Effectiveness of passive ultrasonic irrigation on periapical healing and root canal disinfection: a systematic review

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Key points

The present systematic review evaluated the effectiveness of passive ultrasonic irrigation (PUI) compared with non-activated irrigation (NAI) on root canals' healing and disinfection.

PUI strategy was not able to improve radiographic healing after endodontic treatment or improve bacterial disinfection.

There is no evidence that supports the use of PUI over the NAI in clinical practice. A higher number of standardised randomised clinical trials studies must be conducted comparing PUI and NAI irrigating modalities.

Abstract

Objectives To evaluate the effectiveness of passive ultrasonic irrigation (PUI) compared with non-activated irrigation (NAI) on periapical healing and root canal disinfection.

Data source A comprehensive search without restrictions was performed in the following systematic electronic databases: PubMed, Scopus, Cochrane, Web of Science, ScienceDirect and OpenGrey. Additional studies were sought through hand-searching in the main endodontic journals.

Data selection We included clinical trials that compared PUI and NAI clinical success and root canal disinfection outcomes. The risk of bias was assessed based on the Cochrane Collaboration common scheme for bias assessment. The power analysis of each study was calculated based on the disinfection rates and sample size, and the evidence was qualified using the GRADE tool.

Data synthesis A total of 346 non-duplicated studies were retrieved in the systematic search. One study that assessed the clinical success rate through periapical radiographic healing evaluation and two studies that evaluated root canal disinfection through bacterial growth were considered eligible. These three studies were classified as low risk of bias. The study evaluating radiographic treatment outcome showed no statistical difference (P >0.05). The studies demonstrated large variability among methodology and, in general, low power and moderate evidence. Inconclusive results were reported regarding root canal disinfection when comparing PUI to NAI strategies.

Conclusions Based on the findings, there was no evidence of effectiveness improvement on periapical healing and bacterial disinfection that supports the use of PUI over the NAI in clinical practice.

Introduction

The primary objective of endodontic treatment is to promote an effective root canal disinfection by reducing bacterial load to levels compatible with periradicular tissue healing and preventing microbial recolonisation of the treated canal.[1] Contemporary techniques include mechanical debridement and shaping of the root canals with emphasis on various nickel-titanium (NiTi) rotary or reciprocating systems, intra-canal irrigation with antimicrobial/tissue dissolving agents, and inter-appointment dressings. However, several studies have reported that more than half of the dentinal walls (ranging from 59.6% to 79.9%) have remained unprepared,[2,3,4,5] which is a critical challenge for any available shaping protocol. In fact, mechanical preparation systems are able to act only on the central body of the canal lumen, thus leaving irregularities merely untouched,[5,6] harbouring resident bacteria. Accordingly, irrigation is an essential part of endodontic treatment as it allows for the cleaning of unprepared root canal walls.[1]

In search of new methods to provide additional disinfection for the root canal system and presumably to improve treatment outcome, novel techniques such as ultrasonic irrigation have been proposed.[7] Passive ultrasonic irrigation (PUI) is a non-cutting irrigation protocol that relies on the transmission of acoustic energy from a smooth wire or an oscillating file to an irrigant in the root canal space by means of ultrasonic waves. This irrigation technique induces two physical phenomena: stream and cavitation of the irrigating solution disrupting the vapour lock.[8] The acoustic stream is the rapid movement of the fluid in a circular or vortex shape around the vibrating file.[8] Cavitation is the creation of steam bubbles or the expansion, contraction and/or distortion of pre-existing bubbles in a

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liquid.[8] Transient cavitation only occurs when the file can vibrate freely in the canal or when the file lightly touches the canal walls. When the root canal has already been shaped, the file or wire can move freely and the irrigant can penetrate more easily into the apical part of the root canal system and the cleaning effect will be more powerful.[8,9,10]

PUI has been described as an excellent auxiliary in the process of final cleaning of root canals,[7] increasing the efficiency of irrigant solutions in removing debris, microorganisms and smear layers, especially in areas of anatomical difficulty.[11] However, the assumed additional benefits of PUI over non-activated irrigation (NAI) are mostly based on *in vitro* studies.[11,12,13,14] Moreover, several studies that compared PUI and NAI have methodological limitations, such as the use of different irrigation volumes, time of contact or irrigant type. Furthermore, some studies evaluated the outcomes before and after the PUI protocol as an additional operative step but did not include an NAI control group.[15,16,17,18]

Therefore, considering the inconclusive and contradictory results in the literature, the aim of this systematic review was to answer the focused question: 'does the use of PUI provide better treatment outcomes and root canal disinfection when compared to NAI?'.

