applied sciences

MDPI

Article

Comparative Evaluation of Ultrasonic and Sonic Irrigant Activation Systems: Assessing Extrusion Risk, Debridement, and Biofilm Removal in Distinct Apical Preparation Sizes

Sara Paixão [1], Pedro Sousa Gomes [1,2], Maria Helena Fernandes [1,2], Cláudia Rodrigues [1] and Liliana Grenho [1,2,*]

[1] BoneLab—Laboratory for Bone Metabolism and Regeneration, Faculty of Dental Medicine, University of Porto, 4200-393 Porto, Portugal
[2] Associated Laboratory for Green Chemistry-Network of Chemistry and Technology (LAQV-REQUIMTE), University of Porto, 4200-393 Porto, Portugal
* Correspondence: lgrenho@fmd.up.pt; Tel.: +351-220-901-100; Fax: +351-220-901-101

Abstract: This study aims to compare the effectiveness of ultrasonically and sonically activated irrigation in terms of extrusion risk, root canal debridement, and biofilm removal, considering distinct apical preparation sizes, through an *ex vivo* study in human teeth. Instrumented teeth, to an apical size of 35/.06 or 50/.06, were assigned to three different irrigation procedures: ultrasonically activated irrigation, sonically activated irrigation, and conventional manual irrigation. Apical extrusion risk was evaluated by quantifying irrigant and debris extrusion (n = 10/group). Debris evaluation and smear layer removal from the root canal wall were conducted by scanning electron microscopy (SEM) (n = 5/group), and the elimination of a mature biofilm of *Enterococcus faecalis* was assessed through resazurin assay and SEM (n = 10/group). For statistical analyses, Student's paired t-test and the ANOVA with post-hoc Tukey were used. Activated irrigations exhibited a higher risk of extrusion for the larger apical size, while the risk for manual irrigation remained independent of the apical size. Substantially fewer residual debris and smear layers were observed after the activation of the irrigant, and there was a notable enhancement in biofilm elimination compared to manual irrigation ($p < 0.05$). Notably, the effectiveness of both activated irrigations was more pronounced in root canals prepared to a size 50/.06, with ultrasonic activation showing enhanced improvements. The findings of this study underscore the substantial impact of both ultrasonically and sonically activated irrigation on the effectiveness of root canal disinfection and debridement. This impact is especially prominent with larger apical size, albeit accompanied by an increased risk of extrusion.

Keywords: debridement; disinfection; extrusion; sonic activated irrigation; ultrasonic activated irrigation

Citation: Paixão, S.; Gomes, P.S.; Fernandes, M.H.; Rodrigues, C.; Grenho, L. Comparative Evaluation of Ultrasonic and Sonic Irrigant Activation Systems: Assessing Extrusion Risk, Debridement, and Biofilm Removal in Distinct Apical Preparation Sizes. *Appl. Sci.* **2024**, *14*, 3904. https://doi.org/10.3390/app14093904

Academic Editor: Vittorio Checchi

Received: 28 March 2024
Revised: 23 April 2024
Accepted: 24 April 2024
Published: 3 May 2024

1. Introduction

In contemporary endodontic practice, the instrumentation and irrigation of the root canal system are widely considered critical procedures for successful treatment outcomes [1,2]. Within this context, a broad range of irrigant activating systems has been developed to maximize the efficacy of irrigants following mechanical instrumentation, aiming to improve the debridement and disinfection of the intricate root canal system [3]. This complexity may arise from oval extensions, isthmuses, and apical deltas, which make comprehensive cleaning a challenging task [4–6].

One of the most widely embraced techniques is the use of ultrasonic devices for promoting ultrasonically activated irrigation (UAI), allowing the irrigant delivery into non-instrumented areas and further improving its distribution into the apical area and complex anatomical structures [7]. UAI relies on cavitation and acoustic microstreaming generated by the oscillatory motion of a file, operating at ultrasonic frequencies, usually within the 25–30 kHz range [8,9]. On the other hand, sonic systems offer an alternative approach, operating at lower frequencies (usually within the 1–10 kHz range) than ultrasonic devices

and employing flexible, non-cutting polymer tips. These strategically designed flexible tips are intended to prevent modifications of the root canal's morphology and avoid unintended dentin removal [10,11].

Numerous studies have consistently demonstrated the increased efficacy of both ultrasonic and sonic activation devices when compared to conventional needle irrigation, regarding the removal of pulp tissue remnants [12], the eradication of microorganisms (both planktonic and biofilm) [13–15], and elimination of the smear layer and dentin debris [7,16]. However, despite these valuable insights, it is worth noting that these studies have employed various experimental setups, making it challenging to perform a comprehensive and comparative analysis of their findings. An additional layer of complexity arises from the varied use of distinct apical preparation sizes, further hindering the comparison of data and its direct translation into the clinical setting. The issue of apical preparation size remains a relevant matter in this context, carrying significant clinical implications. However, it remains a topic of ongoing debate as, while several studies endorse the significant enlargement of root canals to ensure effective irrigation, others express caution about larger preparation sizes, citing the potential risk of apical transportation and zipping issues [17,18].

