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Antibacterial Efficacy of Sonic Versus Ultrasonic Irrigation of the Root Canal System: A Systematic Review

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ABSTRACT

Introduction: *Irrigant mechanical agitation has been claimed to enhance the antibacterial efficacy during the root canal treatment. The aim of this study was to systematically review the antibacterial efficacy of ultrasonic compared to sonic agitation of sodium hypochlorite (NaOCl).*

Materials and methods: *Four databases (Cochrane Library, Medline, ScienceDirect and Scopus) were searched to identify systematic reviews, clinical and in vitro trials evaluating biofilm removal following the use of sonic irrigation (SI), passive ultrasonic irrigation (PUI) or both and the conventional syringe and needle irrigation (SNI) on mature permanent teeth or models simulating the root canal. Articles were selected according to the inclusion criteria, data were extracted and the methodological quality was assessed independently by two reviewers.*

Results: *The electronic and hand search retrieved 1028 studies. Two clinical controlled trials, thirteen in vitro controlled trials and one systematic review were included. The risk of bias and quality of the selected studies were qualified as moderate and high according to the JBI (Joanna Briggs Institute) and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) checklists. Overall, both sonic and ultrasonic irrigation improved the bacterial reduction over the conventional irrigation method. However, most of the available evidence could not state significant differences between the antibacterial efficacy of the two methods.*

Conclusion: *It may be concluded that sonic and ultrasonic activation of the irrigants are beneficial in bacterial reduction when compared to conventional needle irrigation, yet, the current data could not find significant differences between the two techniques.*

Keywords

Passive ultrasonic irrigation, Sonic irrigation, Biofilm, Sodium hypochlorite.

Introduction

The occurrence of clinical and radiographic signs related to teeth with previous root canal treatment refers usually to post-treatment apical periodontitis. This latter is mainly caused by bacterial infection that can be persistent from the first root canal therapy or secondary due to a new introduction of bacteria in the root canal trough coronal leakage or obturation failure [1-3].

Because of the complex anatomy of the root canal system, many areas such as isthmuses, lateral canals, apical ramifications and the very apical part of the canal remain untouched during the instrumentation and the conventional irrigation procedures [4,5],

also, bacteria are commonly found at these areas in cases of root canal treatment failure and are able to maintain inflammation of the periapical tissues [6-9]. Actually, there is strong evidence to believe that persistent biofilm during the root canal therapy is the major factor of post- treatment apical periodontitis. Therefore, efficient final irrigation is an important step in the root canal therapy in order to reduce biofilm as much as possible. So far, sodium hypochlorite (NaOCl), introduced in endodontics by Walker in 1936 [10] is still considered the gold star in terms of irrigating solutions as it presents several important properties: antimicrobial efficacy by acting on the bacterial essential enzymatic sites, ability to dissolve organic tissues and acceptable biocompatibility when used in low concentrations (1-6%) [11].

Disinfection of the root canal system is challenging due to the multispecies nature of the biofilm, especially when using conventional irrigation with syringe and needle. In fact, this method seemed to be insufficient to deliver the irrigant in areas hard to reach because of the low velocity and the limited effect beyond the tip of the needle [12]. Therefore, several regimens of mechanical agitation have been developed in order to enhance the disinfection efficacy of NaOCl. Mechanical agitation is mainly achieved via sonic and ultrasonic devices. Passive ultrasonic irrigation (PUI) is used in endodontics for more than 30 years ago [13] and aims to enhance the cleaning effect by producing acoustic streaming and cavitation. This latter occurs when the ultrasonic waves are transmitted from the oscillating file to the irrigant and create growing microbubbles in the fluid until collapse. This leads to a vacuum pressure causing strong shock waves which force the solution into canal ramifications and isthmuses and have a lethal effect on bacteria [14,15]. Such a phenomenon is unlikely to be produced during sonic irrigation (SI) because of the low frequencies of vibration. However, these devices have smooth plastic tips which are safer for the intracanal use [16].

So far, it has been demonstrated that sonic and ultrasonic irrigation can perform in better efficacy over the conventional syringe and needle irrigation (SNI) [17]. Nevertheless, at the time this study was carried out, no previous systematic review allowed the comparison between SI and PUI in terms of biofilm removal.

The aim of our study was to systematically review and critically analyze the current evidence on the bacterial efficacy when SI is compared to PUI.

Material and Methods

The following systematic review was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [18].

Protocol and registration

A protocol of the present systematic review was done according to the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) [19] checklist and was previously published on Open Science Framework. Link to the protocol: https://osf.io/qhv3k.

PICOS Question

The research question was formulated based on PICOS (Population, intervention, comparison, outcomes and study design) format: "Does Passive Ultrasonic Irrigation result in a better biofilm removal when compared to Sonic Irrigation on mature permanent teeth based on controlled trials and systematic reviews?"

- Population: Mature Permanent teeth.
- Intervention: Passive ultrasonic irrigation and Sonic irrigation.
- Comparison: Conventional syringe and needle irrigation.
- Outcomes: Biofilm removal.
- Study design: Controlled trials and Systematic Review on controlled trials.

Eligibility criteria

Studies that met all the following inclusion criteria based on the PICOS question were included in the review:

- Systematic reviews, clinical controlled trials or *in vitro* controlled trials performed on mature permanent teeth without any anterior root canal treatment.
- Systematic reviews, clinical controlled trials or *in vitro* controlled trials performed using models simulating the root canal system.
- Studies evaluating passive ultrasonic irrigation to another irrigation technique in biofilm removal.
- Studies evaluating sonic irrigation to another irrigation technique in biofilm removal.

Studies that met any of the following exclusion criteria were excluded:

- Studies that performed agitation of the irrigant on teeth with root caries, resorption, fractures or fractured instruments within the canal.
- Studies not evaluating the antibacterial effect of irrigation procedures.
- Studies using irrigants other than sodium hypochlorite and EDTA.
- Not standardized instrumentation in the compared groups.
- Studies not including a Passive Ultrasonic Irrigation nor Sonic Irrigation group.
- Studies not including a conventional syringe and needle irrigation group as the control.

The research included all the studies published between January 2010 and January 2021. Only publications in English and those with translations available in English were selected.

Information Sources

An electronic search strategy was conducted for eligible literature from January 2010 to January 2021 on 4 data-bases: Medline, ScienceDirect, Scopus and Cochrane Library.

Search

Appropriate key words and Mesh terms; "therapeutic irrigation", "root canal preparation", "sodium hypochlorite", "ultrasonic therapy", "sonication", "root canal irrigants", "therapeutic irrigation", "sonic agitation", "ultrasonic agitation", "root canal irrigation", "sonic activation", "ultrasonic activation" and

Table 1: Electronic Search Strategy on Medline, ScienceDirect, Scopus and Cochrane Library.

Search strategy	Results			
	PubMed	ScienceDirect	Scopus	Cochrane
Therapeutic Irrigation / instrumentation [Mesh] OR "Therapeutic Irrigation/methods" [Mesh])) AND ("Root Canal Preparation/instrumentation"[Mesh]	19	92	14	17
OR "Root Canal Preparation / methods"[Mesh])) AND "Sodium Hypochlorite" [Mesh]) AND "Ultrasonic Therapy"[Mesh](("Sonication"[Mesh]) AND "Ultrasonic Therapy" [Mesh]) AND "Root Canal Irrigants"[Mesh]	5	54	7	1
(("Sonication" [Mesh]) AND "Ultrasonics"[Mesh]) AND "Root Canal Irrigants"[Mesh]	14	140	24	4
(("Sonication"[Mesh]) AND "Ultrasonic Therapy"[Mesh]) AND "Therapeu tic Irrigation"[Mesh]	6	38	8	1
(("Biofilms"[Mesh]) AND "Root Canal Irrigants"[Mesh]) AND "Ultrasonics"[Mesh]	19	47	55	9
(("Biofilms"[Mesh]) AND "Root Canal Irrigants"[Mesh]) AND "Sonication"[Mesh]	2	83	0	2
"Sonic agitation" AND "Ultrasonic agitation" AND "Root canal irrigation"	40	91	1	0
"Sonic activation" AND "Ultrasonic activation" AND "Root canal Irrigation"	82	135	18	0

"Biofilm" were selected from articles published in endodontic journals and were used in a series of combinations repeated each time in the 4 data bases. The search on ScienceDirect was restricted to Review articles and research articles.
The electronic search strategy is shown in Table 1.

