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The Effectiveness of Laser Assisted Irrigation in Endocanalary Disinfection and Hard Tissue Debris Removal: A Systematic Review

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ABSTRACT

Introduction: The control of the bacterial load during an endodontic treatment is challenging; the usual chemo-mechanical procedures such as conventional needle irrigation (CNI) has not always shown satisfying outcomes. Hence, the necessity of finding complementary methods such as passive ultra-sonic irrigation (PUI), sonic activated irrigation (SAI) and laser assisted irrigation (LAI) in order to achieve the most complete disinfection of the endodontic system.

The aim of our study is to systematically review and critically analyze the current evidence of the effectiveness of laser assisted irrigation when compared to CNI, PUI, SAI.

***Materials and methods:** Electronic search on four data-bases: PubMed, Scopus, ScienceDirect and Cochrane Library was done looking for Systematic reviews, in-vitro and clinical controlled trials assessing the effectiveness of LAI in removing bacteria and dentine debris. Articles were selected, deduplicated than assessed. The studies that met all the inclusion criteria were included and were further screened in order to extract their data and evaluate their methodological quality.*

***Results:** The electronic search yielded a total of 895 potentially eligible record. After the final stage of selection, 25 studies, that are all in-vitro were included; the risk of bias and quality assessment was done through the Preferred Reporting Items for Systematic Reviews and Meta-Analysis and the Joanna Briggs Institute critical appraisal Checklist. All in all, LAI showed better results when compared to CNI in both removal of bacteria and smear layer, yet when compared to PUI and SAI, the studies could not testify significantly for the superiority of an activation method over another.*

***Conclusion:** The use of LAI increased the potential of the irrigant within the endodontic system, especially when compared to CNI. Yet the current data could not give a conclusive judgment of the effectiveness of LAI when compared to PUI and to SAI; eventually further studies need to be performed.*

Keywords

Canal disinfection, Laser therapy, Root canal therapy, Sodium hypochlorite, Ultra-sonic therapy.

Introduction

The dental pulp; the internal tissue of the teeth, is a physiologically sterile connective tissue in which any microbial invasion would lead to a pathological sign. It results mainly in inflammation and

eventually in pulp death and spread of inflammation/ infection to the peri-radicular tissues [1]. It has been firmly established that bacteria are the prime etiological factor in the development and progression of dental pulp and periapical disease [2].

The dynamics of root canal system infections has been studied along the years. Recently, the development of advanced bacterial assessment techniques has led to considerable progress in clarifying the etiopathogenesis of endodontic infections and has shown that most of them are polymicrobial, with prevalence of obligate anaerobic bacteria [3].

Hence, removing these biological agents, disinfecting the root canal system, obtaining a sterile environment, and maintaining this state, are the major objectives of a root canal treatment. In order to execute an endodontic treatment conforming to the "state-of-the-art", it is highly recommended to do a sufficient and convenient chemo-mechanical instrumentation [4]. Biomechanical preparation of the root canal system involves a variety of actions such as instrumentation, irrigation and sometimes the use of an intracanal medicament [5].

Of these three essential steps of root canal therapy, irrigation is a very important determinant, as it has the ability to reach and impact the areas of the root canal wall which are not touched by mechanical instrumentation (apical third, isthmuses, lateral canals, ramifications and anastomosis…), up to 35% of root canal system may be left uninstrumented if we only rely on mechanical preparation [6]. For this matter, ethylenediaminetetraacetic acid and sodium hypochlorite (NaOCL) are the most used irrigants solution and the most reliable ones [7]. The former is a chelating agent with no antibacterial effect, but it facilitates cleansing and the removal of infected tissue. However, the latter is a strong antimicrobial agent with the capacity to 'dissolve' the organic part of pulp residues and dentinal walls [4].

Still, the traditional method of irrigation using a syringe with a needle often fails in adequate delivery and penetration of irrigant solutions within the complex three- dimensional microstructure of the canal system [8], leaving certain organisms, mainly Enterococci who are considered to be very difficult to eliminate [9]. Enlarging canal walls to include isthmus preparation would rather solve this issue, but it will also result in gross enlargement and would go against the principles of minimally invasive endodontics [10].

Consequently, supplementary irrigation methods such as irrigant activation techniques are therefore needed to improve irrigant distribution and to enhance the elimination of endodontic biofilms and dentine debris within the root canal system [11]. Inter alia, there is manual-dynamic activation (MDA), ultrasonically activated irrigation (UAI or passive ultrasonic irrigation PUI), sonically activated irrigation (SAI) and laser activated irrigation (LAI) [8].

The aim of our study is to systematically review and critically analyze the current evidence of the effectiveness of laser assisted irrigation when compared to Conventional needle irrigation, Passive Ultra-sonic Irrigation, and sonic activated irrigation.

Material and Methods

This systematic review has been performed according to the Preferred Reporting Items for Systematic reviews and Meta analysis "PRISMA" guidelines (Appendix A and B).

Protocol and Registration

For this systematic review a protocol was done and was previously published on Open Science Framework under this registration link: https://mfr.osf.io/render?url=https://osf.io/4tqpn/?direct%26mode=render%26action=download%26mode=render

Picos Question

- **Population:** Mature permanent or primary teeth.
- **Intervention:** Laser assisted irrigation (LAI).
- **Comparison:** Passive ultrasonic irrigation (PUI), sonic activated irrigation (SAI) and conventional needle irrigation (CNI).
- **Outcomes:** Canal disinfection and hard tissue debris removal.
- **Study design:** Clinical or in-vitro controlled trials and systematic reviews.

Based on these elements, the research question was constructed as follows:

"During an endodontic treatment, does laser assisted irrigation results in better canal disinfection and hard tissue debris removal when compared to conventional needle irrigation, to passive ultrasonic irrigation and to sonic activated irrigation based on controlled trials and systematic reviews?"

Eligibility Criteria

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

- Systematic reviews or in-vitro or clinical controlled trials performed on mature permanent or primary teeth without any anterior root canal therapy.
- Systematic reviews or in-vitro or clinical controlled trials performed using models simulating the root canal system.
- Studies evaluating Laser assisted irrigation to another irrigation technique in bacteria and hard tissue debris removal.
- Studies that have been published between January 2011 and January 2022.
- Studies published in English and those with translations available in English.