Materials and methods

Protocol and registration

The systematic review protocol was registered on the PROSPERO database (http://www.crd.york.ac.uk) under number CRD42017082331 and it followed the recommendations for the preferred reporting items of systematic review protocol (PRISMA-P).[19]

Search strategy

A systematic search without restrictions was performed by two independent reviewers in the electronic databases PubMed, Scopus, Cochrane, Web of Science, ScienceDirect and OpenGrey from their inception through to 18 November 2017. No filters or limits were applied in the searches, and also no limits regarding language or year of publication. The electronic search strategy was developed using a combination of Medical Subject Heading (MeSH) terms and text. The selection of the descriptors was based on the most cited terms in previous publications related to this theme. The Boolean operators 'AND' and 'OR' were used to create the keywords search (Table 1). The search strategy included no filters, limits or language restriction of publication year. For each database, the following terms were combined: 'ultrasonic irrigation', 'ultrasonic activation', 'Microbial Consortia', 'Microbiota', 'disinfection', 'microbiology', 'bacteria', 'Enterococcus faecalis', 'polymerase chain reaction', 'bacterial reduction', 'culture', 'Periapical Abscess', 'Radiography', 'diagnostic imaging', 'Cone-Beam Computed Tomography', 'periapical lesion', 'periradicular lesion', 'periapical bone destruction', and 'periapical bone loss'. A complementary screening on the references of the selected studies and in the *Journal of Endodontics* and the *International Endodontic Journal* without year-restriction was performed to find any additional work that did not appear in the database search.

Inclusion criteria

The eligibility criteria considered randomised controlled trials (RCTs) or controlled clinical trials (CCTs) that evaluated the periapical healing (clinical true outcome) and disinfection of the root canal system (surrogate outcome), and compared passive with non-activated irrigation protocol in adult patients with fully formed permanent teeth undergoing endodontic treatment.

Exclusion criteria

Studies that did not included NAI as a control group were excluded. In addition, studies with non-standardised root canal preparation within and between NAI and PUI and/or did not use the same volume, composition, concentration and contact time of irrigant solutions for the PUI and NAI groups were also excluded. Reviews, letters, opinion articles, conference abstracts, case reports, serial cases, *in vitro* studies, studies performed on animals, and studies that did not perform the chemo-mechanical step were excluded.

Selection of the studies

Two independent authors selected the studies, examining the retrieved titles and abstracts according to the search strategy. When it was not possible to judge the studies by title and abstract, the full text was obtained for the final decision. Then, the full texts of all potentially eligible studies were evaluated and selected based on the inclusion criteria through the PICOS strategy. Disagreements on inclusion criteria were solved by consensus with a third author following the predefined inclusion criteria. Studies that appeared to be duplicated in the database search were considered only once.

Data extraction

Data collection was obtained by two authors independently and it was performed based on the recommendations of the Cochrane Handbook 5.0.2.[20] The information regarding the details of the study (first author, year and country), sample size, teeth type, clinical procedures (instrument used and irrigating solution), PUI and NAI protocols, and the outcomes (periapical healing and disinfection rates) were analysed. Additionally, the authors were contacted by email to solve eventual missing information.

Quality assessment of the studies

The methodological quality of the studies was performed by two authors independently and was carried out using the Cochrane Collaboration tool[20] for risk assessment of bias.[21] Four key domains were considered for the assessment of the risk of bias: sequence generation; allocation concealment; incomplete outcome data; and selective outcome reporting. Blinding of participants and personnel was not considered key due to the specific devices used during the irrigation protocols. The power analysis for each comparison from included studies was calculated based on the sample size and the percentage of periapical radiographic and CBCT healing that was provided by Liang *et al.*,[22] and calculated the disinfection rates for Herrera *et al.*[23] and Nakamura *et al.*[24] The power analysis is able to measure the effect size that can be detected using a given sample size. For this purpose, a confidence interval of 95% and a two-tailed test, using OpenEpi 3.04.04 software, were adopted.

