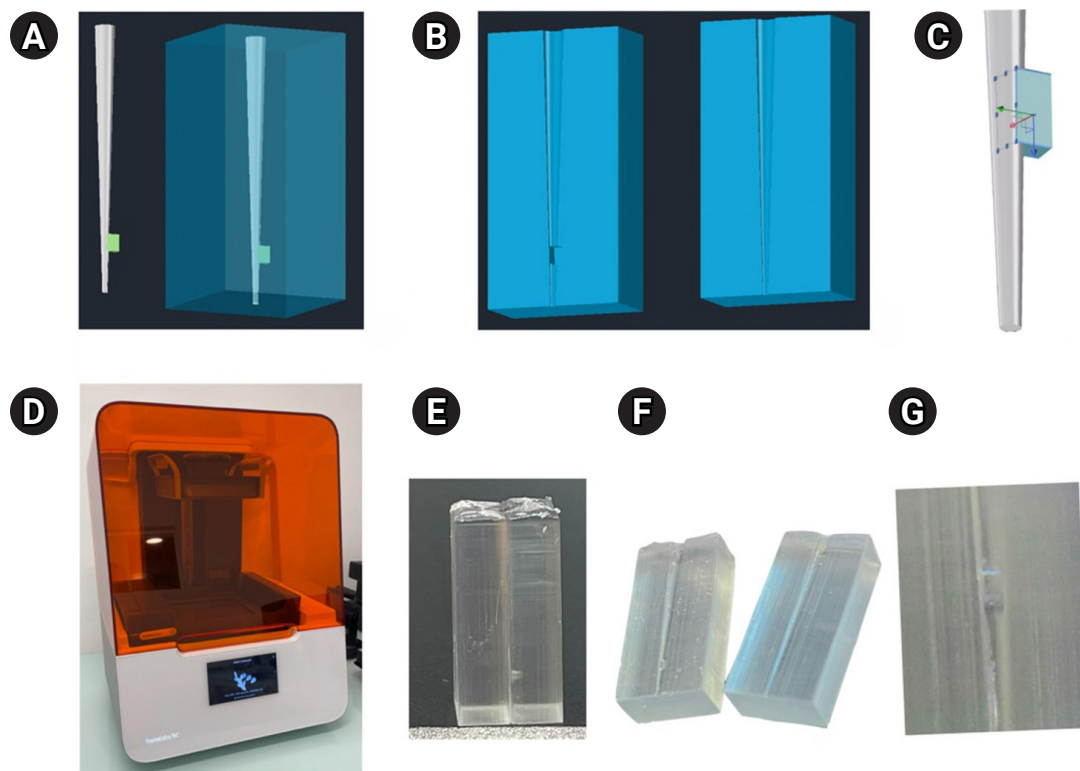
### Sample size calculation

The sample size calculation was based on data from previous studies. Power analysis was conducted using G\*Power 3.1 software for Windows (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) with a significance level of 0.05, statistical power of 80%, and an effect size of 0.4, following Cohen's guidelines. The analysis determined that 30 specimens (10 per group) were required to detect significant differences among the experimental groups. The sample size was also consistent with prior *in vitro* studies evaluating tissue dissolution [9,10].

### Artificial canal design and fabrication

The artificial canal system was designed using AutoCAD software (version 2024, Autodesk Inc.) and fabricated with a professional grade three-dimensional (3D) printer (Imprinter Form 3B; Formlabs, Somerville, MA, USA) at a resolution of 0.05 mm. A biocompatible transparent resin (Biomed Clear Resin, Formlabs) was used to enhance visualization during experiments. The canal design featured an apical diameter of 0.30 mm, a 6% taper, and a total length of 16 mm. Additionally, a 1-mm lateral space was positioned 3 mm from the apex, with dimensions of 0.48 mm at the minor base, 0.54 mm at the major base, and a depth of 0.5 mm [11].

For experimental feasibility, the canal system was printed in two separate parts, allowing precise insertion of pulp tissue into the lateral space. This design also enabled direct observation of tissue dissolution throughout the experiments, providing an optimal setup for evaluating irrigation protocol efficacy (Figure 1).



**Figure 1.** (A–C) Digital renderings of the artificial canal system, highlighting the small lateral canal positioned in the apical third. (D) The professional-grade three-dimensional (3D) printer (Imprinter Form 3B; Formlabs, Somerville, MA, USA) used to fabricate the canal system. (E) The completed 3D-printed canal model, fabricated using biocompatible transparent resin. (F) Demonstration of the model's ability to be opened and reassembled, allowing for the insertion of pulp tissue. (G) Close-up view of the small lateral canal located in the apical third of the model.