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

- Studies that performed activation of the irrigants on teeth with root caries, pathologic resorption, fractures or fractured instruments within the canal.
- Studies not evaluating bacteria or hard tissue debris removal.
- Studies using irrigants other than sodium hypochlorite (NaOCL) or ethylenediaminetetraacetic acid (EDTA).
- Not standardized instrumentation in the compared groups.
- Studies not including Laser assisted irrigation group.

- Studies not including a conventional needle irrigation group as the control group.

Information Sources

Direct electronic research was performed using these online databases: PUBMED, SCIENCE DIRECT, SCOPUS, COCHRANE LIBRARY.

Database Research

Electronic database researches were done using these key words and Mesh terms: "Root canal therapy", "Canal disinfection", "Sodium hypochlorite", "Laser therapy" and "Ultra-sonic therapy", they were used in a series of combinations repeated in each one of the 4 Databases.

The electronic search strategy is shown in Table 1.

Study Selection

Studies selected for this review were all the studies that resulted in the 4 databases in the 2 last combinations, which are 895 records:

- #11: (Root canal therapy) AND (canal disinfection) AND (laser therapy) AND (sodium hypochlorite)
- #12: (Root canal therapy) AND (laser therapy) AND (ultrasonic therapy)

Studies selected were transferred to Zotero reference manager software, where duplication of studies were identified and removed.

Initial screening of studies was done on the basis of title and abstract (and of the full- text exceptionally if the abstract was not clear or available) in order to identify the potentially relevant studies and to exclude the studies with no relevance shown, the off-topic and those that did not meet the inclusion criteria. In case of doubt about a study's relevance, it was included than it was properly assessed in the full-text screening.

All in all, studies that met all the inclusion criteria were included and studies that met any of the exclusion criteria were excluded.

Data Collection Process

Pre-determined data were extracted from the included studies by the two reviewers for evidence synthesis and quality assessment. Then, 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. Aim of the study.
4. Sample size.
5. Type of the samples and canal's anatomy.
6. Instrumentation, apical size, taper.
7. Open or closed system.
8. Method of assessment.
9. Statistical analysis methods
10. Irrigant's solution used, concentration and volume.
11. Devices tested, tips used, parameters.
12. Depth from the working length and working time.
13. Randomization and blinding if applicable.
14. Main outcomes.

Quality Assessment and Risk of Bias in Individual Studies

Due to the lack of a specific method of assessment for in vitro studies, validity of the included trials and systematic reviews was assessed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). Also, methodological quality of the controlled trials was evaluated according to the Joanna Briggs Institute (JBI) critical appraisal Checklist (Appendix E).

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 [12] (Appendix F).

The risk of bias was assessed independently by the reviewers and a cumulative score was calculated for each study.

Table 1: Electronic search strategy on PUBMED, SCIENCE DIRECT, SCOPUS and COCHRANE LIBRARY published between January 2011 and January 2022.

	Combination	Pubmed	Science Direct	Scopus	Cochrane Library	Total
1	(Root canal therapy) AND (canal disinfection)	1510	1320	81	72	2983
2	(Root canal therapy) AND (Laser therapy)	388	1698	34	89	2209
3	(Root canal therapy) AND (sodium hypochlorite)	948	1100	265	126	2439
4	(Canal disinfection) AND (laser therapy)	230	640	17	28	915
5	(Canal disinfection) AND (sodium hypochlorite)	1266	1295	136	73	2770
6	(Laser therapy) AND (sodium hypochlorite)	163	825	50	32	1070
7	(Root canal therapy) AND (canal disinfection) AND (laser therapy)	216	505	2	27	750
8	(Root canal therapy) AND (canal disinfection) AND (sodium hypochlorite)	820	670	32	32	1554
9	(Root canal therapy) AND (Laser therapy) AND (sodium hypochlorite)	139	378	6	29	552
10	(Canal disinfection) AND (laser therapy) AND (sodium hypochlorite)	127	293	10	18	448
11	(Root canal therapy) AND (canal disinfection) AND (laser therapy) AND (sodium hypochlorite)	123	284	21	18	446
12	(Root canal therapy) AND (laser therapy) AND (ultrasonic therapy)	49	394	0	6	449
					TOTAL	16 585