Furthermore, the references list of the selected articles was reviewed for titles suggesting the same topic if they were not identified previously.

Study selection

Duplicates were removed and two reviewers screened titles and abstracts (and the full-text copy in cases where abstracts were not available) of potentially relevant studies independently and excluded off-topic articles that didn't meet the inclusion criteria. In case of doubt or disagreement, the studies were included and the full-texts were assessed for eligibility in the next step. The full texts of the remaining titles were obtained and were evaluated. Studies were included if they met all the inclusion criteria based on the PICOS question. Studies that met any of the following exclusion criteria were excluded.

Data collection process

Pre-determined data were extracted in duplicate from the included studies by the two reviewers for evidence synthesis and quality assessment. Data were arranged in data tables.

Data items

The following data were extracted:

1. First author name and year of publication.
2. Study design.
3. Sample size.
4. Type of samples used.
5. Apical size, taper and if the system was closed.
6. Bacterial inoculation, incubation's period and environment.
7. Irrigant solutions used and volume.
8. Devices tested, controls used, power setting/frequency and depth from the working length.
9. Method of assessment.
10. Randomization and blinding if applicable.
11. Statistical methods adopted and main outcomes.

Quality assessment and risk of Bias in individual studies

Validity of the included trials and systematic reviews was assessed based on the CON- Solidated Standards of Reporting Trials (CONSORT) [20] and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [18] respectively. Also, methodological quality of the clinical controlled trials was evaluated according to the Joanna Briggs Institute (JBI) clinical appraisal Checklist [21]. The critical appraisal tool was adapted to in vitro trials as it was described in a previously published study and only 9 out of 13 items were kept [22] (Table 2). The risk of bias was assessed independently by the reviewers and a cumulative score was calculated for each study. Clinical studies were judged with a low methodologic quality if they had a score of 1, 2, 3 or 4, moderate methodologic quality if they had a score of 5, 6, 7 or 8 and a high methodologic quality if they had a score of 9, 10, 11, 12 or 13, while in vitro studies were judged with a low methodologic quality if they had a score of 1, 2 or 3 points, moderate methodologic quality if they had a score of 4, 5 or 6 points and a high methodologic quality if they had a score of 7, 8 or 9 points.

Table 2: Modified Joanna Briggs Institute critical appraisal tool.

	Yes	No	Unclear
1. Was true randomization used for assignment of participants to treatment groups?			
2. Was allocation to treatment groups concealed?			
3. Were treatment groups similar at the baseline?			
5. Were those delivering treatment blind to treatment assignment?			
6. Were outcomes assessors blind to treatment assignment?			
7. Were treatment groups treated identically other than the intervention of interest?			
10. Were outcomes measured in the same way for treatment groups?			
11. Were outcomes measured in a reliable way?			
12. Was appropriate statistical analysis used?			

The methodological quality of the systematic reviews included was assessed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [18]. Studies were judged with a *low methodologic quality* if they had a score between 1 and 9, *moderate methodologic quality* if they had a score between 10

and 18 and a *high methodologic quality* if they had a score between 19 and 27.

The quality of the studies was assessed independently by two reviewers. In case of disagreement, it was solved through discussion between them.

Results

Due to the variability of the systems being employed, the protocols and the variables being assessed, it was not possible to standardize the research data. Hence, a meta-analysis was not feasible. A narrative synthesis of the available findings was conducted instead.

Study selection

The initial search resulted in a total of 1028 titles, 709 were removed due to duplications. The remaining 319 were screened according to the titles and the abstracts for eligibility and 286 studies were excluded. The publications ranged from January 2010 to January 2021 except one study that dates from 2007. 33 titles were then eligible for full text evaluation by at least one reviewer. 17 studies did not meet the inclusion criteria and were excluded. Reasons for exclusion are presented in Figure 1. Finally, 16 studies were selected for the qualitative synthesis including 2 clinical controlled trials, 13 *in vitro* studies and one systematic review. All the included articles were written in English.

Study characteristics

Of the 13 in vitro studies, three used standardized 3D printed straight root canal models including one with a lateral canal in the apical third [34]. A priori sample size calculation was reported

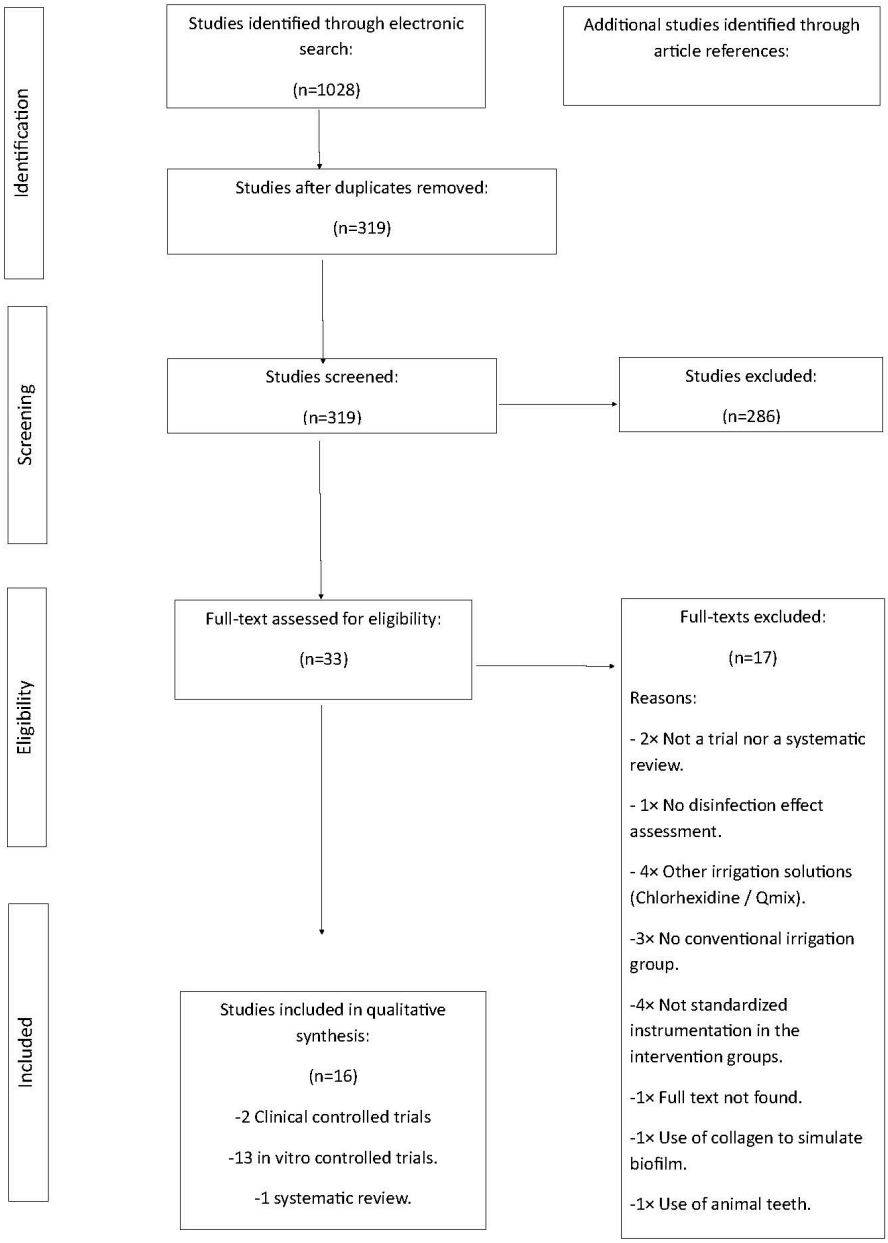

Figure 1: PRISMA flow diagram.