Accordingly, and to the best of the authors' knowledge, no comprehensive study addressed simultaneously the different relevant clinical implications of different irrigant activating systems to different apical preparation sizes. Therefore, the present *ex vivo* study aimed to compare the effectiveness of ultrasonic activation, sonic activation, and conventional manual needle irrigation methods, specifically in the context of two different apical sizes: 35/.06 and 50/.06. Within the confines of a single experimental setting, this study evaluates the performance of these systems across multiple crucial parameters. This evaluation included the quantification of irrigant and debris extrusion, the extent of debris and smear layer removal, as well as the effectiveness in eliminating a mature biofilm of *Enterococcus faecalis*, a commonly isolated pathogen from root canal system in failing endodontic cases [19]. This study is thus structured to facilitate the critical comparison between distinct irrigant activation systems and assess the potential impact of apical preparation sizes on clinically relevant outcomes. The null hypothesis tested was that there are no differences among the irrigant activation systems (ultrasonically activated irrigation, sonically activated irrigation, and conventional manual irrigation).

2. Materials and Methods

2.1. Teeth Selection and Root Canal Preparation

Permanent human anterior and premolar teeth, with single straight roots, fully formed apices without signs of apical resorption, and clinically intact crowns were selected for this study, after approval by the Ethical Committee of the Faculty of Dental Medicine, University of Porto (protocol 5/2018). Tooth and root canal system anatomy was analyzed by digital radiography in the buccal and proximal directions to confirm the presence of a single canal and apical foramen, and the absence of a complex root canal anatomy. Subsequently, the crowns of the teeth were adjusted to a standardized working length (WL) of 18 mm, and access openings were prepared using diamond burs under air-water spray. Patency was established using a size 15 K-file (Dentsply®, Charlotte, NC, USA).

The teeth (N = 230) were divided into two large groups according to the apical preparation size: 35/.06 and 50/.06 taper. Briefly, WL was set at 1 mm short of the apical foramen by visual inspection with a size 15 K-file (Dentsply®, Charlotte, NC, USA). Canals were instrumented using Wave One® Gold files with ISO size 35/.06 taper, or Protaper Next (Dentsply®, Charlotte, NC, USA) X1, X2, X3, X4, X5 to an apical ISO size 50/.06 taper. Copious irrigation with 5.25% sodium hypochlorite (NaOCl) was performed between each preparation cycle, using a 27G-endodontic needle (Monoject®, CardinalHealth™, Dublin, OH, USA). A single experienced endodontist performed all procedures.

2.2. Irrigant Activation

Three irrigant activation procedures were performed, as detailed, in accordance with established protocols [20]. Group Manual Irrigation (Manual): the root canal was irrigated with 1.5 mL of 5.25% NaOCl, for 30 s, in a gentle up-and-down motion, with a 27G-endodontic needle 1 mm short of the WL. The procedure was followed by a 30-s pause, and the cycle was repeated once again. Group Ultrasonically Activated Irrigation (Ultrasonic): the root canal was irrigated with 1.5 mL of 5.25% NaOCl, and the irrigant was activated using R&S® Tri Scaler Compact (R&S, Paris, France), in endodontic mode with power level 6, with a Satelec® ET20 ultrasonic tip 1 mm short of the WL, without binding. The activation was performed for 30 s, followed by a 30-s pause, and the cycle was repeated once again. Group Sonically Activated Irrigation (Sonic): the root canal was irrigated with 1.5 mL of 5.25% NaOCl, and the irrigant was activated using EDDY® tips (VDW, Munich, Germany), at 6 kHz, 1 mm short of the WL without binding. The activation was performed for 30 s, followed by a 30-s pause, and the cycle was repeated once again.

2.3. Apical Extrusion of Irrigant

After instrumentation, teeth were fixed with composite resin in a clear container. The container was filled to the cervical level of the teeth with 1% agarose containing 0.1% (*w*/*v*) M-cresol purple (25 mL, Sigma-Aldrich, Darmstadt, Germany), which undergoes a color change from yellow to purple, in the presence of NaOCl. Subsequently, the teeth, for each apical size (35/.06 or 50/.06), were randomly allocated into three groups (n = 10/group), and root canal irrigations (i.e., Manual, Ultrasonic, Sonic) were performed, as aforementioned. The tooth/gel set-up was examined with transillumination and was photographed in the buccal/lingual direction, from a fixed distance. Each sample was photographed before and 10 min after irrigation. The extent of color change was conducted using Image J software (v. 1.8.0) following color thresholding. Results were reported as the frequency of irrigant extrusion and, for positive samples, the total area of irrigant extrusion (mm^2).

2.4. Apical Extrusion of Debris

To evaluate the apical extrusion of debris, a previously described experimental model was employed [21]. Briefly, the stoppers were separated from 1.5 mL tubes, and their initial weight was determined. Teeth, prepared to an apical size of 35/.06 or 50/.06, were inserted up to the cementoenamel junction, and a 27G needle was placed alongside the stopper to balance the air pressure in/out. Then, each stopper with the tooth and the needle was attached to its tube. The teeth, for each apical size, were randomly divided into three groups (n = 10/group), and the irrigation protocols previously described (i.e., Manual, Ultrasonic, Sonic) were performed. Finally, the stopper, needle, and tooth were separated from the tube, and the debris adhering to the root surface was collected by washing the root with distilled water into the tube. The tubes were stored in an incubator at 68 °C for 5 days and the final weights were measured to ascertain the amount of the extruded debris, obtained by weight difference calculation in milligrams.