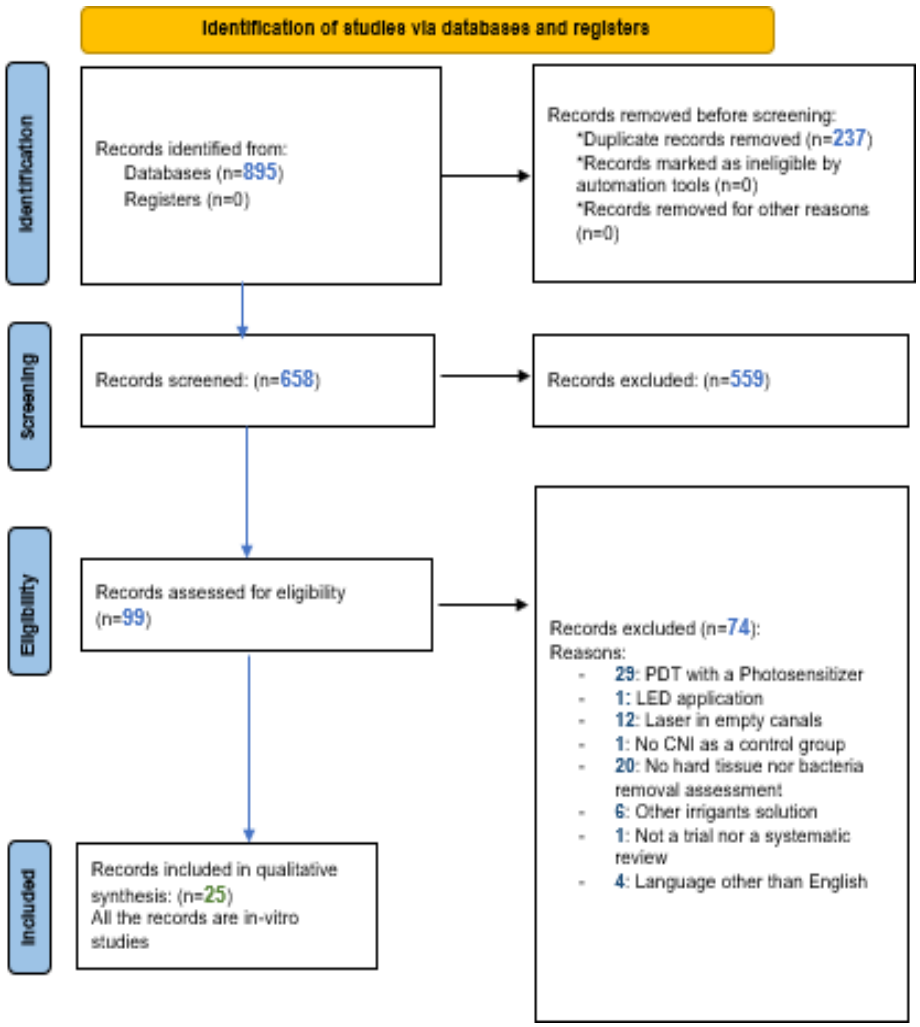

Figure 1: PRISMA flow diagram for studies retrieved through the searching and selection process.

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 out of 13points, 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 out of 9 points.

The methodological quality of the systematic reviews included was assessed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). 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

Study selection

The electronic search process yielded 895 potentially eligible record, including 172 records from PUBMED, 678 from SCIENCE DIRECT, 21 from SCOPUS and 24 from COCHRANE LIBRARY; of which 237 entries were removed after deduplication. After screening of the titles and abstracts, 559 articles were excluded. 99 article were selected for full-text reading. Of these 99 studies, 74 studies were further excluded; the reasons why are reported in the flow chart in (Figure 1). After the final stage of selection, 25 studies that are all in-vitro were included in the systematic review for qualitative analysis.

Study Characteristics

Out of these 25 in-vitro studies, 11 assessed the removal of dentine debris and smear layer within the canals, while 16 articles treated the antibacterial effect of LAI and assessed essentially the removal of Enterococcus faecalis except for one article that assessed the removal of Porphyromonas Gingivalis, Streptococcus Salivarus and Prevotella Intermedia [18]. The sample sizes ranged from 16 [31] to 335 [33]. with only one study that re-used the same samples for every irrigation protocol [30].

Table 2: Details extracted from the articles included in data synthesis.

Study	Type	Smear layer or Bacteria's Removal assessment	Sample size/type	Canal's anatomy	Instrumentation	Apex	Area of interest	Assessment
Lagemann et al. 2014 [13]	*In-vitro*	Smear layer	40/ Human permanent teeth	single patent canals with a standardized length of 12 mm	Protaper ® rotary Ni-Ti files (Maillefer, Dentsply, Tulsa, OK, USA) to size F3 (30) and the WL was 1 mm short of the apex	Open system	Apical, middle and coronal third	***SEM:** scanning electron microscopy at a final magnification of x10000, giving 6 SEM images per root. Images were assessed with a validate image analysis method to quantify smear layer removal. ***Data analysis:** Kruskall-Walli's test with Dunn's post-hoc test.
Korkut et al. 2018 [14]	*In-vitro*	*Smear layer *Enterococcus faecalis	45/Distal roots of human primary mandibular molar	Roots with 10+/-1mm of length, minimal apical resorption (at least 2/3 of the root Remaining) with no visual perforating resorption	Protaper ® rotary system (Dentsply Tulsa Dental, OK) until finishing file F1 to the WL irrigation was done with 2ml of 5% NaOCL between each file	Sealed with flowable composite	*Remaining fluid from the treated canals *SEM: The most visible part of the apex	***Enumeration of E. Faecalis:** plate count technique which consists of an incubation onto three plates of Columbia sheep blood agar for 48h at 35 °C then the identification of the colonies was occasionally done by a biochemical test system (Microgen Bio-products, Camberley, UK) ***Smear layer removal:** by SEM (SU1510; Hitachi, Ibaraki, Japan) at 20kV, and the apical third was evaluated at a x1000 magnification. A total of 20 images were evaluated by three blinded observers using Takeda et al. scoring system ***Statical analysis:** was done using a statistical software (SPSS, Inc, Chicago, IL)
Suer et al. 2020 [15]	*In-vitro*	Enterococcus faecalis	81/ Human mature Permanent mandibular premolar	Single root canals with no curves nor abnormal shapes	NITI FLEX® K files (Dentsply, Germany) up to size #50 using Step-back technique to a WL 1mm short of the apical foramen and Gates Glidden Burs #2,3 and 4 (JS Dental, Ridgefield, USA) to allow the entrance of the fiber to the apical area. Irrigation was done with 0,9% saline solution only	Closed with flowable composite resin (Clearfil Majesty Flow, Kuraray, Medical Inc)	*Remnant fluid from the treated canals *SEM: coronal, middle and apical third of the canals	***Microbiological evaluation:** 10 µl of remnant saline solution used as a final rinse was collected then cultured on sheep blood agar at 37°C for 24h then the colonies were counted ***SEM:** (JSM 6400, JEOL, Tokyo, Japan) to evaluate the smear layer removal ***Statical analysis:** was done using a statistical software program (SPSS, Inc, Chicago, IL)
Tokuc et al. 2019 [16]	*In-vitro*	Enterococcus faecalis	95/ Extracted single rooted premolars	Single straight root canals with a completely formed apex	Stainless steel K- files (Kerr-files, Maillefer, Ballaigues, Switzerland) were used with the step-back technique up to size #55 to a WL 1 mm short of the anatomic apex Irrigation was done with sterile saline solution	Sealed with flowable composite resin	The whole length of the canal	***Bacteriological analysis:** 0,1 ml of dilution was inoculated on TSA and incubated at 37°C for 24h ***Statical analysis:** was performed using SPSS statistics 22 software (IBM Corp, Turkey)
Dai et al. 2018 [17]	*In-vitro*	Enterococcus faecalis	80/ Human mandibular primary incisors without apical foramen resorption		Stainless steel K- files (Dentsply Maillefer Ballaigues, Switzerland) were used with the crown-down technique up to size #30 Irrigation was done with 2 ml of 5,25 % NaOCL		Apical, middle and coronal third	***Bacteriological evaluation:** 10 samples from each group were subjected to CFU- counting evaluations: 100 µl of dilution was inoculated onto BHI agar plates at 37°C for 24h ***SEM:** 5 samples from each group were subjected to SEM (S-4800, Hitachi, Japan) at various magnification from x30 to x500000 and two blinded servers evaluated the smear layer based on a scoring system described by TAKEDA ***CLM analysis:** The remaining samples were stained with a LIVE/DEAD backlight bacterial viability kit (L7012, Life, USA) then visualized under an Olympus confocal laser scanning microscope (FV10 ASW, Olympus, Japan) The digital images were imported into the Image Pro Plus 6.0 Program (Media Cybernetics, USA)