The risk of bias for each entry recording was judged as 'no' to indicate high risk of bias, 'yes' to indicate low risk bias and 'unclear' to indicate either lack of information or uncertainty over the potential risk of bias. When a study was judged as 'unclear' in any of the fields, contact with the authors was made via email in order to obtain more information and to enable the judgement of low or high risk of bias. Since Liang *et al.*[22] did not mention the allocation concealment and Herrera *et al.*[23] did not mention the randomisation and allocation, the authors were contacted by email to solve these domains. During the extraction of the data, in the case of disagreements between reviewers, these were resolved through discussion with an experienced researcher. The strength of the evidence of the included studies was assessed

Table 1 Search strategy in the databases

Database	Search strategy	Findings
Pubmed	#1 ('ultrasonic irrigation' [Title/Abstract] OR 'ultrasonic activation' [Title/Abstract])	367
	#2 ('Microbial Consortia'[MeSH] OR 'Microbial Consortia'[Title/Abstract] OR 'Microbiota'[MeSH] OR 'Microbiota'[Title/Abstract] OR 'disinfection'[MeSH] OR 'disinfection'[Title/Abstract]] OR 'microbiology'[MeSH] OR 'microbiology' [Title/Abstract] OR 'bacteria'[MeSH] OR 'bacteria' [Title/Abstract] OR 'Enterococcus faecalis'[MeSH] OR 'Enterococcus faecalis' [Title/Abstract] OR 'polymerase chain reaction'[MeSH] OR 'polymerase chain reaction'[MeSH] OR 'Microb*'[Title/Abstract] OR ' bacterial reduction' [Title/Abstract] OR 'culture' [Title/Abstract])	2,182,133
	#3 ('Periapical Abscess'[MeSH] OR 'Periapical Abscess'[Title/Abstract] OR 'Radiography'[MeSH] OR 'Radiograp*'[Title/Abstract] OR 'diagnostic imaging' [MeSH] OR 'diagnostic imaging'[Title/Abstract] OR 'Cone-Beam Computed Tomography'[MeSH] OR 'Cone-Beam Computed Tomography'[Title/Abstract] OR 'periapical lesion'[Title/Abstract] OR 'periradicular lesion'[Title/Abstract] OR 'periapical bone destruction'[Title/Abstract] OR 'periapical bone loss'[Title/Abstract]	2,459,460
	# 1 AND # 2	68
	# 1 AND # 3	126
	(# 1 and # 2) OR (# 1 AND # 3)	176
ScienceDirect	#1 TITLE-ABS-KEY(ultrasonic irrigation) OR TITLE-ABS-KEY(ultrasonic activation)	877
	#2 TITLE-ABS-KEY(Microbial Consortia) OR TITLE-ABS-KEY(Microbiota) OR TITLE-ABS-KEY(disinfection) OR TITLE-ABS-KEY(microbiology) OR TITLE-ABS-KEY(bacteria) OR TITLE-ABS-KEY(Enterococcus faecalis) OR TITLE-ABS-KEY (polymerase chain reaction) OR TITLE-ABS-KEY(bacterial reduction) OR TITLE-ABS-KEY(culture)	516,173
	#3 TITLE-ABS-KEY(Periapical Abscess) OR TITLE-ABS-KEY(radiography) OR TITLE-ABS-KEY(diagnostic imaging) OR TITLE-ABS-KEY(Cone-Beam Computed Tomography) OR TITLE-ABS-KEY(periapical lesion) OR TITLE-ABS-KEY(periradicular lesion) OR TITLE-ABS-KEY(periapical bone destruction) OR TITLE-ABS-KEY(periapical bone loss)	46,491
	# 1 AND # 2	54
	# 1 AND # 3	5
	(# 1 and # 2) OR (# 1 AND # 3)	58
Cochrane	#1 TITLE-ABS-KEY(ultrasonic irrigation) OR TITLE-ABS-KEY(ultrasonic activation)	13
	#2 TITLE-ABS-KEY(Microbial Consortia) OR TITLE-ABS-KEY(Microbiota) OR TITLE-ABS-KEY(disinfection) OR TITLE-ABS-KEY(microbiology) OR TITLE-ABS-KEY(bacteria) OR TITLE-ABS-KEY(Enterococcus faecalis) OR TITLE-ABS-KEY (polymerase chain reaction) OR TITLE-ABS-KEY(bacterial reduction) OR TITLE-ABS-KEY(culture)	38,791
	#3 TITLE-ABS-KEY(Periapical Abscess) OR TITLE-ABS-KEY(radiography) OR TITLE-ABS-KEY(diagnostic imaging) OR TITLE-ABS-KEY(Cone-Beam Computed Tomography) OR TITLE-ABS-KEY(periapical lesion) OR TITLE-ABS-KEY(periradicular lesion) OR TITLE-ABS-KEY(periapical bone destruction) OR TITLE-ABS-KEY(periapical bone loss)	32,246
	# 1 AND # 2	5
	# 1 AND # 3	4
	(# 1 and # 2) OR (# 1 AND # 3)	8
OpenGrey - SIGLE	#1 ultrasonic irrigation OR ultrasonic activation	15
	#2 Microbial Consortia OR Microbiota OR disinfection OR microbiology OR bacteria OR Enterococcus faecalis OR polymerase chain reaction OR bacterial reduction OR culture	30,308
	#3 Periapical Abscess OR radiography OR diagnostic imaging OR Cone-Beam Computed Tomography OR periapical lesion OR periradicular lesion OR periapical bone destruction OR periapical bone loss	10,96
	# 1 AND # 2	0
	# 1 AND # 3	0
	(# 1 and # 2) OR (# 1 AND # 3)	0