Table 3: Study details of articles included in data synthesis. MTT, 3-(4,5 dimethylthiazole-2- yl)-2,5-diphenyl tetrazolium; CLSM, Confocal laser scanning microscope; PCR, Polymerase chain reaction; CFU, Colony forming unit; SEM, Scanning electron microscope; TEM, Transmission electron microscope; NR, Not reported; NaOCl, Sodium hypochlorite.

Study	Type	Sample size	Sample	Apical size/ Taper	Apex	Biofilm	Incubation period	Aerobic/ Anaerobic environment	Assessment
Mohmmed et al. 2016 [23]	In vitro	40	3D printed straight root canal model made of clear photopolymer material	30/06	Closed system using wax.	1,1× 10^8 CFU/ ml Enterococcus Faecalis ATCC 19433 grown on the 3 apical mm of each one-half model.	10 days	NR	-Canals were stained using a crystal violet dye and the biofilm removal was recorded during irrigation. The percentage of residual biofilm was quantified using Image-pro Plus 4.5 software.
Neuhaus et al. 2016 [24]	In vitro	24	Extracted straight and curved roots from maxillary premolars, palatal roots from maxillary molars and maxillary front teeth	25/08	Closed system using composite.	Roots were inoculated with 4 different microbes or combinations of microbes: **1**. S. gordonii ATCC 10558 and A. oris ATCC 43146. **2**. S. gordonii ATCC 10558 and F. nucleatum ATCC 25586. **3**. E. faecalis ATCC 29212. **4**. Candida albicans ATCC 76615.	21 days	1-2: Anerobic 3-4: Aerobic	Microbiological samples were taken from each root canal immediately after irrigation procedure, and after 3, 5 and 7days. A quantitative dichotomic analysis was performed to confirm the presence or absence of bacteria.
Zeng et al. 2018 [25]	In vitro	38	Single-rooted premolars. Decoronated to produce standard root length of 15 mm	40/06	Closed system using resin	Roots were sterilized then inoculated with 1× 10^8 cells/ml of Enterococcus Faecalis ATCC-29212	21 days	Aerobic	-Root canals were sampled before and after irrigation. -Bacterial metabolic activity was evaluated on 12 teeth from each group using MTT solution and the percentage of intracanal bacterial load was calculated. -3 teeth from each canal were split longitudinally, stained using a LIVE/DEAD BackLight bacteria viability kit and the percentage of "dead" bacteria was assessed under CLSM.
Bago et al. 2012 [26]	In vitro	114	Extracted human teeth with single root canal. Teeth were decoronated to obtain a standard root length of 12 mm.	30/09	Closed system using a composite resin	Teeth were sterilized then inoculated with Enterococcus Faecalis ATCC 29212	7 days	NR	Root canals were sampled, before and after irrigation and incubated for 48H at 37°C before colony-forming units (CFUs) grown count. The presence of E. Faecalis was also confirmed by PCR.
Pedulla et al. 2019 [27]	In vitro	140	Extracted human teeth with a single straight root canal. Teeth were decoronated to obtain a standard root length of 16 mm.	40/04	Closed system using cyanoacrylate	All teeth were sterilized then 130 inoculated with 1,5×10^8 CFU/ml of Enterococcus Faecalis 29212	21 days	Anaerobic	-Root canals were sampled twice; immediately after irrigation and 24h after, then CFUs count was performed. -Turbidity of the samples was also recorded as an indicator of bacterial growth.
Pasqualini et al. 2010 [28]	In vitro	112	Extracted human teeth with a single root canal. Teeth were decoronated to obtain a standard root length of 15 mm.	30/09	Closed system using sticky wax.	All teeth were sterilized with ethylene oxide then canals were infected with 30µL of Enterococcus Faecalis ATCC 29212	2 hours	Aerobic	Samples were taken from the root canals and were then incubated at 37°C for 24 hours under aerobic conditions. CFUs grown were then counted.
Al-Obaida et al. 2019 [29]	In vitro	50	Extracted anterior human teeth. Teeth were decoronated to obtain a standard root length of 16 mm.	30/04	Open system.	All teeth were sterilized in an autoclave at 121°C then 45 root canals were infected with 1× 10^8 cells/ml of Enterococcus Faecalis	21 days	Aerobic	Roots were split longitudinally, stained using a LIVE/ DEAD BackLight bacteria viability kit and the percentage of "dead" bacteria was assessed under CLSM at each third.
Rödig et al. 2018 [30]	In vitro	65	Extracted human teeth with single and straight root canals. Teeth were decoronate d to obtain a standard Root length of 15 mm.	40/02	Closed system using acrylic resin.	All teeth were sterilized in an autoclave at 121°C then the root canals were infected with 1,5× 10^8 cells/ml of Enterococcus Faecalis ATCC 29212	72 hours	Aerobic	Samples were taken from the root canals and incubated aerobically for 24 hours. Colony-forming units (CFUs) grown were then counted.
Mohmmed et al. 2017 [31]	In vitro	18	Straight root canals of 18mm length were manufactured using 3D printer.	30/06	Closed system using sticky wax	Models were sterilized using gas plasma with hydrogen peroxide, then the 3 apical mm of one-half root canal were immersed with 1.1 × 10^8 CFU/ ml of Enterococcus Faecalis	10 days	Anaerobic	-Viability of bacterial cells was assessed under CLSM. - Effect of NaOCl on the residual surface biofilm was assessed under SEM. - Effect of NaOCl on the residual surface biofilm and individual cells was assessed under TEM.