2.5. Removal of Debris and Smear Layer

The teeth, prepared to an apical size of 35/.06 or 50/.06 and irrigated according to the procedures mentioned (i.e., Manual, Ultrasonic, Sonic) (n = 5/group), were longitudinally split using a chisel. The morphology of the canal surface was accessed by scanning electron microscopy (SEM, FEI Quanta 400 FEG/ESEM) without any additional manipulation. Prior to imaging, the specimens were sputter-coated (SPI-Module) with a thin film of gold/palladium. To evaluate the superficial debris and smear layer, a previously published four-score index system was adopted [22]. Succinctly, the index used for debris evaluation ranged between score 1: none to slight presence of superficial debris covering up to 25% of the dentinal surface; score 2: little to moderate presence of debris covering between 25–50% of the surface; score 3: moderate to heavy presence of residual debris covering between

50–75% of the surface; score 4: heavy amount of aggregated or scattered debris covering over 75% of the surface. The index used for smear layer evaluation ranged between score 1: little or no smear layer, covering less than 25% of the specimen with tubules visible and patent; score 2: little to moderate or patchy amounts of smear layer, covering between 25–50% of the specimen with many tubules visible and patent; score 3: moderate amounts of scattered or aggregated smear layer, covering between 50–75% of the specimen with minimal to no tubules visible or patent; score 4: heavy smear layer covering over 75% of the specimen with no tubule orifices visible or patent. The evaluation was conducted by two calibrated observers (S.P. and L.G.).

2.6. Removal of Bacterial Biofilm

The apical foramen of instrumented teeth (35/.06 or 50/.06) was sealed with self-cure glass ionomer, and the root surfaces were covered with two layers of nail varnish. To initiate the experiment, previously sterilized teeth had their root canals filled up to the orifice level with a suspension of *E. faecalis* ATCC 29212 (ca. 10^8 cells/mL). These teeth were individually submerged in tubes containing brain heart infusion broth (BHI, Liofilchem, Roseto degli Abruzzi (TE), Italy) and incubated at 37 °C for 21 days, with regular renewal of the culture medium every two days. After incubation, the BHI broth in the canal space was aspirated, and the canal was washed with phosphate buffer saline (PBS, Sigma Aldrich, Darmstadt, Germany). Subsequently, the teeth, for each apical size, were randomly gathered into three groups (n = 10/group), and root canal irrigations (i.e., Manual, Ultrasonic, Sonic) were performed. An additional control group (C) was created in which the root canals were not subjected to any irrigation procedure.

To determine the remaining metabolic active bacteria in the root canals after each procedure, the resazurin assay was conducted. Briefly, root canals were filled with BHI broth with 10% resazurin (0.1 mg/mL, Sigma-Aldrich, Darmstadt, Germany) and incubated for 3 h at 37 °C. The medium in the canal space was then collected, and its fluorescence intensity (excitation: 530 nm; emission: 590 nm) was measured in a microplate reader (Synergy HT, BioTek, Santa Clara, CA, USA). Results were presented as relative fluorescence units (RFU).

Additionally, an assessment of the biofilm remaining on the root canal after each irrigation procedure was accessed by SEM. Briefly, teeth were fixed with 1.5% glutaraldehyde (Sigma-Aldrich) for 30 min, longitudinally split, and dehydrated in sequential graded ethanol solutions. Lastly, teeth were sputter-coated with Au-Pd alloy and visualized by SEM.

2.7. Statistical Analysis

Results are presented as mean ± standard deviation for two groups (apical size): 35/.06 or 50/.06; and three subgroups (irrigation procedures): ultrasonically activated irrigation, sonically activated irrigation, and conventional manual irrigation. For statistical analyses, Student's paired t-test and the one-way analysis of variance (ANOVA) with post-hoc Tukey HSD were used in IBM® SPSS® Statistics software (v. 28.0.0.0, Chicago: SPSS Inc. USA). The level of significance was set at $p < 0.05$.

3. Results

3.1. Apical Extrusion of Irrigant and Debris

Regarding the extrusion tests, each experimental group exhibited a similar tendency for both irrigant and debris extrusion (Figure 1). Specifically, the manual group demonstrated some degree of extrusion but was unrelated to the apical preparation size. Conversely, for sonic and ultrasonic activation, a greater occurrence of irrigant and debris extrusion was observed in teeth with an apical preparation of 50/.06, in contrast to 35/.06 (Figure 1a,c). Notably, significant differences in debris extrusion were identified between these preparations (Figure 1c, $p < 0.05$). Although extrusion was more pronounced in the activation protocols with larger apical preparations compared to the manual group, no significant

differences were observed. In a more in-depth analysis, teeth exhibiting positive irrigant extrusion were further examined to evaluate the extent of this extrusion. It was found that the size of the apical preparation influenced the extent of extrusion across all groups (Figure 1b). A more extensive area of extrusion was observed for an apical size of 50/.06, compared to 35/.06. Representative images illustrating the extrusion areas are presented in Figure 1b—inset images.