### Pulp specimen preparation

Bovine pulp tissue was collected post-slaughter from mandibular incisors of food-production animals, and the study was not classified as an animal study. Fresh, intact anterior mandibular teeth were extracted within 36 hours of slaughter and immediately stored in a 0.1% thymol solution to preserve tissue integrity. The crowns were sectioned at the cementoenamel junction using a high-speed diamond bur (Komet; Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) under continuous irrigation to prevent thermal damage. Pulp tissue was carefully extracted, rinsed with distilled water to remove any debris, and individually stored in 1.5-mL Eppendorf tubes containing 1 mL of distilled water at  $-20^{\circ}\text{C}$  until experimentation.

Before the experiments, pulp samples were thawed at room temperature for 30 minutes and incubated at  $37^{\circ}\text{C}$  in a water bath for 15 minutes to simulate clinical conditions. The specimens were then standardized to

$1 \times 0.5 \times 0.5$  mm under  $8\times$  magnification (SOM 32; Karl Kaps GmbH, Asslar, Germany), using millimeter graph paper and a surgical scalpel blade (Braun, Tuttlingen, Germany) for precise cutting and measurement. Each standardized specimen was then gently inserted into the lateral canal using micro-tweezers and positioned under stereomicroscopic visualization to guarantee accurate placement without compression, folding, or deformation. This meticulous approach ensured reproducible tissue volumes and uniform conditions across all samples.

### Groups and experimental setup

All samples were weighed using a high-precision micro-balance (accuracy of 0.00001 g, Explorer Semi-Micro; Ohaus Corporation, Parsippany, NJ, USA) at three stages: before the experiment, after pulp insertion, and after each irrigation cycle. The following groups were tested:

Group A: Pulp tissue was inserted into the lateral

space, and the canal was assembled. The apex was sealed with wax to simulate a closed system. NaOCl 3% (CanalPro; Coltene/Whaledent Inc., Cuyahoga Falls, OH, USA) was delivered at 1 mL/min using a syringe pump (Pilote A2; Fresenius Vial SAS, Brézins, France) with a 30-gauge side-vented needle (CanalPro Sideport Tips, Coltene/Whaledent Inc.) positioned 1 mm short of the working length. After irrigation, the canal was rinsed with 1 mL/min sterile saline, dried with sterile paper points, weighed, and opened for examination. This cycle was repeated until the pulp tissue was completely dissolved.

Group B: Pulp tissue was inserted into the lateral space, and the canal was assembled. The apex was sealed with wax to simulate a closed system. NaOCl 3% (0.1 mL) was manually delivered by an experienced operator using a syringe to fill the canal, followed by a 3-minute waiting period. The canal was then rinsed with sterile saline at 1 mL/min, dried with sterile paper points, weighed, and observed. This process was repeated until complete tissue dissolution.

Group C (control): The protocol for group B was fol-

lowed, but sterile saline was used instead of NaOCl.

A total of 10 canals were prepared per group (Figures 2 and 3), and 10 irrigation cycles were performed per experiment.

### Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. One-way analysis of variance was then performed to evaluate differences in pulp tissue dissolution and the time required for complete dissolution among the groups, followed by Tukey honestly significant difference *post hoc* analysis for pairwise comparisons. A significance level of 0.05 was adopted.