using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool.[25]

Results

Selection of the studies

The database screening resulted in 346 studies after removal of duplicates, as exhibited in the flow diagram (Fig. 1). After title and abstract reading, nine studies[15,16,17,18,22,23,24,26] matched the inclusion criteria. No additional study was added following a manual search of the references of these nine studies. After reading the complete articles, two studies were excluded due to the absence of a control group,[17,18] one study due to the assessment of pain and root canal filling as the main outcome,[16] two because of the use of different irrigant volumes for PUI and NAI,[15,26] and one study was excluded due to including an additional irrigation with 2% chlorhexidine as well as 1% NaOCl for PUI protocol group.[27] It was not necessary to discuss these decisions with the third author to resolve disagreements, as the two independent reviewers agreed with the included studies.

Characteristics of the included studies

The details of the three included studies are exhibited in Table 2. All studies were randomised clinical trials, the minimum number of enrolled teeth was 24 and the maximum number was 84. The tooth type varied as Nakamura *et al.*[24] included multi-rooted teeth, whereas Herrera *et al.*[23] and Liang *et al.*[22] included only single-rooted teeth.

Regarding the clinical procedures, the instrumentation protocol also presented discrepancies, since Nakamura *et al.*[24] used reciprocating instruments while the other authors used continuous rotary systems.[22,23] Moreover, Herrera *et al.*[23] used 2% chlorhexidine gel as an irrigating solution, while others authors[22,24] used NaOCl in different concentrations (2.5% and 5.25%). PUI protocols also varied among the studies as Liang *et al.*[22] applied PUI for ten seconds after the use of each instrument, while Nakamura *et al.*[24] and Herrera *et al.*[23] used PUI for 30 seconds with different application protocols.

Among these three studies, only Liang *et al.*[22] evaluated clinical measure as an outcome. These authors assessed the periapical radiographic and cone-beam computed tomographic healing ten to 19 months after endodontic treatment, after the PUI protocol, and did not find any statistical difference when compared to NAI protocol (P >0.05). The other authors[23,24] evaluated the disinfection rates of the PUI protocol. Herrera *et al.*[23] used culture procedures to evaluate the colony forming units (CFU) reduction of obligate anaerobes and facultative anaerobes bacteria, and Nakamura *et al.*[24] assessed the total bacteria count using the quantitative polymerase chain reaction (qPCR) technique. Herrera *et al.*[23] found similar results (P >0.05) for disinfection when comparing PUI to NAI protocols. Nakamura *et al.*[24] used quantitative polymerase chain reaction, a molecular technique, to assess the reduction of the number of total bacteria, and found that PUI was more effective than NAI (P <0.05).