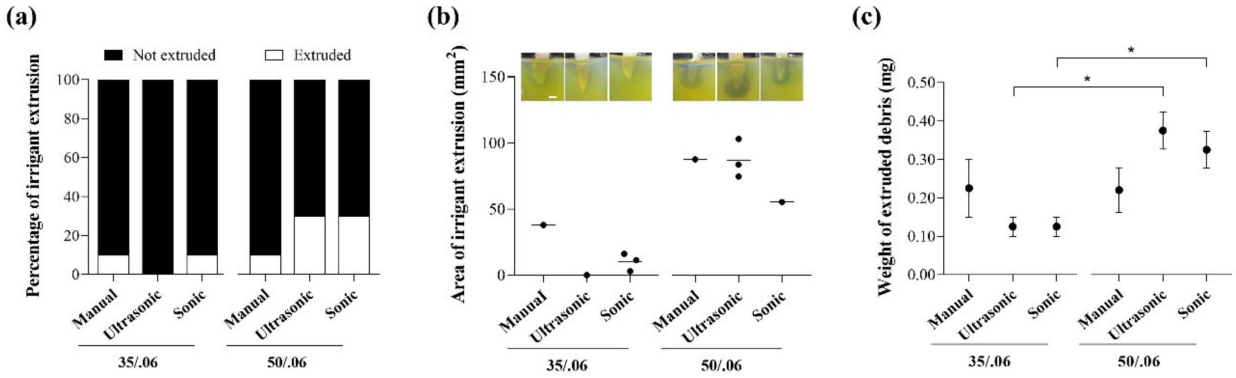

Figure 1. (**a**) Frequency of irrigant extrusion under different root canal irrigation procedures and apical preparation sizes. (**b**) Extension of irrigant extrusion in positive samples, and representative images of irrigant extrusion (inset, scale bar 0.5 cm). (**c**) Mass of apically extruded debris under different root canal irrigation procedures and apical preparation sizes. * Significantly different between apical sizes, $p < 0.05$.

3.2. Removal of Debris and Smear Layer

The assessment of debris and smear layer removal upon irrigation procedures was analyzed by SEM, and both the representative images and the number of samples indexed for each score are presented in Figure 2. In the manual group, regardless of the apical size, the entire root canal walls presented superficial debris and a smear layer, without any visible openings of the dentinal tubules. After ultrasonic activation, root canals at 35/.06 display the minimal presence of debris and a thin smear layer covering the walls, with a low frequency of visible/patent dentinal tubules. In root canals at 50/.06, the presence of debris and smear layer was minimal, and the identification of patent dentinal tubules was substantial. As for sonic activation, root canals prepared at the smaller apical size presented minimal to moderate debris and smear layers, with many dentinal tubules exposed. In contrast, root canals with larger apical size exhibited a reduction in superficial debris, while the smear layer persisted at a minimal to moderate level, with numerous identifiable dentinal tubules. Overall, specimens with an apical size of 50/.06 that underwent sonic or ultrasonic activation showed reduced debris and smear layers compared to those subjected to manual irrigation.

3.3. Removal of Bacterial Biofilm

Concerning the removal of a 21-day bacterial biofilm, all three irrigation procedures significantly reduced the metabolic activity of the established biofilm, regardless of the apical preparation size, compared to the control group without irrigation (Figure 3a, $p < 0.05$). Additionally, ultrasonic and sonic activation induced a significant reduction compared to manual irrigation, for both apical sizes ($p < 0.05$). For an apical size of 35/.06, no significant differences were observed between ultrasonic and sonic groups. Conversely, for 50/.06, the ultrasonic activation induced a significantly greater reduction compared to sonic activation ($p < 0.05$). Furthermore, a significantly higher reduction in the metabolic activity of the biofilm was achieved in the apical size 50/.06, over the 35/.06, for both ultrasonic and sonic systems ($p < 0.05$). For manual irrigation, the reduction was similar for both apical sizes. Representative images of the biofilm remaining on the root canal surface

after each procedure, focusing on teeth instrumented to an apical size 50/.06, are presented in Figure 3b.