Merigo et al. 2021 [18]	*In-vitro*	*Porphyromonas gingivalis *Streptococcus salivarus *Enterococcus faecalis *Prevotella intermedia	73/ Human single rooted and caries-free teeth, without previous canal treatment or canal filling and a root size no longer than 16 mm lengthwise	Single and straight canal	Wave One Primacy Reciprocating File 025.08 (Maillefer, Ballaigues, Switzerland) to a WL 0,5 mm short of the apex The catheterism was done with an ISO K-file n° 10 (MMG 10 L21 ref 20,106,008 Micro-Mega, Besancon, France) coated with Glyde File prep Root canal Conditioner (Dentsply Maillefer, Ballaigues, Switzerland) Irrigation was done with 2,5% NaOCL by an endodontic syringe (Monoject Kendall, Endodontic needle syringe, Tyco-Healthcare, Mansfield, MA, USA) and an ultra-fine needle (Tip Ultradent ref/UP 1349 20 NAVITIP 21 mm, South Jordan, UT, USA)	-	-	***Bacterial evaluation:** culture and incubation onto a Columbia agar plate with 5% sterile horse blood for 72h ***SEM:** (JEOL JSM-5310- LV scanning microscope, JEOL Ltd, Tokyo, Japan) with magnifications from x50 to x500 first, then a finer SEM analysis was operated by coating specimens with gold to obtain magnification from x1000 to x10000
Cheng et al. 2016 [19]	*In-vitro*	Enterococcus faecalis	155/Intact and caries-free single rooted permanent human teeth with no previous coronal restoration or root canal treatment	Straight root canals	ISO 021 round bur at slow speed under cooling water was used to enlarge the canals. Irrigation was done with 5% NaOCL	-	Different depths inside the dental tubules (100, 200, 300, 400 and 500 µm)	***Bacterial evaluation:** culture and incubation onto BHI agar plate at 37°C for 24h, then the CFU were recorded ***SEM:** (S-4800, Hitachi) used to observe the specimens and to quantify the depth to which the bacteria had invaded the dental tubules ***Statical analysis:** SPSS statistics package for Windows (version 13.0)
Cheng et al. 2017 [20]	*In-vitro*	Enterococcus faecalis Collected from teeth with an apical periodontitis	115/ Permanent, intact, caries- free and single rooted human teeth	Straight root canals with mature root apical	Preparation was done with NI-TI rotary instruments (Sybron Endo) up to K3 (#40/04) to a WL 1 mm short to the apical foramen, using crown-down technique. Irrigation was done with a 27-G side vented needle and NaOCL 5,25%	Sealed with light curing flowable composite resin (3M China Ltd)	Middle and apical third of the canal for SEM	***Bacterial evaluation:** One hundred microliters of each dilution were spread onto BHI agar plates at 37°C for 48h ***SEM:** (S-4800, Hitachi, Japan) every 1mm one microscopic field was selected starting from the coronal to the apical end of the canal ***Statical analysis:** SPSS statistics package for Windows (version 13.0; SPSS, Inc., Chicago, IL)
Wang et al. 2018 [21]	*In-vitro*	Enterococcus faecalis	70/Single rooted teeth free of dental caries	-	Canals were enlarged to 1,5mm in diameter with a Gates Glidden drill #6 (Tusla Dentsply, Tusla, OK) at 350 rpm with cooling water and each dentin block was fractured into two semicylindrical halves	-	Cross et longitudinally sectioned specimens for SEM	***CLSM examination:** a live/dead Baclight ™ Bacterial Viability Kit (L-13152, Molecular Probes; Invitrogen, Inc, Carlsbad, CA) following the manufacturer's instruction was used, then the specimens were observed with a CLSM (FluoView 1000, Olympus, Japan), and the FV10-ASW 3.1 Viewer software was used to calculate the dead cell volume *SEM: (S-4800, Hitachi, Japan) at magnifications of x500, x10000 and x50000 ***Statical analysis:** SPSS 17.0 (SPSS, Inc., Chicago, IL)
Zhu et al. 2013 [22]	*In-vitro*	*Smear layer *Enterococcus faecalis	96/Single rooted Human teeth	Single canals	Canals were enlarged to an apical size of #40 using stainless steel K-files (Dentsply Maillefer, Ballaigues, Switzerland) and rotary NI-TI BioRace instruments (BR5, 4% taper, FKG dentaire, La Chaux-de-Fonds, Switzerland) Irrigation was done with 3% NaOCL	Sealed with a flowable composite resin (3M Dental products, St Paul, MN)	Apical, middle and coronal third	***Microbial analysis:** 50 µl of aliquots were plated onto BHI agar plates at 37°C for 48h, then the CFU were counted ***SEM:** S3400N (Hitachi, Tokyo, Japan) at various magnification ranging from x300 to x5000 and a smear layer score was calculated for each specimen ***Data analysis:** Kruskal- Wallis and the Mann- Whitney Wilcoxon U tests were used
Pedullà et al. 2012 [23]	*In-vitro*	Enterococcus faecalis	148/ Single rooted Human teeth	-	Canals were instrumented to the canal terminus using stainless steel K- files from size #10 to #15 followed by rotary Mtwo NI- TI instruments up to size #25 and 0,06 taper (Sweden & Martina, DueCarrare-Pd, Italy) Irrigation was done with 5% NaOCL	Sealed with Super-EBA (Harry J; Bosworth Co., Skokie, IL, USA)	Culture and incubation for 48h on blood agar	***Bacterial evaluation:** Dilutions from 10^4 to 10^6 were plated on blood agar and incubated at 37°C for 48h ***Data analysis:** was done with a statistical software (MedCalc Software, Mariakerke, Belgium)