Study quality assessment

The three included studies[22,23,24] were classified as low risk of bias (Fig. 2). All of them were randomised clinical trials and presented a control group. The blindness of the participants and personnel was not possible, since the PUI protocols included special irrigating apparatus not allowing the blindness. However, Liang *et al.*[22] and Nakamura *et al.*[24] performed the assessment of the outcomes blindly. After being contacted, Herrera *et al.*[23] confirmed that the study was randomised as well as the allocation concealment, justifying the low risk of bias for both of these domains (Fig. 2). Liang *et al.*[22] did not mention the allocation concealment in the study or respond to the authors' email, and for this reason the domain remained unclear. Two included studies presented low effect size considering the power analysis (Table 2). Nakamura *et al.*[24] was the lowest one with 1.15%, Liang *et al.*[22] presented 19.89%, and Herrera *et al.*[23] presented the highest effect size with 98.02%. In addition, the GRADE tool demonstrated the moderate quality of the evidence (Table 3).

Fig. 1 Systematic review flow diagram

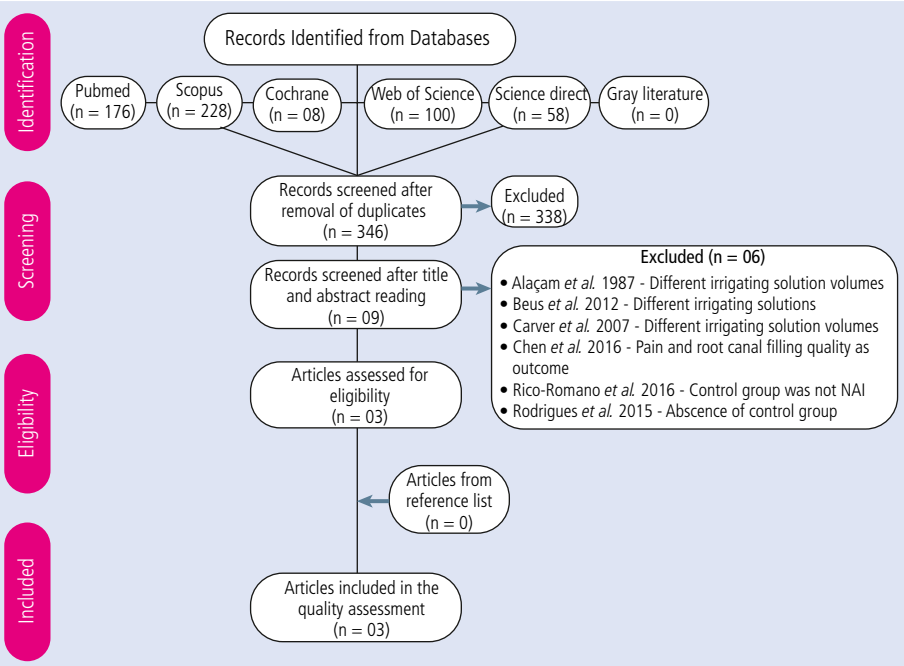
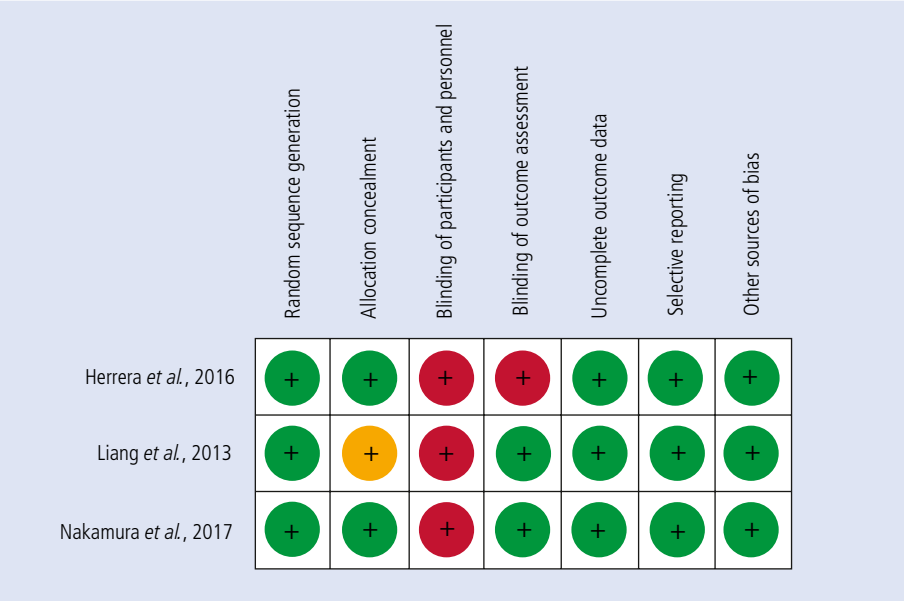