Azim et al. 2016 [32]	In vitro	78	Extracted mandibular premolars and distal roots of mandibular molars with a single root canal. Teeth were decoronated to obtain a standard root length of 18mm.	25/04	Closed system using composite resin	All teeth were sterilized in an autoclave at 121°C then the root canals were infected with 1× 10^8 cells/ml of Enterococcus Faecalis ATCC 47077	21 days	Aerobic	-Bacterial metabolic activity was evaluated using MTT solution and the percentage of intracanal bacterial load was calculated. - Roots were split longitudinally, stained using a LIVE/DEAD BackLight bacteria viability kit and the percentage of "dead" bacteria was assessed under CLSM at 50µm, 100µm and 150µm from the canal surface.
Maden et al. 2017 [13]	In vitro	80	Extracted canines with a single root canal. Teeth were decoronated to obtain a standard root length of 15mm.	30/09	Closed system using composite resin.	69 Root canals were infected with 1,8 × 10^8 CFU/ml Enterococcus Faecalis ATCC 29212	21 days	Aerobic	Samples were taken from each root canal twice then were incubated at 37°C for 24h. CFUs were counted.
Li et al. 2020 [33]	In vitro	30	Extracted premolars with straight root canals. Teeth were decoronated to obtain a standard root length of 12mm.	30/09	Closed system using flowable composite	All root canals were infected with 1× 10^8 cells per ml of Enterococcus Faecalis 29212	21 days	Aerobic	Roots were split longitudinally, stained using a LIVE/DEAD BackLight bacteria viability kit and the percentage of "dead" bacteria was assessed under CLSM at 300µm
Mohmmed et al. 2018 [34]	In vitro	43	A straight root canal model of 18 mm manufactured using 3D printing by the assembly of two similar half root and containing a lateral canal at the 3 apical mm.	30/06	Open system.	All models were sterilized using gas plasma with hydrogen peroxide vapor, then the 3 apical mm of one half of each root canal were infected with 1,1 × 10^8 Enterococcus Faecalis ATCC 19433	10 days	NR	-Biofilm removal at the 3 apical mm was recorded during the irrigation and the percentage of residual biofilm was calculated. -Roots were also examined under SEM to assess the appearance of bacteria at the 3 apical mm.
Carver et al. 2007 [35]	In vivo	31	Adult patients with mandibular molar presenting a pulpal necrosis, an acute or chronic periapical periodontitis and a radiolucentcy on the mesial root periapex of 2×2mm minimum.	30/04 or 30/06	Closed apex	-	-	-	Three bacterial samples were taken from each root canal before instrumentation, after instrumentation and after irrigation, and were incubated for 7 days. CFUs were counted under an operating microscope at 10× magnification.
Huffaker et al. 2010 [36]	In vivo	84	Patients with any tooth with apical periodontitis and negative cold test.	-	Closed apex	-	-	Anaerobic	Samples were taken from each root canal before and after the irrigation procedure and were then incubated anaerobically for 7 days. Bacterial growth was then observed.

Table 4: Characteristics of Use for Conventional Needle Irrigation. WL: Working length; NaOCl: Sodium hypochlorite; EDTA: Ethylene diamine tetra acetic acid; NR: Not reported.

Study	Needle	End type	Gauge	Irrigant/Volume	Depth from the WL	Time
Mohmmed et al. 2016 [23]	Monoject	Side-cut-open-end	27	NaOCl 2,5%/ 9ml	3 mm	30 sec
Neuhaus et al.2016 [24]	Endo-irrigation needle KerrHawe	NR	30	NaOCl 1,5%/ 6ml EDTA (17%)/1ml	0 mm	NR
Zeng et al.2018 [25]	ProRinse Endodontic Irrigation Probe	Closed-ended	30	NaOCl 3%/ 1,5ml	1 mm without binding	30 sec
Bago et al.2012 [26]	BD Microlance	NR	30	NaOCl 2,5%/5ml	2 mm	60 sec
Pedulla et al.2019 [27]	Max-I-Probe	NR	30	NaOCl 3%/3ml.	Deep without binding	60 sec
Pasqualini et al. 2010 [28]	NR NR	NR NR	30 30	NaOCl 5%/2ml NaOCl 5%/2ml	2 mm 2 mm	15 sec 30 sec
Al-Obaida et al. 2019 [29]	NR	Side-vented	30	NaOCl 2,5%/NR	2 mm	NR
Rödig et al. 2018 [30]	Endo-EZE	Side-vented	30	NaOCl 1%/2ml	1 mm	30 sec
Mohmmed et al. 2017 [31]	NR	Side-cut open-ended	27	NaOCl 2,5%/9ml	3 mm	30 sec
Azim et al. 2016 [32]	NR	Side-vented	30	EDTA 17%/2ml NaOCl 6%/3ml	2mm	1 minute 3 cycles of 30 sec
Maden et al. 2017 [13]	Max-I-Prob	NR	30	NaOCl 5,25%/5ml	2 mm	60 sec
Li et al. 2020 [33]	NR	Side-vented	30	NaOCl 5,25%/2ml EDTA 17%/2ml Sterile water/2ml	1mm	60 sec
Mohmmed et al./2018 [34]	Monoject	Side-cut open-ended	27	NaOCl 2,5%/9ml	NR	30 sec
Carver et al./ 2007 [35]	NR	NR	NR	NaOCl 6%/15ml	Deep without binding	NR
Huffaker et al./ 2010 [36]	Monoject	Side-vented	27	NaOCl 5%/NR	NR	2 cycles of 30 seconds

Table 5: Characteristics of Use for Sonic Irrigation. WL: Working length; Cpm: Cycles per minute; NR: Not reported; NaOCl: Sodium hypochlorite; EDTA: Ethylene diamine tetra acetic acid.

Study	Irrigant/Volume	Device	Power setting/ Frequency	Tip/Taper	Depth from the WL	Agitation time
Mohmmed et al. 2016 [23]	NaOCl 2,5%/ 9ml	EndoActivator	10000 cpm	25/04	2 mm	30 sec
Neuhaus et al. 2016 [24]	NaOCl 1,5%/ NR	SonicFlex	6 kHz	EDDY 25/04	0 mm	3 cycles of 20 seconds
Zeng et al. 2018 [25]	NaOCl 3%/1,5ml	Proxeo ZA-55-LM	6 kHz	EDDY 25/04	1 mm	30 sec
Bago et al. 2012 [26]	NaOCl 2,5%/5ml	EndoActivator	10000 cpm	24/04	2 mm	30 sec
Pedulla et al.2019 [27]	NaOCl 3%/3ml	EndoActivator	167,67 Hz	25/04	NR	3 cycles of 20 seconds
Pasqualini et al.2010 [28]	NaOCl 5%/5ml	EndoActivator	10000 cpm	15/02	2 mm	15 sec 30 sec
Al-Obaida et al. 2019 [29]	NaOCl 2,5%/NR	EndoActivator NR	NR NR	NR EDDY 25/04	2mm	1 min
Rödig et al. 2018 [30]	NaOCl 1%/2ml	EndoActivator	10000 cpm	25/04	1mm	30 sec
Mohmmed et al. 2017 [31]	NaOCl 2,5%/9ml	EndoActivator	High power setting	25/04	2 mm	30 sec
Azim et al. 2016 [32]	EDTA 17%/2ml NaOCl 6%/3ml	Not activated EndoActivator	- NR	- 15/02	- 1 mm	- 3 cycles of 30sec
Maden et al. 2017 [13]	NaOCl 5,25%/5ml	EndoActivator	167 Hz	25/04	2mm	60 sec
Li et al. 2020 [33]	NaOCl 5,25%/2ml EDTA 17%/2ml Sterile water/2ml	EndoActivator	10000 cpm	25/04	1 mm	60 sec
Mohmmed et al. 2018 [34]	NaOCl 2,5%/9ml	EndoActivator	NR	25/04	2 mm	30 sec
Huffaker et al. 2010 [36]	NaOCl 5%/NR	EndoActivator	10000 cpm	NR	NR	2 cycles of 30sec

Table 6: Characteristics of Use for Ultrasonic Irrigation. WL: Working length; Cpm: Cycles per minute; NR: Not reported; NaOCl: Sodium hypochlorite.