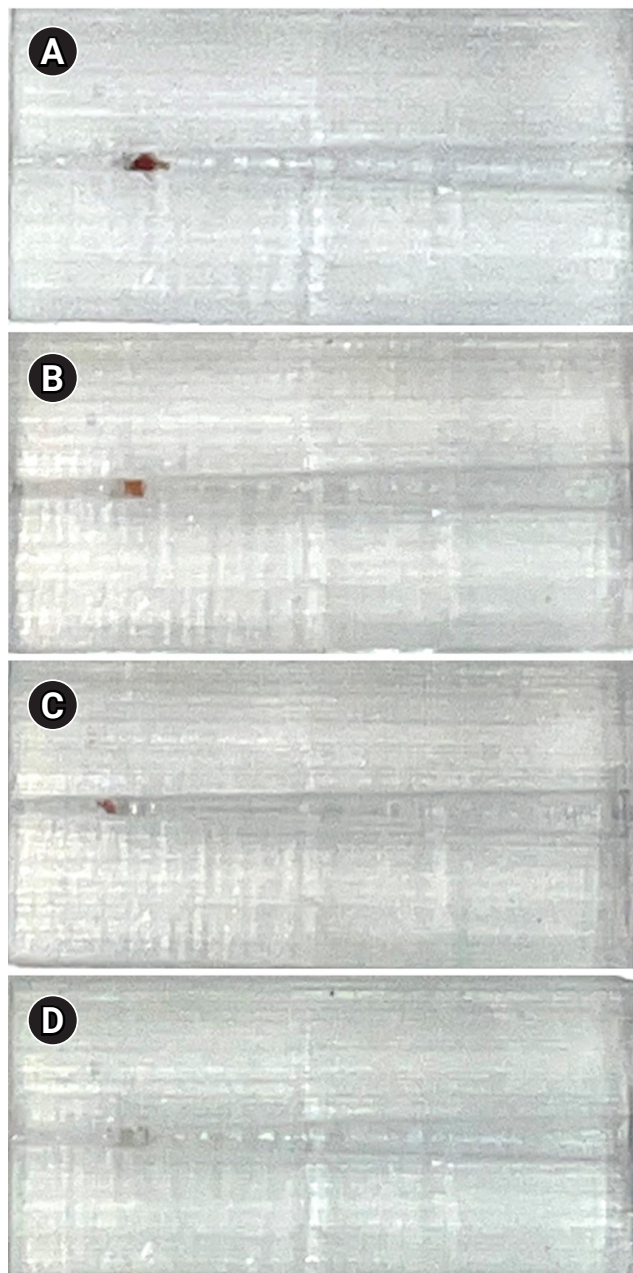
## RESULTS

Complete pulp tissue dissolution was achieved in all samples of groups A and B. However, the volume of NaOCl required and the time for complete dissolution differed significantly between the two groups ( $p < 0.05$ ). In contrast, no tissue dissolution was observed in any sample from group C, where sterile saline was



**Figure 2.** (A–C) High-precision microbalance (Explorer Semi-Micro; Ohaus Corporation, Parsippany, NJ, USA) used to weigh samples at different experimental stages. (D, E) Syringe pump (Pilote A2; Fresenius Vial SAS, Brézins, France) used for controlled delivery of sodium hypochlorite during the experiments.

used as the irrigant. In group A, complete dissolution was consistently observed after the 6th irrigation cycle, corresponding to a total NaOCl volume of  $6.0 \pm 0.66$  mL per test. The average time required for complete dissolution in this group was  $6.0 \pm 0.66$  minutes. In group B,



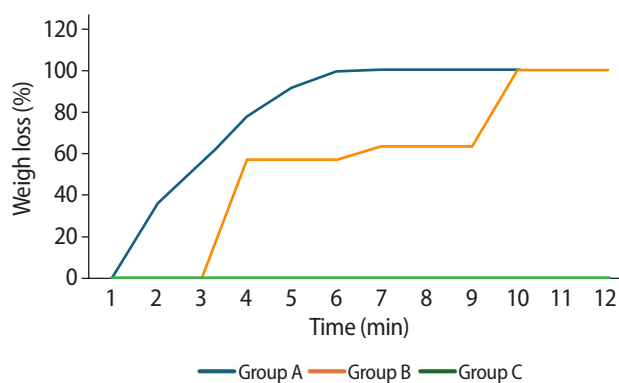
**Figure 3.** Visualization of pulp tissue within the small lateral canal located in the apical third of the artificial canal model. (A) Intact pulp tissue before irrigation. (B) Partially dissolved pulp tissue. (C) Near-complete tissue dissolution. (D) Complete dissolution of pulp tissue.

complete dissolution occurred after the 4th cycle, with a total NaOCl volume of  $0.4 \pm 0.06$  mL per test and a mean dissolution time of  $12.6 \pm 1.8$  minutes (Figure 4).

## DISCUSSION

The present study evaluated the impact of irrigation time and volume on NaOCl's tissue dissolution capacity, demonstrating that both factors significantly influence dissolution efficiency. Complete pulp dissolution was achieved in all samples exposed to NaOCl, though the required volume and time varied between groups. Group A, which received continuous irrigation at a flow rate of 1 mL/min, achieved complete dissolution in a shorter time but required a higher total volume of NaOCl. In contrast, group B, which utilized intermittent delivery with a resting phase, achieved the same outcome using significantly less NaOCl, albeit over a longer period. As a result, the null hypothesis was rejected. These findings highlight the dynamic interaction between irrigation parameters and chemical efficacy, emphasizing the need for refined irrigation protocols to enhance clinical outcomes.