Fig. 2 Risk of bias summary according to the Cochrane collaboration tool



Discussion

Ultrasonic activation might improve both mechanical and chemical aspects of the irrigation procedure, which has been demonstrated in several *in vitro* studies.[11,12,28] Therefore, PUI was proposed to improve endodontic outcomes, such as periradicular healing and bacterial reduction.[16,17,18,22,23,24,27] However, clinical studies showed conflicting results regarding PUI.[22,23,24] Within this background, and all the attention that this irrigation protocol has gained in endodontics, the present systematic review of clinical studies focused on assessing the impact

Table 2 Qualitative analysis and characteristics of the included studies

Authors; year; country	Sample size (n)	Tooth type	Sample size	Clinical procedures	Control protocol	PUI protocol	Outcome	Power analysis
Liang *et al.* 2013[22] China	84	Single-root teeth	Control n = 43 PUI n = 41	Instrumentation: Rotary system (FKG Dentaire) Irrigating solution: 5.25% NaOCl	The canals were filled with 5.25% NaOCl with flow rate of approximately 0.2 mL/sec. It was used 2 mL 15% EDTA solution for one minute. Canals were finally flushed three times with 2 mL 5.25% NaOCl at a flow rate of 0.2 mL/sec.	In the ultrasonic group, after every other instrument, the irrigant was also activated by using a #20 stainless steel parallel-shaped noncutting instrument (IrriSafe; Satelec Acteon) with an ultrasound for ten seconds. It was used 2 mL 15% EDTA solution for one minute. A volume of 2 mL 5.25% NaOCl was delivered three times, after which the irrigant was ultrasonically activated for ten seconds.	CBCT absence and reduction of the radiolucency together were observed in the ultrasonic group in 95.1% and in the control group in 88.4% of the cases. *no statistical difference ($p > 0.05$)	19.89%
Herrera *et al.* 2016[21] Brazil	24	Maxillary single-rooted teeth with one root canal per root	Control n = 12 PUI n = 12	Instrumentation: Gates-Gliden burs (Dentsply Maillefer) and Mtwo rotary nickel-titanium system (VDW) Irrigating solution: 2% chlorhexidine gel (CHX) and saline solution	1 mL of EDTA for 30 seconds; then, EDTA was aspirated and refreshed, repeating the procedure twice	The root canal was filled with 1 mL of EDTA for 30 seconds using an ultrasonic tip (E5; Helse, Brazil) inserted 2 mm short of the root canal length. Power setting of the ultrasonic device was 30% (repeated twice)	Bacteria counting (UFC): non-selectively obligate anaerobes and facultative anaerobes bacteria. Median (min–max). After chemo-mechanical preparation: 1.5 x 104 (2.2 x 103 - 3 x 104) After EDTA control: 2.4 x 103 (1.9 x 102 - 4.3 x 103) After EDTA with PUI: 8.4 x 103 (2.3 x 102 - 1.1 x 104) *no statistical difference ($p > 0.05$)	98.02%
Nakamura *et al.* 2017[23] Brazil	50	Single-rooted teeth or in one root with a single canal from multi-rooted teeth	Control n = 25 PUI n = 25	Instrumentation: R40 or R50 Reciproc instruments (VDW) Irrigating solution: 2.5% NaOCl	The root canal was filled with 2 mL of 2.5% NaOCl, inserted up to 2 mm short of the working length. The procedure was repeated twice. A volume of 2 mL of 17% EDTA was added in the canal root for 30 seconds. The procedure was repeated twice. A volume of 2 mL of 2.5% NaOCl was added. The procedure was repeated twice	Irrigant was activated by a smooth wire with 0.2 mm diameter and.01 taper (Irrisonic; Helse, Brazil) driven by a piezoelectric ultrasonic device set at 10% power. The root canal was filled with 2 mL of 2.5% NaOCl, inserted up to 2 mm short of the working length, and then activated for 30 seconds (repeated twice). A volume of 2 mL of 17% EDTA was activated for 30 seconds (repeated twice). A volume of 2 mL of 2.5% NaOCl and activated for 30 seconds (repeated twice)	Bacteria counting (qPCR): total bacteria Media (min–max) Mean (standard deviation) Control: after chemo-mechanical preparation 3.53 X 10^4 (0–3.05 X 10^6) After irrigation – 1.08 X 10^4 (0–3.38 X 10^5) PUI: after chemomecanical preparation 1.41 X 10^4 (0–5.67 X 10^5) After irrigation: 4.29 X 10^3 (0–2.22 X 10^4) *significant reduction in PUI protocol ($P < 0.05$)	1.15%