Study	Irrigant/Volume	Device	Power setting or Frequency	File Size/ Taper	Depth from the WL	Activation time
Mohmmed et al. 2016 [23]	NaOCl 2,5%/ 9ml	Satelec P5 Newtron	Power 7	IrriSafe 20/02	2 mm	30 sec
Neuhaus et al./2016 [24]	NaOCl 1,5%/NR	VDW Ultra	Power set at 20%	Irrisafe tip (size and tip not reported)	1 mm	3 cycles of 20 seconds
Pedulla et al./2019 [27]	NaOCl 3%/3ml NaOCl 3%/3ml	Suprasson P5 Booster EndoUltra	30 KHz 40 KHz	Ultrasonic file ISO 15 (Satelec Acteon) Ultrasonic file ISO 15/02	2 mm 2 mm	3 cycles of 20 seconds 3 cycles of 20 seconds
Al-Obaida et al./2019 [29]	NaOCl 2,5%/NR	EndoUltra	NR	NR	2 mm	1 min
Rödig et al./ 2018 [30]	NaOCl 1%/2ml NaOCl 1%/2ml	VDW Ultra VDW Ultra	30% of the maximum value 30% of the maximum value	IRRI K 15/NR An ultrasonically activated needle (Endo- EZE)	1 mm 1 mm	30 sec 30 sec
Mohmmed et al./ 2017 [31]	NaOCl 2,5%/9ml	Satelec P5	Power setting 7	Irrisafe 20/02	2 mm	30 sec
Maden et al./2017 [13]	NaOCl 5,25%/5ml	MiniEndo SybronEndo	Power setting 4	DT-007 EMS SA	2mm	60 sec
Li et al./2020 [13]	NaOCl 5,25%/2ml EDTA 17%/2ml Sterile water/2ml	Satelec Acteon	Power setting 6	20/-	1 mm	60 sec
Mohmmed et al. 2018 [34]	NaOCl 2,5%/9ml	Satelec P5 Newtron	Power setting 7	IrriSafe 20/02	2 mm	30 sec
Carver et al. 2007 [36]	NaOCl 6%/15ml	MiniEndo	Maximum power setting	25-G needle connected to the MiniEndo handpiece	Deep without binding	1min

Table 7: Outcomes. PUI, Passive ultrasonic irrigation; SI, Sonic irrigation; SNI, Syringe and needle irrigation; PIPS, Photon-induced photoacoustic streaming; CLSM, Confocal laser scanning microscope; MTT, 3-(4,5 dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium.

Study	Intervention groups	Control	Main Outcomes
Mohmmed et al. 2016 [23]	SI: EndoActivator PUI: Satelec P5 Newtron Gutta Percha Agitation	SNI	Biofilm removal: The greatest removal was associated with the PUI group (90.13%) followed by the SI group (88.72%). Both techniques removed significantly more biofilm in comparison with the conventional irrigation, but no significant differences were detected between them.
Neuhaus et al. 2016 [24]	SI: SonicFlex + EDDY PUI: VDW Ultra	SNI Negative control (without any irrigation)	Neither S. gordonii/A. Oris nor S. gordonii/F. nucleatum were detectable after SI and PUI immediately and after 7 days. Both EDDY and PUI were less effective against E. Faecalis and C. Albicans; immediately after irrigation all samples were negative, but after 3 days at least 50% of the samples were positive. There were no significant differences between the EDDY and PUI groups.
Zeng et al. 2018 [25]	SI: Proxeo ZA-55-LM + EDDY	SNI Negative control (sterile specimens, not irrigated) Positive control (contaminated specimens, not irrigated)	MTT assay: No statistically significant differences were detected between EDDY and SNI in bacterial reduction (87.8 ± 4.4% and 91.2 ± 3.9%, respectively). CLSM evaluation: The EDDY group was significantly more efficient than SNI in killing bacteria within 0-50μm and 50-100μm. However, further down the dentinal tubules, significant difference could not be found.
Bago et al. 2012 [26]	SI: EndoActivator Pulsed diode laser irrigation Irradiation with a diode laser Irradiation with a diode laser using 3D Endoprobe	SNI Positive control (NaCl irrigation)	SI was significantly more effective than the SNI in reducing Enterococcus Faecalis populations.
Pedulla et al. 2019 [27]	SI: EndoActivator using NaOCl PUI: Suprasson P5 Booster using NaOCl PUI: EndoUltra using NaOCl SI: EndoActivator using bi-distilled water PUI: Suprasson P5 Booster using bi- distilled water PUI: EndoUltra using bi-distilled water	SNI using NaOCl SNI using bi-distilled water	The bacterial reduction was significantly high in both PUI groups using NaOCl without any significant difference between them, whereas no significant differences were found between the SI group and the SNI with NaOCl. Bi-distilled water did not reduce the bacterial load.

Pasqualini et al. 2010 [28]	SI: EndoActivator for 15 sec SI: EndoActivator for 30 sec	SNI for 15 sec SNI for 30 sec Positive control (irrigation with sterile water)	The most important bacterial load reduction was assigned to the SI group that performed for 30 seconds. However, no significant differences were found among the groups.
Al-Obaida et al. 2019 [29]	SI: EndoActivator SI: EDDY PUI: EndoUltra	SNI Positive control (Irrigation with saline solution) Negative control (sterile specimens, not irrigated)	Overall, The EndoActivator device resulted in the greatest percentage of "dead" bacteria in comparison with the EDDY and the EndoUltra devices. All the agitation methods performed better than the SNI.
Rödig et al. 2018 [30]	SI: EndoActivator PUI: VDW Ultra with intermittent flush PUI: VDW Ultra with continuous flush	SNI Infection control group (saline)	The bacterial load reduction in the SNI group, SI group, PUI with intermittent and continuous flush groups were 99,61%, 98,58%, 99,23% and 99,86% respectively. No significant differences were detected between the groups.
Mohmmed et al./ 2017 [31]	SI: EndoActivator PUI: Satelec P5	SNI Control group (not irrigated)	Reduction in the amount of biofilm was significantly greater in the agitation groups compared to the SNI group. Further significant differences could not be found among the agitation groups.
Azim et al./2016 [32]	SI: EndoActivator XP Endo Finisher PIPS	SNI	MTT assay results: Pairwise comparisons showed no significant difference in the bacterial reduction efficiency between the EndoActivator group and the SNI group. CLSM evaluation: The EndoActivator group detected significantly more dead bacteria at all levels of the coronal and the apical thirds and also at 50µm of the middle third.
Maden et al. 2017 [13]	SI: EndoActivator PUI: MiniEndo SybroEndo Low level electric current agitation	SNI	Both SI and PUI techniques reduced significantly more bacteria than the conventional method, however, there was no significant difference between the two methods.
Li et al./2020 [33]	SI: EndoActivator PUI: Satelec Acteon M3 Max File	SNI Blank control group (Specimens were not instrumented after the inoculation) Post-instrumentation baseline group (Specimens were not irrigated after the instrumentation)	In the apical third: Bacterial inhibition was significantly the greatest in the PUI group, whereas no significant difference was found between SNI and SI groups. In the middle and coronal third: Bacterial inhibition was the greatest in the PUI group, followed by the EA and finally CNI. Pairwise comparisons showed significant differences between all the groups.
Mohmmed et al. 2018 [34]	SI: EndoActivator PUI: Satelec P5 Newtron	SNI	Images analysis: Results showed that the greatest removal of E. Faecalis was attributed to the PUI group, followed by the SI group and finally the SNI group. Pairwise comparisons showed statistically significant differences between all the groups.
	Gutta percha agitation		SEM analysis: **At 3mm form the apex**: Entire biofilm elimination was associated with PUI and SI groups. **At 2mm from the apex**: The greatest biofilm deformation was associated with the PUI group, followed by SI group. **At 1mm from the apex**: The biofilm structure was intact in the SNI group. The destruction of biofilm was noticed in both ultrasonic and sonic groups (PUI>SI). However, unharmed bacterial cells were still identified in both groups.
Carver et al. 2007 [35]	PUI: MiniEndo in continuous flush	SNI	The results showed a significant reduction of positive culture in the PUI in comparison with the SNI group.
Huffaker et al. 2010 [36]	SI: EndoActivator	SNI	No significant difference was found in the disinfection ability between SI and the SNI group.

Table 8: Quality assessment and results of the clinical and *in vitro* studies. JBI, Joanna Briggs Institute.