Olivi et al. 2014 [24]	*In-vitro*	Enterococcus faecalis	26/Single rooted human maxillary centrals and laterals incisors and maxillary or mandibular canines	-	Canals were prepared with NI- TI rotary files (Profile GT, Dentsply Tulsa Dental, Tulsa, Okla.) using crown-down technique up to size #20 and 0,06 taper Apical preparation was done with master pical file size #25 and 0,06 taper Irrigation was done with 5% NaOCL	Sealed using a three-step bonding system (Adper Scotchbond, 3M ESPE, St. Paul, Minn) and a flowable composite (Ena Flow, Micerium, Genova, Italy)	The entire root canal area	***Bacterial evaluation:** Culture and incubation for 48h at 37°C ***SEM:** (Philips XL30/CP, Philips, Eindhoven, Netherlands) at 20kilovolts ***Statical analysis:** using a statistical software (JMP 10, SAS, Cary, N.C.)
Arslan et al. 2013 [25]	*In-vitro*	Smear layer	60/Single rooted, non-carious human maxillary central incisors	Single rooted canals with an intact apical tip	The instrumentation was done using Protaperrotary instruments (Dentsply Maillefer, Ballagues, Switzerland) up to size #40 (F4) Irrigation was done with 2 ml of 5% NaOCL	Sealed with boxing wax	Middle and apical third	***SEM:** (EVO LS10; Zeiss, Oberkochen, Germany) at a magnification of x3000 and Adobe Photo-shop software was used to count the open dentinal tubules ***Statical analysis:** SPSS software (SPSS Inc, Chicago, IL)
Aldeen et al. 2018 [26]	*In-vitro*	Dentine debris	54/Single rooted, caries-free human teeth	Single canal without any calcification or resorption	Protaper universal rotary instruments (Dentsply Maillefer, Ballagues, Switzerland) up to F2 (size 25) and WL 1 mm short from the apical foramen Irrigated was done with 2ml of 5,25% NaOCL	-	Apical and coronal third	***Dental debris removal evaluation:** Digital Camera (Nikon D80; Nikon Co, Tokyo, Japan) and a stereomicroscope (Meiji Techno D80; Saitama, Japan) were used to take images at x20 magnifications and the evaluating was done using a defined scoring system from 0 to 3 ***Statical analysis:** SPSS Statistics 17 software (IBM, SPSS Inc, Chicago, USA)
Wang et al. 2017 [27]	*In-vitro*	Smear layer	100/ Permanent human, single rooted mandibular premolars	Straight root canals without any internal or external resorption, calcification, or previous root canal treatment	The canals were instrumented with sequential M3 NI-TI rotary instruments (#19/02, #20/04, #25/04, #25/06 and #35/04) (YiRui, China) using the crown down technique to a WL defined as 1 mm short of the apical foramen Irrigation was done with 0,5% NaOCL	-	Coronal, middle and apical third	***SEM:** (S-4800; Hitachi, Japan) 40 images at a magnification of x1000 were obtained and were evaluated using a scoring system of 5 scores
Licata et al. 2015 [28]	*In-vitro*	Enterococcus faecalis	52/ Human single rooted teeth without curves or abnormal shapes root	Single, straight canals	The roots were prepared with Ni-Ti files and a 30.06 gauging final apical preparation (Mtwo file system: VDM GmbH, Munich, Germany). Irrigation was done with 5,25% NaOCL and 17% EDTA	Apex closed by bonding	-	***Bacterial assessment:** An aliquot of 100 µl of broth was subcultured onto two vancomycin- resistant enterococco (VRE) agar base plates, whilst the remaining broth was incubated at 37°C for 4 days ***Statical analysis:** a statistical software program (StatView 5.0.1., SAS Institute, Inc. Cary, NY)
Aydin et al. 2020 [29]	*In-vitro*	Enterococcus faecalis	72/ Human mature single rooted incisors with a maximum root curvature of 10°	Single canals with a closed apex	The canals were instrumented manually, respectively, with ISO sizes 15-20 K- files and Protaper universal rotary Ni-Ti instruments (Dentsply Maillefer, Ballaigues, Switzerland) S1, S2 and SX were used for the coronal and middle third of the canal and the apical finishing process was completed using F1, F2 and F3 files to a WL defined 1mm short of the main foramen Irrigation was done with 1ml of 2,5% NaOCL	Root ends were covered with cyanoacrylate	-	***Bacterial assessment:** A dilution of 10 µl was pipetted onto the Mueller-Hinton agar (MHA) and incubated at 37° for 48h ***Statical analysis:** Kruskal-Wallis H test was applied
Deleu et al. 2015 [30]	*In-vitro*	Dentine debris	25/ Human maxillary straight rooted canines	-	The canals were prepared to an ISO size 30 with 6% taper using profile series (Dentsply Maillefer, Ballaigues, Switzerland) up to the WL defined as 1 mm short of the apical foramen Irrigation was done with 2,5% NaOCL	-	-	***Dentine debris removal:** pictures at 13.6 magnification were taken using a digital camera mounted on an operating microscope (OPMI Pico, Carl Zeiss, Göttingen, Germany), then the pictures were scored using a scoring system from 0 to 3 ***Statistical analysis:** Kruskal-Wallis and the Mann-Whitney U test were applied