of PUI on endodontic treatment healing and/or root canal disinfection.

The reduced number of included studies can be highlighted as the main limitation of the current systematic review. A total of 346 studies were obtained from the electronic search. After the eligibility criteria and discarding of any duplicates, only three were included.[22,23,24] It is important to emphasise that these three included studies were classified as low risk of bias and were well designed, even though they were not absolutely comparable due to important discrepancies in the methodology design. However, two studies presented a low effect size after power analysis performance.[22,24] The power analysis of the included studies demonstrated that only Herrera *et al.*[23] was adequately powered to find significant results, since the power of this study was higher than 95%. The limited sample size was an important reason for the low power of the studies. The

Table 3 Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system of rating quality of evidence and grading strength of recommendations in systematic reviews

Certainty assessment							Summary of considerations
No of participants (studies)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Impact
158 (3 RCTs)	Serious*	Not serious	Not serious	Not serious	None	Moderate	It was considered to judge the risk of bias the all domains (random sequence generation, allocation concealment, blinding outcome assessment, incomplete outcome data, selective reporting and other sources of bias). For this intervention (passive ultrasonic irrigation or non-activated irrigation), it was not possible to blind the participants and personnel

*from three included studies, one did not blind the outcome assessment

findings presented here reinforce the need for the conducting of powered studies in this field. In addition, only Liang *et al.*[22] considered a clinical true outcome when evaluating the periapical radiographic and CBCT healing, since Nakamura *et al.*[24] and Herrera *et al.*[23] evaluated disinfection rates as the outcome. In these cases of incomparable methodologies and limited number of included studies, meta-analysis is not recommended. Several studies were excluded due to non-standardised root canal preparation and particularly due to differences in the type, volume and total contact time of the irrigant solution. Therefore, studies lacking standardisation of these variables introduced a known confounder in the comparison and were not considered suitable to answer the current review question.[15,16,17,18,26]