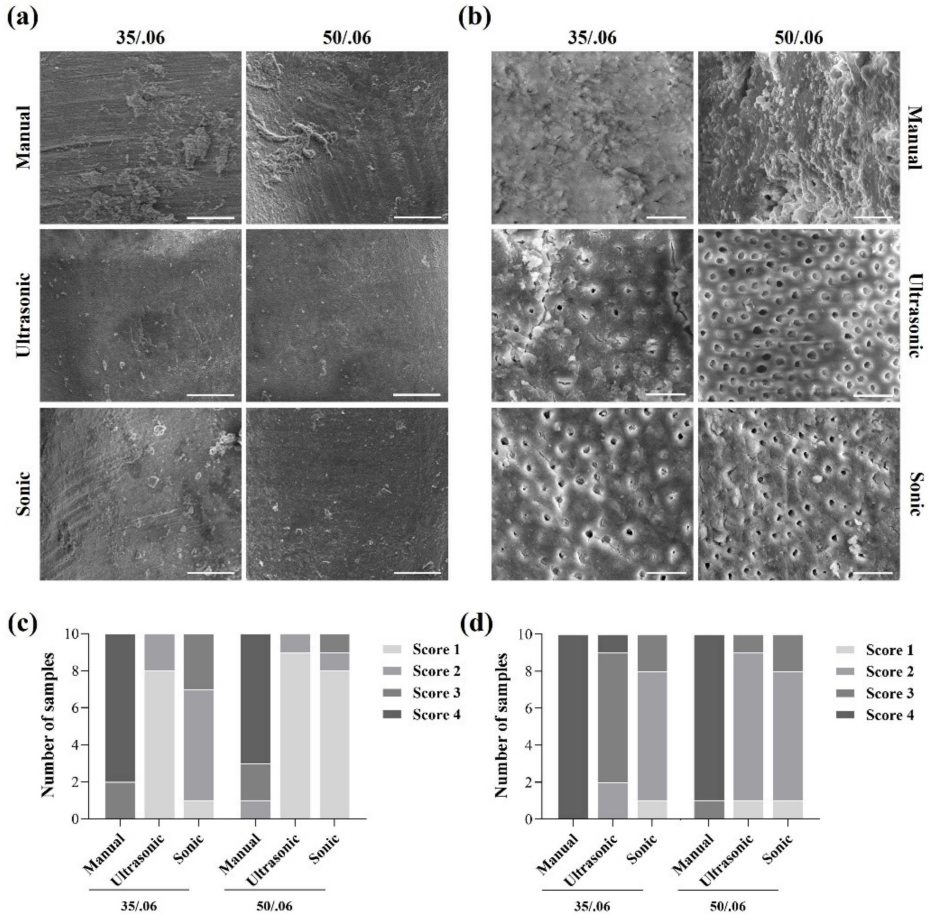

Figure 2. Representative SEM images of the root canal walls at (**a**) lower magnification for debris evaluation (bars 100 μm); and at (**b**) higher magnification for smear layer evaluation (bars 10 μm). Number of samples indexed for each score in evaluating (**c**) residual debris and (**d**) smear layer, after selected irrigation procedure, in apical sizes of 35/.06 and 50/.06.

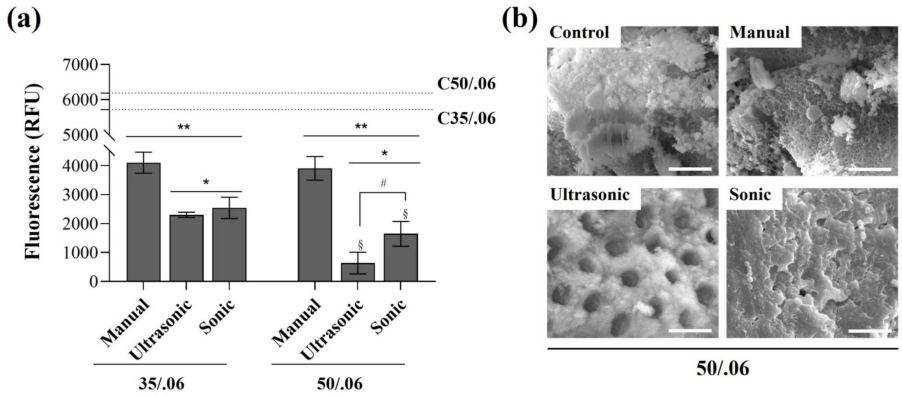

Figure 3. (**a**) Metabolic activity of the 21-day *E. faecalis* biofilm after irrigation procedure, quantified by the resazurin assay. * Significantly different from the Manual, for the same apical size; ** Significantly different to the control (C), for the same apical size; # Significant differences between Ultrasonic 50/.06 and Sonic 50/.06 groups; § Significant differences between the same irrigation procedure at different apical sizes; for all $p < 0.05$. (**b**) Representative SEM images of the root canal walls after irrigation, in an apical size of 50/.06 (bars 5 μm).

4. Discussion

In contemporary endodontics, a variety of irrigation delivery and activating devices are available to improve the disinfection and debridement of the root canal system during treatment. A survey among members of the American Association of Endodontists reported that nearly half of them used these devices, with 48% using ultrasonic activation and 34% opting for sonic activation, to improve irrigation efficacy [23]. While published investigations have addressed and compared the effectiveness of ultrasonic and sonic irrigant activation against conventional manual needle irrigation, these studies focused on distinct experimental settings, neglecting the impact of the apical canal preparation size on the irrigating outcomes [7,13–16]. In this context, the present study addresses, through multiple clinically relevant parameters, the efficacy of different irrigation procedures on two apical sizes, i.e., 35/.06 and 50/.06, by conducting an *ex vivo* investigation in human teeth.

One of the most significant risks associated with the irrigation procedure remains the extrusion of debris and irrigant solution into the periapical region, which may cause post-operative undesirable outcomes, as periapical inflammation, postoperative pain, and, ultimately, compromise the success of root canal treatment [24–26]. Although all irrigation procedures have an associated risk of apical extrusion, the extension of this extrusion may differ according to the instrumentation techniques and devices employed [27,28]. In the present study, the extrusion risk with conventional needle irrigation did not appear to be influenced by the apical size. On the contrary, both ultrasonic and sonic activation procedures exhibited a higher risk of extrusion, involving both irrigant and debris, particularly within the larger apical size, i.e., 50/.06. Notably, these values tended to be higher in the ultrasonic group. These results are consistent with a previous study [27], which similarly observed a reduction in the frequency of extrusion in teeth with a lower apical preparation size (i.e., 35/.06, compared to 50/.06). Similarly, another study argues that larger apical preparations might increase the risk of canal transportation and perforation, which raises concerns in considering larger canals size preparations [18].