Study	JBI 1	JBI 2	JBI 3	JBI 4	JBI 5	JBI 6	JBI 7	JBI 8	JBI 9	JBI 10	JBI 11	JBI 12	JBI 13	Total	Methodologic quality
Mohmmed et al. 2016 [23]	1	1	1		1	0	1		1		1	1		8/9	High
Neuhaus et al. 2016 [24]	0	0	1		0	1	1		1		1	1		6/9	Moderate
Zeng et al. 2018 [25]	0	0	1		0	1	1		1		1	1		6/9	Moderate
Bago et al. 2012 [26]	1	0	1		0	0	1		1		0	1		5/9	Moderate
Pedulla et al. 2019 [27]	1	0	1		0	0	1		1		1	1		6/9	Moderate
Pasqualini et al. 2010 [28]	1	0	1		0	0	1		1		0	1		5/9	Moderate
Al-Obaida et al. 2019 [29]	0	0	1		0	1	1		1		0	0		4/9	Moderate
Rödig et al. 2018 [30]	1	0	1		0	0	1		1		1	1		6/9	Moderate
Mohmmed et al. 2017 [31]	1	1	1		0	1	1		1		1	1		8/9	High
Azim et al. 2016 [32]	0	0	1		0	1	1		1		1	1		6/9	Moderate
Maden et al. 2017 [13]	0	0	1		0	0	1		1		1	1		5/9	Moderate
Li et al. 2020 [33]	1	0	1		1	1	1		1		1	1		8/9	High
Mohmmed et al. 2018 [34]	1	1	1		0	0	1		1		1	1		7/9	High
Carver et al. 2007 [35]	1	0	1	0	0	1	1	1	1	1	1	1	1	10/13	High
Huffaker et al. 2010 [36]	1	0	0	0	1	1	1	0	1	1	1	1	1	9/13	High

only once by Li et al. [33]. The sample sizes ranged between 18 and 140 and none of the studies reused the same samples in the different intervention groups. Straight root canals were used in six studies, while only one study used curved root canals but did not specify their outcomes according to the curvatures [24]. No information was reported about the root canal curvature in 6 studies. Root length was standardized in all the in vitro studies except in one [24] and it ranged between 12 and 18mm. Teeth were apically closed using wax (n=3) or more commonly resin (n=8), except in two studies; irrigation was performed on open systems [29,34]. Apical size and taper before irrigation ranged between 25 and 40 and 02 and 09 respectively. This information was not reported in one clinical study because the apical sizes and tapers were variant and dictated clinically by the canal curvature and the initial size of each tooth [36].

In all the *in vitro* trials, root canals were inoculated with a single species Enterococcus Faecalis biofilm. However, Neuhaus el al. [24] had grown in addition multispecies biofilm. The incubation period ranged between 2 hours and 21 days and the environment was varying. In the two included clinical studies [35,36], samples were taken from each root canal after the irrigation procedure and polymicrobial biofilm was assessed.

Six studies carried out CFUs count, four used CLSM to calculate "dead" bacteria after staining with LIVE/DEAD viability kits, two assessed the bacterial metabolic activity using MTT solutions, two assessed the residual biofilm under SEM and two others recorded the biofilm removal and quantified the residual biofilm after each second of irrigation. PCR, turbidity test and bacterial growth were also used as methods of assessment in some studies.

Irrigation

NaOCl was used in concentrations between 1 and 6% and EDTA in 17% concentration. The total volume of irrigant delivered per root canal ranged from 1,5ml to 15ml for NaOCl, and from 1 to 2ml for EDTA. Irrigation was performed through needles between 27 and 30 G, however, the gauge was not reported in one study [35]. In general, needles and the agitating instruments were inserted to the same depth, which varied between 1 and 3 mm from the WL, except in one study that reported a difference [24]. 30 seconds was the most reported contact time (Table 4).

Eight studies tested both PUI and SI, six studies tested SI compared to conventional irrigation and one study reported about PUI in comparison with conventional irrigation.

EndoActivator and EDDY were the two sonic systems described in the included studies. The EndoActivator performed at 160 Hz using the yellow and red tips (15/02 and 25/04 respectively). Whereas the EDDY mounted on different sonic handpieces performed at a higher frequency (6000 Hz). Both sonic systems used smooth tips made in polymer. Furthermore, the EDDY tip was inserted once to the WL [24].

Several ultrasonic scalers and stainless-steel tips were tested and performed at frequencies ranging around 30 kHz. Also, EndoUltra, a cordless ultrasonic device, was reported in two studies and vibrated at a greater frequency which is 40 kHz [27,29]. In most of the studies, ultrasonic irrigation was performed with an intermittent flush; root canals were filled with the irrigant first then an oscillating tip was inserted. However, needles connected to the devices and ultrasonically driven in a continuous flushing and activation process were reported twice [30,35].

One included systematic review reported about the antibacterial efficacy of PUI compared to SNI as a secondary outcome. The review included 19 *in vitro* studies where root canals were mostly inoculated with a single species Enterococcus faecalis biofilm. Culture-based technique and CLSM were the two assessment methods used [37].

Risk of bias within studies

A summary of the methodological quality assessment across the studies is presented in Table 8. None of the studies met all the criteria. After data extraction, the quality of the studies was assessed according to the JBI checklist as it was described previously. The included studies were classified as moderate methodologic quality

and high methodologic quality. Furthermore, the included systematic review was ranked as moderate methodologic quality [37].

Summary measures

Three studies concluded that PUI was significantly more efficient than SI against the intracanal biofilm and five others could not detect any statistically significant differences between the two techniques. Also, most of the studies consented that both agitation techniques were significantly more efficient than SNI. However, Huffaker et al. evaluated the efficacy of SI clinically and it could not significantly differ from SNI in terms of biofilm reduction [36]. This outcome was also confirmed by another *in vitro* trial [27]. Moreover, the EndoActivator was found to reduce more bacteria when compared to devices working in higher frequencies in a study conducted by Al-Obaida et al. [29].

Pedulla et al. also tested the effectiveness of the mechanic agitation devices using bi-distilled water in comparison with agitated NaOCl. Bi-distilled water was shown to be not effective against biofilm even when it's agitated [27].

Furthermore, Căpută et al. systematically reviewed the antibacterial efficacy of PUI compared to SNI, and concluded that PUI was significantly more effective in the majority of the studies (n=10). However, no statistically significant differences could have been found between the two techniques in eight other studies. SNI seemed to be more effective in reducing the bacterial load in one included study [37].

Discussion

The present systematic review discussed the efficacy of passive ultrasonic irrigation compared to sonic irrigation and the efficacy of both techniques in biofilm removal during the root canal therapy. In order to provide a comprehensive answer to the PICOS question, available systematic reviews, clinical and *in vitro* studies were reviewed despite their different level of evidence. The type of each study was clearly mentioned and was taken in consideration during the synthesis.

The included studies tested either sonic or ultrasonic devices or both and the conventional irrigation method using syringe and needle as control.

During the study selection, a number of articles were excluded due to their internal validity issues, mainly the nonstandardized protocols of irrigation among the different groups and the absence of a control group. External validity issues were also considered as reasons to exclude trials with non-representative samples such as animal teeth and trials using unusual irrigant solutions in the protocol.

Overall, the outcomes confirmed the superiority of the agitation devices over the conventional irrigation in terms of bacterial reduction. Also, most of the studies could not detect any significant differences between PUI and SI, however, a moderate level of evidence showed significant superiority for PUI.

All of the included studies tested the mechanical agitation devices on grown single species of Gram-positive facultative Enterococcus faecalis biofilm. Indeed, Enterococcus faecalis has been implicated as a main reason of endodontic treatment failures and has been often identified in teeth with post-treatment apical periodontitis [38-40]. This was also confirmed in our review by the study of Neuhaus et al. who found out that Enterococcus Faecalis was resistant to PUI and SI immediately after the irrigation process and in long term [24]. Furthermore, Enterococcus Faecalis is able to grow biofilm under different conditions [41].