Previous studies have established that NaOCl concentration, temperature, and contact time are critical factors influencing its tissue-dissolving capacity [9,10,12]. However, the relative importance of irrigation volume versus exposure time has been less explored. While the results of the present study may seem intuitive, they carry significant clinical relevance by demonstrating that



**Figure 4.** Graphic showing the percentage of pulp tissue weight loss over time in all groups. Group A, continuous 3% NaOCl at 1 mL/min; group B, intermittent 3% NaOCl, 0.1 mL with 3-min rest; and group C, saline control.

efficient tissue dissolution can be achieved through two distinct strategies: continuous irrigation with a larger volume or prolonged exposure with a minimal amount of irrigant. The findings suggest that while higher volumes accelerate tissue dissolution by continuously replenishing the active solution—and possibly through a flow and reflux effect—extended exposure to a smaller volume can achieve similar outcomes over a longer duration. These results align, to some extent, with the ‘shaping for cleaning’ concept, which emphasizes that mechanical instrumentation mainly facilitates irrigation rather than being solely responsible for debridement.

Advances in rotary and reciprocating instrumentation have reduced canal preparation time, increasing reliance on irrigation to remove residual pulp tissue and biofilms [7]. This study reinforces the need to optimize irrigation parameters, particularly volume and contact time, to maximize NaOCl efficacy. Although continuous irrigation ensures constant exposure to fresh irrigant, it is impractical for clinicians to maintain prolonged irrigation at high volumes during routine endodontic procedures. Therefore, this study highlights an alternative approach: even small volumes of NaOCl—sufficient to fill the root canal—can effectively dissolve tissue when given enough contact time. This strategy not only preserves chemical efficacy but also reduces operator fatigue and the excessive consumption of irrigant [9]. Optimizing irrigation protocols to balance efficiency, practicality, and clinician ergonomics is essential for improving the overall success of root canal disinfection [13–15].

A key strength of this study is the use of a controlled artificial canal system, which allowed for direct comparisons of different irrigation protocols under standardized conditions. The model included a lateral canal positioned in the apical third, with dimensions deliberately chosen to accommodate a measurable volume of pulp tissue and enable reproducible placement and dissolution assessment in this anatomically challenging area. Although these dimensions are slightly larger than typical anatomical lateral canals, they are consistent with previously validated experimental models [11] and ensured methodological reliability. Additionally, the use of bovine pulp tissue provided a consistent and reproducible substrate for dissolution analysis, ensur-

ing methodological reliability. The precise weighing of samples before and after each irrigation cycle further strengthened the accuracy of the findings, allowing for a quantifiable assessment of NaOCl’s effectiveness across different irrigation strategies. By eliminating anatomical variability and other uncontrollable clinical factors, this experimental design minimized potential biases and provided greater control over key variables, ensuring a more reliable evaluation of the specific effects of irrigation time and volume.

As with all *in vitro* studies, certain limitations must be acknowledged. The artificial canal system, while providing a controlled and reproducible environment, does not fully replicate the anatomical complexities of human root canals, such as variations in dentinal tubules, the presence of a smear layer, or biofilm interactions that could influence NaOCl penetration and efficacy [11]. Additionally, the experimental setup did not account for potential fluctuations in NaOCl concentration over time, nor the presence of organic and inorganic debris that could affect its reactivity. Another limitation is the absence of activation techniques, such as sonic or ultrasonic agitation, which are known to enhance irrigant effectiveness by improving penetration and tissue contact [13–15]. The use of a 3% NaOCl solution, although not the most concentrated option available, was deliberate, as it reflects a widely used commercial product and ensures quality control and stability throughout the experiments. Consequently, the findings primarily reflect the passive dissolution potential of NaOCl under controlled conditions. Nonetheless, the standardized methodology used in this study enabled precise isolation of the effects of irrigation time and volume, minimizing confounding variables and providing valuable insights into NaOCl dynamics that can inform future research and clinical practice.

Further research should investigate the combined effects of NaOCl volume, exposure time, and agitation methods to determine the most efficient irrigation protocol for clinical scenarios. Future studies using micro-computed tomography could provide a 3D assessment of tissue dissolution in anatomically complex canal systems. Additionally, evaluating alternative irrigants and NaOCl concentrations under similar conditions could further refine best practices for endodontic

irrigation. It is important to note that this study focused exclusively on tissue dissolution and did not assess NaOCl's effectiveness against bacterial biofilms. Since biofilm removal is a critical aspect of root canal disinfection, future research should explore how different irrigation protocols influence biofilm disruption and eradication. Incorporating microbiological models would offer a more comprehensive understanding of NaOCl's role in both tissue and biofilm dissolution, ultimately improving clinical recommendations.