Lloyd et al. 2014 [31]	*In-vitro*	Dentine debris	16/ Human mandibular molars (only the mesial root is used)	-	Instrumentation was completed to size 30/.06 (Profile Vortex; Dentsply Tulsa Dental Specialties, Tusla, OK) using a crown-down fashion to a WL defined as 0,5mm short of the canal terminus Irrigation used 10 ml of 6% NaOCL	-	-	***Dentine debris removal's assessment:** the canal volumes were reconstructed from micro-computed tomographic scans (Varian Medical Systems, Palo Alto, CA) before and after treatment, then the 3D data sets were analyzed and compared using 2- way analysis of variance and Tukey method
Guidotti et al. 2014 [32]	*In-vitro*	Smear layer	48/ Human single-rooted teeth	Canals with no anatomic curves or alterations	Canals were firstly treated with manual K- files from size 06 to 20, then were prepared by rotary Ni-Ti Protaper™ Dentsply Maillefer, Ballaigues, Switzerland) using S1, S2, F1, F2 and F3 following the sequence recommended by the manufacturer Irrigation was done with 1 ml of 2.5% NaOCL	-	Coronal, middle and apical third	***SEM:** (JEOL JSM-5310LV 35, Jeol Ltd, Tokyo, Japan) with an initial magnification of x35, then in every third the most representative area was photographed at a magnification of x500; The images were evaluated using the scoring system first described by Ciucchi et al. then modified by Bertrand et al. ***Statistical analysis:** Kruskal-Wallis and the Mann-Whitney U test was applied
Cheng et al. 2017 [33]	*In-vitro*	Enterococcus faecalis	335/ Permanent, human, caries-free, intact, single rooted teeth with no previous coronal restoration or canal treatment	Straight root canals	Canals were first negotiated and instrumented to a #10 K-file (Dentsply Maillefer); next they were prepared with K3 Ni-Ti rotary instruments (Sybron Endo) to an apical width of #15/0.04 using a crown-down technique to a WL defined as 1mm short of the apical foramen Irrigation was done with 5ml od 5,25% NaOCL and 5ml of 17% EDTA	-	-	***SEM:** (S-4800, Hitachi) every 1mm one microscopic field was selected starting from the coronal to the apical end of the canal ***Bacterial reduction:** 1000 µl of each dilution was spread onto BHI agar plates and were incubated at 37°C for 48h, then the CFUs were counted ***Statical analysis:** SPSS statistics package for Windows (version 13.0; SPSS, Inc., Chicago, IL)
Mancini et al. 2018 [34]	*In-vitro*	Smear layer	80/Single rooted, intact mandibular premolars with mature root apices and roots longer than 15 mm, with no caries, cracks, endodontic treatment or restorations	Root canals without any curvatures greater than 5° or calcification	Canals were shaped by means of Protaper Ni-Ti rotary instruments (Dentsply Maillefer) until F4 reached the WL Irrigation was done using 3 ml of 5,25% NaOCL	Sealed with flowable composite	*Tip of the tooth *1, 3, 5, 8 mm from the apex	***SEM:** (SUPRA 35; Carl Zeiss SMT, Oberkochen, Germany) used to take 5 micrographs in the same position inside the canal at 3 different magnifications: x300, x1000 and x3000. The micrographs were evaluated using a scoring system from 1 to 5 ***Statistical analysis:** Kruskal-Wallis and the Mann-Whitney U test was applied
Seet et al. 2012 [35]	*In-vitro*	Enterococcus faecalis	58/Fully formed, caries-free single rooted teeth with closed apices and no severe resorption	Single canals	Mechanical instrumentation of the root canal was performed with K3 rotary Ni-Ti files (Sybron Endo) using crown-down technique The file sizes were #25/0.10, #25/0.08, #25/0.06, #35/0.04, #35/0.06 and 40/0.06 Irrigation was done using 20.0 ml of 17% EDTA	Sealed with Cavit then two coats of varnish were painted over.	Apical, middle and coronal third	***SEM:** (Philips XL 30, field emission SEM; Eind-hoven, The Netherlands) at a variety of magnifications
Cretella et al. 2017 [36]	*In-vitro*	Enterococcus faecalis	128/ human single rooted teeth	single canals	Canals were prepared using Protaper Ni-Ti rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) Irrigation was performed with 3ml of 5,25% NaOCL	Sealed with composite resin		***Bacterial reduction:** The bacterial load of each sample was counted by CFUs and the count was carried out with the Uro-Quick system then was scored according to a 3-point scale: 0/1/2
Bahrololoomi et al. 2017 [37]	*In-vitro*	Enterococcus faecalis	60/ Human anterior primary teeth with root resorption less than 1/4	-	Canals were prepared and shaped with K-files up to #50 (Mani, Japan) to 9mm of WL and irrigated with 5,25% NaOCL	Sealed with temporary cement	-	***Bacterial reduction evaluation:** 1 µl of each sample was cultured onto blood agar at 37°C for 24h *Statistical analysis: SPSS software version 21 was used with Mann-Whitney test

Table 3: Characteristics of use for Conventional Needle Irrigation (CNI).