On the other hand, when root canal preparation was increased to a size 50/.06, a significant reduction in residual debris and smear layer was observed, in contrast to a size 35/.06, particularly after activation of the irrigant. Similarly, it has been reported that a basic preparation to a size 25/.06 produced significantly less clean root canal walls than a size 40/.04 [29]. An enlargement of the apical preparation has been advanced for improved cleaning, through better acoustic streaming and penetration of irrigants, which is a critical aspect considering that the remaining tissue and debris can negatively impact endodontic treatment. Such debris can interfere with the adhesion of root-filling materials and provide a niche and nutritional source for microorganisms, potentially contributing to the development of secondary infection [30,31]. Additionally, both sonic and ultrasonic activation procedures significantly enhanced the removal of dentin debris and smear layer, compared to conventional manual needle irrigation, regardless of the apical size. These results are in accordance with most of the literature, which advocates that irrigant activation exhibits enhanced canal debridement efficacy over the use of needle irrigation alone [7,16,32]. Comparing both activation methods, ultrasonic activation demonstrated superior debris and smear layer removal than sonic activation. The outperformance of ultrasonic activation may be justified by the higher driving frequency of ultrasound compared to that of the sonic device [9]. Theoretically, a higher frequency results in an increased flow velocity, which allows the irrigant to reach otherwise inaccessible regions inside the complex root canal system and increases the shear stress that disrupts debris [33,34]. However, it is worth noting that potent ultrasonic irrigation may also entail some limitations. It has been shown that even in noncomplicated root canal geometries, ultrasonic instruments, in the mean, come in contact with the wall during 20% of the activation time [35]. File-to-wall contact dampens the energy and constrains the file movement, which may lead to the accidental removal of small amounts of dentin, changing root canal morphology, even with a noncutting design [36]. Instead, sonic activation with soft polymer tips minimizes the risk of unintentional dentin removal and root canal alterations [37]. The resulting surface

alterations may also hamper the proper adhesion of filling materials and offer favorable niches for bacterial adhesion and proliferation.

Microbial biofilms play a central role in the development of pulpal and periapical diseases and, accordingly, the reduction of the microbial load in the root canal is a major clinical aim and a relevant parameter to be evaluated in experimental studies of endodontic irrigation [38,39]. In this context, *E. faecalis* is the primary pathogen isolated, in part due to its ability to bind to dentin and invade dentinal tubes, where it can survive for long periods [19]. Perseverance of microorganisms within the root canal system is the major cause of post-treatment failure [40]. In the present investigation, both sonic and ultrasonic activation of the irrigant presented a significantly higher capability in the elimination of a mature biofilm of *E. faecalis* than manual irrigation, regardless of the apical size, which is in accordance with published data [10]. These differences may be explained by the fact that conventional irrigation provides far lower fluid dynamics compared to the investigated activation techniques [41]. For an apical size of 35/.06, antibacterial efficacy was similar for both sonic and ultrasonic activation procedures, which is in accordance with other studies [14]. However, for an apical size of 50/.06, ultrasonic activation exhibited significantly improved results, compared to sonic activation. The enhanced effectiveness of ultrasonic activation has been associated with its acoustic streaming and cavitation effects that increase shear stress and, consequently, enhances the disruption of the biofilm [14]. Overall, an improved performance in eliminating a 21-day biofilm was attained for the larger apical size for all the assayed procedures, in accordance with other studies that advocate the enlargement of the apical sizes for a more significant reduction in the root canal biomass and increased effectiveness of the irrigation procedure [12,42].

The experimental models used in this study have some limitations. The use of straight, single-rooted teeth precludes possible variations found in clinical scenarios, such as WL loss or nonstandard preparation and irrigation in curved root canals. However, this experimental system provided a standardized and reproducible setup to determine the potential relationship between root canal preparation size and irrigating procedure, through an integrative analysis of clinically relevant parameters. The next step will involve investigating the replicability of the results of the present study in multi-rooted teeth, with curved roots and/or more complex root canal anatomies.

5. Conclusions

Considering the study's findings, the null hypothesis was rejected, and several significant conclusions emerged: (1) The employment of both sonic and ultrasonic activation techniques is associated with a higher risk of extrusion, particularly for larger apical preparation sizes; however, (2) these methods offer significantly higher efficiency in removing debris and smear layer, and (3) demonstrate a superior capacity for biofilm elimination. Notably, (4) ultrasonic activation applied to root canals at 50/.06 exhibited the best-combined outcomes across all evaluated parameters.