## CONCLUSIONS

This study demonstrated that continuous irrigation with 3% NaOCl consistently dissolved pulp tissue in 6 minutes using 6.0 mL, whereas intermittent irrigation achieved complete dissolution in 12 minutes using only 0.4 mL. These findings suggest that both volume and contact time play critical roles in tissue dissolution, and clinicians may tailor irrigation protocols based on procedural priorities such as time efficiency or chemical conservation.

## CONFLICT OF INTEREST

Emmanuel João Nogueira Leal da Silva is the Associate Editor of *Restorative Dentistry and Endodontics* and was not involved in the review process of this article. The authors declare no other conflicts of interest.

## FUNDING/SUPPORT

The authors have no financial relationships relevant to this article to disclose.

## AUTHOR CONTRIBUTIONS

Conceptualization, Methodology: Iandolo A, Abdellatif D, Belladonna F, Silva EJNL. Data curation: Mancino D, Coussens C, Louvrier A, Euvrard E. Formal analysis: Iandolo A, Abdellatif D, Mancino D, Coussens C, Louvrier A, Belladonna F, Euvrard E, Silva EJNL. Funding acquisition, Project administration: Silva EJNL. Investigation: Mancino D, Rolin G, Coussens C, Louvrier A, Euvrard E. Supervision: Belladonna F, Silva EJNL. Writing – original draft: Iandolo A, Abdellatif D, Mancino D, Belladonna F, Silva EJNL. Writing – review & editing: Iandolo A, Abdellatif D, Belladonna F, Silva EJNL. All authors read and approved the final manuscript.

## DATA SHARING STATEMENT

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

## REFERENCES

1. Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T. Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;94:756-762.
2. Zehnder M. Root canal irrigants. *J Endod* 2006;32:389-398.
3. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. *J Endod* 2004;30:785-787.
4. Heling I, Rotstein I, Dinur T, Szewc-Levine Y, Steinberg D. Bactericidal and cytotoxic effects of sodium hypochlorite and sodium dichloroisocyanurate solutions in vitro. *J Endod* 2001;27:278-280.
5. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999;12:147-179.
6. Macedo RG, Wesselink PR, Zaccheo F, Fanali D, Van Der Sluis LW. Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH. *Int Endod J* 2010;43:1108-1115.
7. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. *J Endod* 2004;30:559-567.
8. De Deus G, Silva EJNL, Souza E, Versiani MA, Zuolo M. Shaping for cleaning the root canals: a clinical-based strategy. Cham, Switzerland: Springer Nature; 2022.
9. Iandolo A, Dagna A, Poggio C, *et al.* Evaluation of the actual chlorine concentration and the required time for pulp dissolution using different sodium hypochlorite irrigating solutions. *J Conserv Dent* 2019;22:108-113.
10. Del Carpio-Perochena A, Monteiro Bramante C, Hungaro Duarte M, *et al.* Effect of temperature, concentration and contact time of sodium hypochlorite on the treatment and revitalization of oral biofilms. *J Dent Res Dent Clin Dent Prospects* 2015;9:209-215.
11. Christofzik D, Bartols A, Faheem MK, *et al.* Shaping ability of four root canal instrumentation systems in simulated three-dimensional-printed root canal models. *PLoS One* 2018;13:e0201129.
12. Krah-Sinan AA, Adou-Assoumou M, Xavier Djolé S, Diemer F, Gurgel M. The effects of sodium hypochlorite on organic matters: influences of concentration, renewal frequency and contact area. *Iran Endod J* 2020;15:18-22.
13. van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive

ultrasonic irrigation of the root canal: a review of the literature. *Int Endod J* 2007;40:415-426.

14. Boutsoukis C, Arias-Moliz MT. Present status and future directions - irrigants and irrigation methods. *Int Endod J* 2022;55 Suppl 3:588-612.
15. Nagendrababu V, Jayaraman J, Suresh A, Kalyanasundaram S, Neelakantan P. Effectiveness of ultrasonically activated irrigation on root canal disinfection: a systematic review of in vitro studies. *Clin Oral Investig* 2018;22:655-670.