Three included studies used clear 3D printed models as samples for bacterial inoculation and the irrigation procedure [23,31,34]. These materials are different from natural root canals because of the absence of porosity due to dentinal tubules and may have a different behavior towards the attachment and growth of biofilm, nevertheless, harmless bacterial cells were still detected in the apical third of these models. All the same, 3D printing allows the production of very similar and standardized samples wich increases the level of randomization. These models made in transparent materials also allow direct visualization of previously stained canals using crystal violet dye and recording of the bacterial removal during the irrigation process and thus the evaluation of the bacterial reduction in a time-dependent way. The effect of crystal violet dye on the oxidative ability of NaOCl has been discussed, however, measuring of the available chlorine and pH demonstrated that the dye was neutral towards NaOCl [23].

The incubation period before the irrigation process varied between 2 hours and 21 days. According to the findings of Stojicic et al., biofilm resistance to disinfecting agents increases between 2 and 3 weeks of incubation. Otherwise, younger biofilm may be easier to remove during the endodontic irrigation [42]. In our review, almost half of the studies incubated the biofilm for periods under 2 weeks (n=6).

Curved root canals were reported only once [24], while all the studies that provided information about the canal curvature mentioned the use of straight root canals. It has been argued that sonic and ultrasonic devices may be more efficient when used within straight root canals where the instrument can oscillate freely unlike the curved ones [43,44]. Hence, the bacterial reduction may be easier in straight root canals, however, the volume of available evidence concerning biofilm removal from curved root canals is considerably low and further investigations are needed.

Rödig et al. compared the efficacy of continuous ultrasonic irrigation using a flow rate of 4ml/ min to intermittent ultrasonic irrigation on extracted human teeth [30]. No statistically significant differences could be found between the two methods. It is important to note that the study used a safe flow rate to avoid apical extrusion of the irrigant [45].

In addition, Pedulla et al. tested the agitation of NaOCl in comparison with bi-distilled water using sonic and ultrasonic devices [27]. Significant bacterial reduction has been demonstrated

in the NaOCl groups, while the bacterial load in the bi-distilled groups after irrigation has not decreased. These findings confirm that the mechanical agitation is not sufficient against bacteria unless the chemical effect of NaOCl is added [46]. Nevertheless, the mechanical agitation is also needed to increase the bactericidal properties of NaOCl. In effect, ultrasonics are able to accelerate the reaction rate of NaOCl through acoustic streaming and cavitation. Moreover, it has been showed that temperature rise occurs during the ultrasonic activation which enhances NaOCl properties [47-49].

Teeth were decoronated in nine studies to obtain a standardized working length. Although this method allows to enhance the similarity of the samples among the different groups, but it also facilitates the access of the instruments to the root canals which is different from the clinical situation.

Unlike the *in vitro* studies reviewed, the biofilm treated in the two clinical studies was different [35,36]. In these later, the authors investigated the effectiveness of sonic and ultrasonic irrigation on teeth with a primary root canal infection which means on a different anaerobic Gram-negative polymicrobial biofilm [50]. Huffaker et al. concluded that there was no significant difference in the bacterial reduction between the sonic and the conventional irrigation groups which is not in line with the findings of the included *in vitro* trials [36]. This may be due to the difference in resistance of Enterococcus faecalis and the Gram-negative biofilm [50].

Most of the studies assessed the bacterial reduction via CFUs count considered for too long as the gold standard method. However, the reliability of the aforementioned technique is debatable. Actually, this method lacks of accuracy due to the use of the logarithmic scale which is not able to detect small differences [51].

Limitations

The results of the present review are mainly based on *in vitro* studies, only two clinical studies were included. In addition, most of the *in vitro* trials focused on single species Enterococcus faecalis biofilm reduction which is not representative of the polymicrobial intracanal biofilm. Moreover, further studies should assess the effectiveness of PUI and SI on curved root canals.

Conclusion

Within the limitations of this systematic review, it can be concluded that the use of sonic or ultrasonic devices for final irrigation during the endodontic treatment is highly recommended in order to enhance the antibacterial effect of NaOCl. Nevertheless, the current data could not detect enough significant differences between the two mechanical agitation techniques.

References

1. Siqueira J.F, Rôças I.N, Ricucci D, et al. Causes and management of post-treatment apical periodontitis. British Dental Journal. 2014; 216: 305-312.
2. Siqueira J.F, Rôças I.N. Clinical Implications and Microbiology of Bacterial Persistence after Treatment Procedures. Journal of Endodontics. 2008; 34: 1291- 1301.
3. Siqueira J.F, Rôças I. N, Alves F. R. F, et al. Periradicular status related to the quality of coronal restorations and root canal fillings in a Brazilian population. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2005; 100: 369-374.
4. Vertucci F.J. Root canal anatomy of the human permanent teeth. Oral Surgery, Oral Medicine, Oral Pathology. 1984; 58: 589-599.
5. Siqueira Junior J.F, Rôças I. das N, Marceliano-Alves M. F, et al. Unprepared root canal surface areas: causes, clinical implications, and therapeutic strategies. Brazilian Oral Research. 2018; 32: e65.
6. Vieira A.R, Siqueira J.F, Ricucci D, et al. Dentinal Tubule Infection as the Cause of Recurrent Disease and Late Endodontic Treatment Failure: A Case Report. Journal of Endodontics. 2012; 38: 250-254.
7. Nair P.N.R, Sjögren U, Krey G, et al. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: A long-term light and electron microscopic follow-up study. Journal of Endodontics. 1990; 16: 580-588.
8. Ricucci D, Siqueira J.F. Apical Actinomycosis as a Continuum of Intraradicular and Extraradicular Infection: Case Report and Critical Review on Its Involvement with Treatment Failure. Journal of Endodontics. 2008; 34: 1124-1129.
9. Carr G.B, Schwartz R.S, Schaudinn C, et al. Ultrastructural Examination of Failed Molar Retreatment with Secondary Apical Periodontitis: An Examination of Endodontic Biofilms in an Endodontic Retreatment Failure. Journal of Endodontics. 2009; 35: 1303-1309.
10. Cárdenas-Bahena Á, Sánchez-García S, Tinajero-Morales C, et al. Use of sodium hypochlorite in root canal irrigation. Opinion survey and concentration in commercial products. Rev Odont Mex. 2012; 16: 252-258.
11. Estrela C, Estrela C.R.A, Barbin E.L, et al. Mechanism of action of sodium hypochlorite. Brazilian Dental Journal. 2002; 13: 113-117.
12. Haapasalo M, Shen Y, Wang Z, et al. Irrigation in endodontics. British Dental Journal. 2014; 216: 299-303.
13. Maden M, Ertuğrul İ.F, Orhan E.O, et al. Enhancing antibacterial effect of sodium hypochlorite by low electric current-assisted sonic agitation. PLOS ONE. 2017; 12: e0183895.
14. Weller R.N, Brady J.M, Bernier W.E. Efficacy of ultrasonic cleaning. Journal of Endodontics. 1980; 6: 740-743.
15. Martin H, Cunningham W. Endosonics - The ultrasonic synergistic system of endodontics. Dental Traumatology. 1985; 1: 201-206.
16. Plotino G, Cortese T, Grande N. M, et al. New Technologies to Improve Root Canal Disinfection. Brazilian Dental Journal.

2016; 27: 3-8.