Author Contributions: Conceptualization, S.P., C.R. and L.G.; Formal analysis, S.P. and L.G.; Funding acquisition, M.H.F.; Investigation, S.P. and L.G.; Methodology, S.P., P.S.G. and L.G.; Resources, M.H.F. and C.R.; Supervision, L.G.; Visualization, S.P.; Writing—original draft, S.P.; Writing—review and editing, S.P., P.S.G., M.H.F., C.R. and L.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Committee of the Faculty of Dental Medicine, University of Porto (protocol 5/2018, 1 February 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: This work received support and help from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020), through national funds.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Park, E.; Shen, Y.; Haapasalo, M. Irrigation of the apical root canal. *Endod. Top.* **2012**, *27*, 54–73. [CrossRef]
2. Waltimo, T.; Trope, M.; Haapasalo, M.; Orstavik, D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. *J. Endod.* **2005**, *31*, 863–866. [CrossRef]
3. Virdee, S.S.; Farnell, D.J.J.; Silva, M.A.; Camilleri, J.; Cooper, P.R.; Tomson, P.L. The influence of irrigant activation, concentration and contact time on sodium hypochlorite penetration into root dentine: An *ex vivo* experiment. *Int. Endod. J.* **2020**, *53*, 986–997. [CrossRef] [PubMed]
4. Paque, F.; Ganahl, D.; Peters, O.A. Effects of root canal preparation on apical geometry assessed by micro-computed tomography. *J. Endod.* **2009**, *35*, 1056–1059. [CrossRef]
5. Siqueira Junior, J.F.; Rocas, I.D.N.; Marceliano-Alves, M.F.; Perez, A.R.; Ricucci, D. Unprepared root canal surface areas: Causes, clinical implications, and therapeutic strategies. *Braz. Oral Res.* **2018**, *32* (Suppl. S1), e65. [CrossRef] [PubMed]
6. Fornari, V.J.; Hartmann, M.S.M.; Vanni, J.R.; Rodriguez, R.; Langaro, M.C.; Pelepenko, L.E.; Zaia, A.A. Apical root canal cleaning after preparation with endodontic instruments: A randomized trial *In Vivo* analysis. *Restor. Dent. Endodont.* **2020**, *45*, e38. [CrossRef] [PubMed]
7. Urban, K.; Donnermeyer, D.; Schafer, E.; Burklein, S. Canal cleanliness using different irrigation activation systems: A SEM evaluation. *Clin. Oral Investig.* **2017**, *21*, 2681–2687. [CrossRef] [PubMed]
8. van der Sluis, L.W.M.; Versluis, M.; Wu, M.K.; Wesselink, P.R. Passive ultrasonic irrigation of the root canal: A review of the literature. *Int. Endod. J.* **2007**, *40*, 415–426. [CrossRef] [PubMed]
9. Caputa, P.E.; Retsas, A.; Kuijk, L.; de Paz, L.E.C.; Boutsioukis, C. Ultrasonic irrigant activation during root canal treatment: A systematic review. *J. Endod.* **2019**, *45*, 31–44. [CrossRef] [PubMed]
10. Eggmann, F.; Vokac, Y.; Eick, S.; Neuhaus, K.W. Sonic irrigant activation for root canal disinfection: Power modes matter! *BMC Oral Health* **2020**, *20*, 102. [CrossRef] [PubMed]
11. Jiang, L.M.; Verhaagen, B.; Versluis, M.; van der Sluis, L.W.M. Evaluation of a sonic device designed to activate irrigant in the root canal. *J. Endod.* **2010**, *36*, 143–146. [CrossRef] [PubMed]
12. Lee, O.Y.S.; Khan, K.; Li, K.Y.; Shetty, H.; Abiad, R.S.; Cheung, G.S.P.; Neelakantan, P. Influence of apical preparation size and irrigation technique on root canal debridement: A histological analysis of round and oval root canals. *Int. Endod. J.* **2019**, *52*, 1366–1376. [CrossRef] [PubMed]
13. Zeng, C.; Willison, J.; Meghil, M.M.; Bergeron, B.E.; Cutler, C.W.; Tay, F.R.; Niu, L.N.; Ma, J.Z. Antibacterial efficacy of an endodontic sonic-powered irrigation system: An *In Vitro* study. *J. Dent.* **2018**, *75*, 105–112. [CrossRef] [PubMed]
14. Neuhaus, K.W.; Liebi, M.; Stauffacher, S.; Eick, S.; Lussi, A. Antibacterial efficacy of a new sonic irrigation device for root canal disinfection. *J. Endod.* **2016**, *42*, 1799–1803. [CrossRef] [PubMed]
15. Azim, A.A.; Griggs, J.A.; Huang, G.T.J. The Tennessee study: Factors affecting treatment outcome and healing time following nonsurgical root canal treatment. *Int. Endod. J.* **2016**, *49*, 6–16. [CrossRef] [PubMed]
16. Haupt, F.; Meinel, M.; Gunawardana, A.; Huelsmann, M. Effectiveness of different activated irrigation techniques on debris and smear layer removal from curved root canals: A SEM evaluation. *Aust. Endod. J.* **2020**, *46*, 40–46. [CrossRef] [PubMed]
17. Baugh, D.; Wallace, J. The role of apical instrumentation in root canal treatment: A review of the literature. *J. Endod.* **2005**, *31*, 333–340. [CrossRef] [PubMed]
18. Wu, M.K.; R'oris, A.; Barkis, D.; Wesselink, P.R. Prevalence and extent of long oval canals in the apical third. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2000**, *89*, 739–743. [CrossRef] [PubMed]
19. Alghamdi, F.; Shakir, M. The influence of *Enterococcus faecalis* as a dental root canal pathogen on endodontic treatment: A systematic review. *Cureus* **2020**, *12*, e7257. [CrossRef] [PubMed]
20. Guven, Y.; Uygun, A.D.; Arslan, H. Efficacy of EDDY, ultrasonic activation, XP-endo Finisher and needle irrigation on the removal of mTAP from artificially created grooves in root canals. *Aust. Endod. J.* **2021**, *47*, 639–644. [CrossRef] [PubMed]
21. Myers, G.L.; Montgomery, S. A Comparison of weights of debris extruded apically by conventional filing and canal master techniques. *J. Endod.* **1991**, *17*, 275–279. [CrossRef]
22. Plotino, G.; Ozyurek, T.; Grande, N.M.; Gundogar, M. Influence of size and taper of basic root canal preparation on root canal cleanliness: A scanning electron microscopy study. *Int. Endod. J.* **2019**, *52*, 343–351. [CrossRef] [PubMed]
23. Dutner, J.; Mines, P.; Anderson, A. Irrigation trends among american association of endodontists members: A web-based survey. *J. Endod.* **2012**, *38*, 37–40. [CrossRef] [PubMed]
24. Gernhardt, C.R.; Eppendorf, K.; Kozlowski, A.; Brandt, M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. *Int. Endod. J.* **2004**, *37*, 272–280. [CrossRef] [PubMed]
25. Karamifar, K.; Tondari, A.; Saghiri, M.A. Endodontic periapical lesion: An overview on the etiology, diagnosis and current treatment modalities. *Eur. Endod. J.* **2020**, *5*, 54–67. [CrossRef]