Study	Sample size	Needle	End type	Gauge	Irrigant + volume	Distance from WL	Time
Lagemann et al. 2014 [13]	10	-	-	-	1% NaOCL The final flush used 15% EDTAC for 2 min and 1% NaOCL for 3 min		
Korkut et al. 2018 [14]	10 (5 inoculated and 5 non-inoculated)	-	-	-	NaOCL 5%	2mm short of the WL	1 min
Suer et al. 2020 [15]	25	-	-	-	NaOCL 5% - 2 ml		1 min
Tokuc et al. 2019 [16]	15	-	Side perforated		NaOCL 5% - 2 ml	At the WL	2 min
Dai et al. 2018 [17]	20	-	-	-	NaOCL 5,25% - 5 ml	-	1 min
Merigo et al. 2021 [18]	6	Ultra-fine needle (Tip Ultradent ref: UP 1349 20 Navitip 21mm, South Jordan, UT, USA)	-	-	NaOCL 2,5% - 4 ml	-	1 min
Cheng et al. 2016 [19]	15	Patterson Dental Supply	Side- vented	27-G	NaOCL 5,25% - 5 ml	-	1 min
Cheng et al. 2017 [20]	15	Patterson Dental Supply, Saint Paul, MN	Side- vented	27-G	NaOCL 5,25% - 5 ml	The needle was moved back and forth to 1mm short of the WL and 1mm below the orifice of the canal	1 min
Wang et al. 2018 [21]	20	-	-	-	NaOCL 5,25% - 1 ml	-	1 min and 3min (10 samp les each)
Zhu et al. 2013 [22]	16 (8 for the antibacterial evaluation 8 for smear layer removal evaluation)	Ultradent, South Utah, USA	-	30-G	*NaOCL 3% - 10 ml *EDTA 17% - 10 ml *NaOCL 3% (5ml) + EDTA 17% (5ml)	1mm short of the WL	-
Pedullà et al. 2012 [23]	32	Max-I-Probe, Dentsply Rinn, Elgin, IL, USA	-	30-G	NaOCL 5% -3 ml	As close as possible to the WL	30 sec
Olivi et al. 2014 [24]	10	Max-I-Probe (Dentsply Rinn, Elgin, Ill.)	-	-	NaOCL 5% -3 ml	Needle placed in the middle one-third of the root canal without any activation	2 cycles of 30 sec each + resting time of 30 sec
Arslan et al. 2013 [25]	10	Navitip; Ultradent, South Jordan, UT	Side port	31-G	EDTA 15% - 5ml then NaOCL 5% - 5ml then distilled water - 5 ml as a final rinse	Tip 1mm short of the WL and moved back and afterwards	120 sec
Aldeen et al. 2018 [26]	15	Navitip; Ultradent products Inc., South Jordan, USA	Side vented	30-G	NaOCL 5,25% - 6 ml	1mm short to the WL and moved slowly up and down over a distance of 4mm in the apical third of the canal	60 sec
Wang et al. 2017 [27]	10 for NaOCL 10 for EDTA 10 for NaOCL + EDTA	Patterson Dental Supply	Side vented	27-G	NaOCL 5,25% - 5 ml EDTA 17% - 5 ml NaOCL 5,25% + EDTA 17% - 5ml each	Needle placed 1mm short of the WL then moved back and forth	60 sec
Licata et al. 2015 [28]	13	-	-	27- G	NaOCL 5,25% and EDTA 17%	-	-
Aydin et al. 2020 [29]	10	KerrHawe SA, Bioggio, Switzerland	Side vented	30-G	NaOCL 2,5% - 5 ml	Needle moved within the root canal to a distance 1mm short of the WL, then moved up and down slightly in the canal without contacting the root canal walls	60 sec
Deleu et al. 2015 [30]	20	Monoject; Sherwood Medical, St Louis, MO, USA	-	27-G	NaOCL 2,5% - 4 ml	Needle placed 1mm short of the WL and moved up and down at the apical half of the canal with a flow rate of 0,3ml/s	-
Lloyd et al. 2014 [31]	7	ProRinse, Dentsply Tusla Dental Specialties	Side vented Luer- Lock needle	30-G	EDTA 17% - 4 ml then NaOCL 6% - 10 ml	Needle placed 1mm short of the WL	60 sec for EDTA + 30 sec for NaO Cl
Guidotti et al. 2014 [32]	12	Luer-lock Vista ™ syringe	-	27-G	17% EDTA	Continuous flow of irrigation with a “coming- and-going” movement	2 min
Cheng et al. 2017 [33]	5 groups with different apical terminal working width (#15, #20, #25, #30, #40) of 20 canals each = 100	Patterson Dental Supply	Side vented	27-G	NaOCL 5,25% - 5ml followed by Sodium thiosulfate -5ml and NS	Needle moved back and forth	60 sec for each irrigants
Mancini et al. 2018 [34]	10	Navitip; Ultradent, South Jordan, UT	-	30-G	17% EDTA- 3ml for 1 min followed with 5,25% NaOCL -3ml	Needle inserted at 1mm from the WL	1 min
Seet et al. 2012 [35]	-	Monoject, Tyco Healthcare, Mansfield, MA, USA	-	27-G	4% NaOCL – 5 ml	Needle placed 2 mm from the apex	60 sec
Cretella et al. 2017 [36]	24	-	-	-	NaOCL 5,25% - 5ml	-	3 min
Bahrololoomi et al. 2017 [37]	30	-	-	-	NaOCL 5,25%	-	-

Table 4: Characteristics of use for Laser Assisted Irrigation (LAI).