Ideally, randomised controlled clinical trials evaluating the long-term radiographic success of root canal treatment would provide the most reliable evidence, in order to determine whether PUI is more effective than NAI. For this reason, the success rate of root canal therapy was defined as the true clinical outcome during the design phase of this systematic review. However, only one relevant clinical study comparing the clinical success of the two methods was retrieved during the search.[22] Additionally, the healing rates may be affected by various parameters, while a one-year or more follow-up study to assess the role of PUI and NAI may require confounding of other factors. Thus, these trials are scarce in the literature. Therefore, this systematic review included clinical studies that evaluated surrogate outcomes, such as root canal disinfection rates. Studies evaluating surrogate outcomes found controversial results, since Herrera *et al.*[23] did not observe a statistical difference between PUI and NAI protocols, while Nakamura *et al.*[24] found a higher decontamination rate after the PUI protocol. This difference could be attributed to several differences among the studies such as the irrigation protocol and laboratory methods. Regarding the irrigation protocol, Herrera *et al.*[23] used 2% chlorhexidine gel and saline solution while Nakamura *et al.*[24] used 2.5% NaOCl. Moreover, Herrera *et al.*[23] performed PUI using 17% EDTA for 30 seconds three times, while Nakamura *et al.*[24] performed PUI for 30 seconds six times, twice with 2.5% NaOCl, twice with 17% EDTA, and then twice with 2.5% NaOCl. This difference could be also attributed to the analytic method tool differences and the bacteria population assessed. Herrera *et al.*[23] evaluated the CFU reduction of obligate anaerobes and facultative anaerobes bacteria, while Nakamura *et al.*[24] quantified the reduction rates through qPCR and evaluated the total bacteria. Although one of the objectives of endodontic treatment is to reduce the bacterial load inside root canal system, the clinical success depends on varied factors such as immunological response, which plays a key role in infection remission and bone healing.[29] In this sense, it is imperative to analyse clinical studies that evaluate true outcomes. It is also important to point out that the only clinical study included herein that assessed the influence of PUI on periradicular tissue healing failed to find differences between the irrigation protocols, demonstrating that there was no improvement in radiographic healing when PUI was performed.[22]

In the present systematic review, all studies evaluated the true clinical and surrogate outcomes in single-rooted teeth[22,23,24] or in one root with a single canal from multi-rooted teeth.[23] This is an important limitation of this study's findings because the results cannot be applied truly to multi-rooted teeth. The morphologic complexity of these teeth tends to be more challenging for effective root canal disinfection. Moreover, both microbiological studies present limitations because they have collected samples using absorbent paper points. This technique may reveal bacteriological conditions only in the main root canal, as absorbent paper points do not reach microorganisms located in dentinal tubules, lateral canals and apical ramifications. Moreover, the sampling collection method using paper points may not be able to obtain samples that can really represent the bacterial population of the root canal system of infected teeth, which is crucial for the improvement of treatment protocols.

Systematic reviews of RCTs are useful to provide solid scientific evidence to support, or not, the usage of materials and operative techniques in the dental practice.[30] The Cochrane Handbook[20] presents a guide to the risk of bias assessment of RCTs in order to evaluate their methodological quality. The judgement of the overall risk of bias included the assessment of each domain individually. In this context, the particularities of the studies helped the authors to decide the importance of the domains and to choose which one must be considered key.[20] Randomisation is an important tool that guarantees unpredictable exposure allocation and reduces bias selection; for this reason, this domain in particular was chosen as a key one. Two of the selected studies[23] stated that random distribution of participants was performed, and used a software-based method for allocation; however, Herrera *et al.*[20] did not describe details of randomisation and it was necessary to contact the authors, who reported randomisation by shuffling envelopes. In the same way, the allocation concealment, considered a key domain, is important to determine whether intervention allocations could have been

foreseen. The blinding of participants and personnel is an important measurement, however, in some studies, it was not possible. For this reason, in case the PUI protocols included specials devices, the blindness of the participants and personnel was not considered a key domain. However, the blinding of the outcome assessment was considered a key domain, since it was reasonable to perform this during the clinical parameters assessment or bacterial disinfection analysis. Only Liang *et al.*[20] and Nakamura *et al.*[20] performed the assessment of the outcomes blindly. The report of incomplete outcome domain describes the dropout of participants and sample lost. This domain was considered key since the high rates of dropout could create a disproportion among the studied groups; in this systematic review, small sample loses were observed. Selective reporting was included as a key domain. It evaluates if the reports present a suggestion of selective outcome and followed the pre-existing protocol. Finally, the other bias was considered a key domain in order to include any additional bias that could not be mentioned in the previously mentioned domains.

Conclusions

This systematic review highlighted the need for randomised clinical trials comparing PUI and NAI irrigating modalities. Furthermore, the available clinical studies have different clinical protocols and evaluating outcomes, which make them less comparable within and between groups as previously demonstrated. Thus, based on the presented findings, there is no evidence that supports the use of PUI over NAI in clinical practice, in order to improve periapical healing or bacterial disinfection.

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