26. Boutsioukis, C.; Arias-Moliz, M.T. Present status and future directions—Irrigants and irrigation methods. *Int. Endod. J.* **2022**, *55* (Suppl. S3), 588–612. [CrossRef] [PubMed]
27. Mitchell, R.P.; Baumgartner, J.C.; Sedgley, C.M. Apical extrusion of sodium hypochlorite using different root canal irrigation systems. *J. Endod.* **2011**, *37*, 1677–1681. [CrossRef] [PubMed]
28. Liapis, D.; De Bruyne, M.; De Moor, R.; Meire, M. Postoperative pain after ultrasonically and laser-activated irrigation during root canal treatment: A randomized clinical trial. *Int. Endod. J.* **2021**, *54*, 1037–1050. [CrossRef]
29. Plotino, G.; Grande, N.M.; Tocci, L.; Testarelli, L.; Gambarini, G. Influence of Different Apical Preparations on Root Canal Cleanliness in Human Molars: A SEM Study. *J. Oral Maxillofac. Res.* **2014**, *5*, e4. [CrossRef] [PubMed]
30. Fornari, V.J.; Silva-Sousa, Y.T.C.; Vanni, J.R.; Pecora, J.D.; Versiani, M.A.; Sousa-Neto, M.D. Histological evaluation of the effectiveness of increased apical enlargement for cleaning the apical third of curved canals. *Int. Endod. J.* **2010**, *43*, 988–994. [CrossRef] [PubMed]
31. Wang, Z.J.; Shen, Y.; Haapasalo, M. Root canal wall dentin structure in uninstrumented but cleaned human premolars: A scanning electron microscopic study. *J. Endod.* **2018**, *44*, 842–848. [CrossRef] [PubMed]
32. Rodig, T.; Koberg, C.; Baxter, S.; Konietschke, F.; Wiegand, A.; Rizk, M. Micro-CT evaluation of sonically and ultrasonically activated irrigation on the removal of hard-tissue debris from isthmus-containing mesial root canal systems of mandibular molars. *Int. Endod. J.* **2019**, *52*, 1173–1181. [CrossRef]
33. Boutsioukis, C.; Lambrianidis, T.; Kastrinakis, E. Irrigant flow within a prepared root canal using various flow rates: A Computational Fluid Dynamics study. *Int. Endod. J.* **2009**, *42*, 144–155. [CrossRef] [PubMed]
34. Kanter, V.; Weldon, E.; Nair, U.; Varella, C.; Kanter, K.; Anusavice, K.; Pileggi, R. A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontology* **2011**, *112*, 809–813. [CrossRef]
35. Boutsioukis, C.; Verhaagen, B.; Walmsley, A.D.; Versluis, M.; van der Sluis, L.W.M. Measurement and visualization of file-to-wall contact during ultrasonically activated irrigation in simulated canals. *Int. Endod. J.* **2013**, *46*, 1046–1055. [CrossRef]
36. Retsas, A.; Koursoumis, A.; Tzimpoulas, N.; Boutsioukis, C. Uncontrolled removal of dentin during *in vitro* ultrasonic irrigant activation in curved root canals. *J. Endod.* **2016**, *42*, 1545–1549. [CrossRef] [PubMed]
37. Plotino, G.; Grande, N.M.; Mercade, M.; Cortese, T.; Staffoli, S.; Gambarini, G.; Testarelli, L. Efficacy of sonic and ultrasonic irrigation devices in the removal of debris from canal irregularities in artificial root canals. *J. Appl. Oral Sci.* **2019**, *27*, e20180045. [CrossRef] [PubMed]
38. Sjogren, U.; Figdor, D.; Persson, S.; Sundqvist, G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int. Endod. J.* **1997**, *30*, 297–306. [CrossRef] [PubMed]
39. Liu, C.; Li, Q.; Yue, L.; Zou, X. Evaluation of sonic, ultrasonic, and laser irrigation activation systems to eliminate bacteria from the dentinal tubules of the root canal system. *J. Appl. Oral Sci.* **2022**, *30*, e20220199. [CrossRef] [PubMed]
40. Haapasalo, M.; Udnæs, T.; Endal, U. Persistent, recurrent, and acquired infection of the root canal system post-treatment. *Endod. Top.* **2003**, *6*, 29–56. [CrossRef]
41. Swimberghe, R.C.D.; Coenye, T.; De Moor, R.J.G.; Meire, M.A. Biofilm model systems for root canal disinfection: A literature review. *Int. Endod. J.* **2019**, *52*, 604–628. [CrossRef] [PubMed]
42. Rollison, S.; Barnett, F.; Stevens, R.H. Efficacy of bacterial removal from instrumented root canals In Vitro related to instrumentation technique and size. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontology* **2002**, *94*, 366–371. [CrossRef] [PubMed]