17. Virdee S. S, Seymour D. W, Farnell D, et al. Efficacy of irrigant activation techniques in removing intracanal smear layer and debris from mature permanent teeth: a systematic review and meta-analysis. International Endodontic Journal. 2017; 51: 605-621.
18. Moher D, Liberati A, Tetzlaff J, et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Medicine. 2009; 6: e1000097.
19. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews. 2015; 4.
20. Moher D, Schulz KF, Altman D. CONSORT Group (Consolidated Standards of Reporting Trials). The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA. 2001; 285: 1987-1991.
21. http://joannabriggs.org/research/criticalappraisal-tools.ht
22. Ethem Yaylali I, Kececi A.D, Ureyen Kaya B. Ultrasonically Activated Irrigation to Remove Calcium Hydroxide from Apical Third of Human Root Canal System: A Systematic Review of In Vitro Studies. Journal of Endodontics. 2015; 41: 1589-1599.
23. Mohmmed S. alarab, Vianna M.E, Penny M.R, et al. A novel experimental approach to investigate the effect of different agitation methods using sodium hypochlorite as an irrigant on the rate of bacterial biofilm removal from the wall of a simulated root canal model. Dental Materials. 2016; 32: 1289-1300.
24. Neuhaus K.W, Liebi M, Stauffacher S, et al. Antibacterial Efficacy of a New Sonic Irrigation Device for Root Canal Disinfection. Journal of Endodontics. 2016; 42: 1799-1803.
25. Zeng C, Willison J, Meghil M, et al. Antibacterial efficacy of an endodontic sonicpowered irrigation system: An in vitro study. Journal of Dentistry. 2018; 75: 105- 112.
26. Bago I, Plečko V, Gabrić Pandurić D, et al. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. International Endodontic Journal. 2012; 46: 339-347.
27. Pedullà E, Genovese C, Messina R, et al. Antimicrobial efficacy of cordless sonic or ultrasonic devices on Enterococcus faecalis- infected root canals. Journal of Investigative and Clinical Dentistry. 2019; 00: e12434.
28. Pasqualini D, Cuffini A.M, Scotti N, et al. Comparative Evaluation of the Antimicrobial Efficacy of a 5% Sodium Hypochlorite Subsonic-activated Solution. Journal of Endodontics. 2010; 36: 1358-1360.
29. Al-Obaida M.I, Moukaddem R, Allahem Z, et al. Comparison of bacterial removal from dentinal tubules with different irrigant agitation techniques: An in vitro study. The Saudi Dental Journal. 2019; 31: 431-436.
30. Tina Rödig, Felix Zimmermann, Frank Konietschke, et al. Comparison of the antibacterial efficacy of sonic- and two ultrasonic activated irrigation techniques in reducing intracanal Enterococcus faecalis populations. Quintessence Int. 2018; 49: 689-697.
31. Mohmmed S.A, Vianna M.E, Penny M.R, et al. Confocal laser scanning, scanning electron, and transmission electron microscopy investigation of Enterococcus faecalis biofilm degradation using passive and active sodium hypochlorite irrigation within a simulated root canal model. Microbiology Open. 2017; 6: e00455.
32. Azim A.A, Aksel H, Zhuang T, et al. Efficacy of 4 Irrigation Protocols in Killing Bacteria Colonized in Dentinal Tubules Examined by a Novel Confocal Laser Scanning Microscope Analysis. Journal of Endodontics. 2016; 42: 928-934.
33. Li Q, Zhang Q, Zou X, et al. Evaluation of four final irrigation protocols for cleaning root canal walls. International Journal of Oral Science. 2020; 12.
34. Mohmmed S.A, Vianna M.E, Penny M. R, et al. Investigations into in situ Enterococcus faecalis biofilm removal by passive and active sodium hypochlorite irrigation delivered into the lateral canal of a simulated root canal model. International Endodontic Journal. 2017; 51: 649-662.
35. Carver K, Nusstein J, Reader A, et al. In Vivo Antibacterial Efficacy of Ultrasound after Hand and Rotary Instrumentation in Human Mandibular Molars. Journal of Endodontics. 2007; 33: 1038-1043.
36. Huffaker S. K, Safavi K, Spangberg L. S. W, et al. Influence of a Passive Sonic Irrigation System on the Elimination of Bacteria from Root Canal Systems: A Clinical Study. Journal of Endodontics. 2010; 36: 1315-1318.
37. Căpută P. E, Retsas A, Kuijk L, et al. Ultrasonic Irrigant Activation during Root Canal Treatment: A Systematic Review. Journal of Endodontics. 2019; 45: 31-44.e13.
38. Sundqvist G, Figdor D, Persson S, et al. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1998; 85: 86-93.
39. Sedgley C.M, Lennan S.L, Clewell D.B. Prevalence, phenotype and genotype of oral enterococci. Oral Microbiology and Immunology. 2004; 19: 95-101.
40. Marcos Sergio Endo, Fernanda Graziela Corrêa Signoretti, Ariane Cássia Salustiano Marinho, et al. PCR identification of endodontic pathogens and DNA quantification in samples from teeth with posttreatment apical periodontitis. Clinical and Laboratorial Research in Dentistry. 2014; 20: 197.
41. George S, Kishen A, Song P. The Role of Environmental Changes on Monospecies Biofilm Formation on Root Canal Wall by Enterococcus faecalis. Journal of Endodontics. 2005; 31: 867-872.
42. Stojicic S, Shen Y, Haapasalo M. Effect of the Source of Biofilm Bacteria, Level of Biofilm Maturation, and Type of Disinfecting Agent on the Susceptibility of Biofilm Bacteria to Antibacterial Agents. Journal of Endodontics. 2013; 39: 473- 477.
43. Ahmad M, Pitt Ford, T.R. Crum L. A, et al. Ultrasonic debridement of root canals: Acoustic cavitation and its

relevance. Journal of Endodontics. 1988; 1410: 486-493.

44. Van der Sluis L. W.M, Versluis M, Wu M. K, et al. Passive ultrasonic irrigation of the root canal: a review of the literature. International Endodontic Journal. 2007; 40: 415-426.
45. Chang J.W.W, Cheung A.W.T, Cheung G.S.P. Effect of root canal dimensions, injection rate, and needle design on the apical extrusion of an irrigant: an in vitro study. Journal of Investigative and Clinical Dentistry. 2014; 6: 221-227.
46. Pedullà E, Genovese C, Campagna E, et al. Decontamination efficacy of photon-initiated photoacoustic streaming (PIPS) of irrigants using low-energy laser settings: an ex vivo study. International Endodontic Journal. 2012; 45: 865-870.
47. Macedo R. G, Verhaagen B, Wesselink P. R, et al. Influence of refreshment/activation cycles and temperature rise on the reaction rate of sodium hypochlorite with bovine dentine during ultrasonic activated irrigation. International Endodontic Journal. 2013; 47: 147-154.
48. Leonardi D. P, Grande N. M, Tomazinho F. S. F, et al. Influence of activation mode and preheating on intracanal irrigant temperature. Australian Endodontic Journal. 2019; 45: 373-377.
49. Sirtes G, Waltimo T, Schaetzle M, et al. The Effects of Temperature on Sodium Hypochlorite Short-Term Stability, Pulp Dissolution Capacity, and Antimicrobial Efficacy. Journal of Endodontics. 2005; 31: 669-671.
50. Sakko M, TjÄDerhane L, Rautemaa-Richardson R. Microbiology of Root Canal Infections. Primary Dental Journal. 2016; 5: 84-89.
51. M.J. Miller. A fresh look at USP<1223>validation of alternative microbiological methods and how the revised chapter compares with PDA TR33 and the proposed revision to Ph. Eur. 5.1.6. Pharmceut Rev. 2005; 18: 22-35.