Study	Sample size	Device	Active medium	Parameters	Tip	Distance from WL	Delivery system	Irrigant + volume	Time
Lagemann et al. 2014 [13]	10	Ezlase, Biolase, San Clemente, CA, USA	Diode (940 nm – pulsed mode)	– Mode: chopped – Peak power: 8 W – Average power: 4.0 W Pulse duration: 50 ms – Pulse frequency: 10 Hz	Plain 200 µm diameter, 14 mm long, E2 type tip	Tip placed 1mm short of the WL and kept stationary for the first 2 sec then withdrawn at a rate of 1mm/s for the remaining 8 sec	10 cycles of 10 sec with 5 sec rest in between	15% EDTA C	10*10 sec + 5 sec rest time in between
Korkut et al. 2018 [14]	10	Light Walker AT; Foton a	Er: YAG (2940 nm – PIPS)	– Mode: PIPS – Wave length: 2940 nm – Frequency: 15 Hz – Energy: 20 mJ – Pulse duration: 50 µs – Coaxial air-water spray feature set to off	400 µm quartz PIPS tip	Tip placed in the coronal reservoir and kept stationary		NAOC L 5%	1 min of activation
Suer et al. 2020 [15]	25	-	Er, Cr: YSGG	– Power: 0,75 W – Output power: 0,45 W – Frequency: 20 Hz – 0% water and 0% air	Fiber tip	Tip inserted all the way down to the apex and the canal was irradiated from the apical to cervical region with helicoidal movements	One lasing cycle = 4 irradiations of 10 sec each with 5 sec intervals	NAOC L 2,5%	4*10 sec + 5 sec intervals in between
Toku c et al. 2019 [16]	15	Waterlase Iplus™ MD; Biolase Technology	Er, Cr: YSGG 2780 nm	– Wave length: 2780 nm – Power: 1,25 W – Repetition rate: 50 Hz – Pulse duration: 50 µs – Water and air spray set to off	A 200 µm diameter and 21 mm length fiber radial firing tip (RFT)	5 mm away from WL and kept stationary	4 irradiations of 10 sec each with 5 sec intervals	NAOC L 5% - 0,5ml	4*10 sec + 5 sec intervals
Dai et al. 2018 [17]	20	Lamb da Dental Laser, LAMB DA Scientific a SPA, Italy	Diode Laser (810 nm – Continuous mode)	– Wave length: 810 nm – Power: 2,0 W – Continuous mode	A 200 µm diameter optical tip	1 mm short from WL	4 irradiations of 5 sec each with 10 sec intervals	NAOC L 5,25% - 1,25 ml	4*5 sec + 10 sec intervals
Merigo et al. 2021 [18]	14	Waterlase Iplus, Biolase Technology Inc., Irvine, CA, USA	Er, Cr: YSGG (2780 nm – pulsed mode)	– Wave length: 2780 nm – Near infra-red pulsed mode – Output Power: 1,5 W – Frequency: 15 Hz – Fluence: 318,471 J/cm$_2$ – Pulse duration: 60 µs – Peak power:1666,67 w – Without water and air spray	Flexible optical Germanate lead fiber with 200 µm diameter and 25mm length	Tip introduced into the canal in parallel to the walls, then slowly moved up and down at a speed of 5mm/s with a circular movement from the apical end of the canal; Additional parietal movements with moving up only (4mm/s) and sliding against root canal walls were also performed	Continuo us dynamic irrigation	NAOC L 2,5% - 4 ml	60 sec of irradiation
Chen g et al. 2016 [19]	90	Fotona	Er: YAG (2940 nm – PIPS mode)	– Wavelength: 2940 nm – Pulse mode: SSP (super short pulse) – Pulse energy: 20 mJ – Output Power: 0,3 or – 0,5 or 1,0 W – Frequency: 15 Hz (for 0,3W) 25 Hz (for 0,5W) 50 Hz (for 1,0W)	PIPS optical tip (Fotona) with a diameter of 300 µm	Tip placed at the orifice of the root canal	Irradiation for 20s or 30s with 15s intervals	NAOC L 5,25%	
Cheng et al. 2017 [20]	15	Fotona, Ljubljna, Slovenia	Er: YAG (2940 nm – PIPS mode)	– Wavelength: 2940 nm – Frequency: 25 Hz – Power: 0,5 W – Pulse energy: 20 mJ – Pulse mode: SPP with 50µs	PIPS optical tip (Fotona) with a diameter of 300 µm	Tip was placed 1mm below the orifice of the canal	Laser was activated for 30s with 15s intervals	NAOC L 5,25% - 5ml	30 sec of irradiation

Wang et al. 2018 [21]	10	Waterlase, Biolase, San Clemente, CA, USA	Er, Cr: YSGG (2780nm)	– Wavelength: 2780 nm – Frequency: 20 Hz – Power: 0,75 W – Pulse energy: 37,5 mJ – Pulse duration: 60 µs – Water 0% and air 5%	RFT3 conical fiber tip with 415 µm diameter and 17 mm length	The fiber tip was pulled out parallel to the root canal wall and returned to the orifice at a speed of 1mm/s	15 cycles of 1min or 3 min with 15 sec intervals	NAOC L 5,25% - 1 ml	3min cumulative time 20*15 +15 sec intervals
	10	Fotona M021 - 3AF/ 3, Ljublj ana, Slovenia	Er: YAG (2940 nm – SSP mode)	– Wavelength: 2940 nm – Frequency: 15 Hz – Power: 0,3 W – Pulse energy: 20 mJ – Pulse duration: 50 µs (SSP mode)	A PIPS fiber tip with a 600 µm diameter and 9 mm length	The fiber was immersed 1mm below the irrigation solution	15 cycles with 15 sec intervals	NAOC L 5,25% - 1 ml	
Zhu et al. 2013 [22]	16: 8 for bacte ria's assessment and 8 for smear layer removal evaluation	Fidelis, Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm – PIPS mode)	– Wavelength: 2940 nm – Frequency: 15 Hz – Pulse energy: 20 mJ – Pulse duration: 50 µs – Coaxial water spray set to off	A 12 mm long and 400 µm diameter quartz tip	Tip placed in the coronal access opening of the pulp chamber and kept stationary	-	NaOC l 3% - 3ml	1 min
Pedu llà et al. 2012 [23]	32	Fidelis AT, Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm – free- running emission mode)	– Wavelength: 2940 nm – Frequency: 15 Hz – Pulse energy: 20 mJ – Pulse duration: 50 µs – Mode: Free running emission – Coaxial water spray set to off	A 12 mm long and 400 µm diameter quartz tip	Tip placed in the coronal reservoir only	Laser irradiation by cycles	NaOCL 5%	30 sec
Olivi et al. 2014 [24]	10	Light Walker AT; Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm – PIPS mode)	– Wavelength: 2940 nm – Frequency: 15 Hz – Average power: 0,3 W – Pulse energy: 20 mJ – Pulse duration: 50 µs – Coaxial air-water spray set to off	A 9 mm long and 600 µm diameter quartz tip	Tip placed in the coronal access opening only and kept stationary	2 cycles of 30 sec each with a resting time of 30 sec	NaOCL 5%	60 sec of irradiation + 30s rest time
Arsla n et al. 2013 [25]	10	Doctor smile, Lamb da Scientifica Srl, Vicenza, Italy	Diode laser (808 nm – pulsed mode)	– Wavelength: 808 nm – Power source: 20 W – Pulse energy: 2 W – Pulse mode: 10 ms on/ 10 ms off	Fiber optic cable with a 300 µm size	2mm short of the WL and the tip was withdrawn gently from the apical to the coronal region with a helical movement	15% EDTA agitated: *1ml for 10 s *2ml for 20s *3ml for 30s *4ml for 40s	EDTA 15% was activated, then a final flush of 15% EDTA then 5% NaOCL then distilled water	120 sec of exposition
Aldeen et al. 2018 [26]	15	Light Walker AT; Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm- pulse mode)	– Wavelength: 2940 nm – Frequency: 20 Hz – Pulse energy: 40 mJ – Pulse duration: 50 µs – Water and air turned off	A 14 mm long and conical 400 µm diameter quartz tip (Fotona)	Fiber placed in the coronal artificial pulp chamber	2 cycles of 30 sec each with a resting time of 30 sec	5,25% NAOC L – 6 ml	1min of activation and 30 sec of rest
Wang et al. 2017 [27]	10 for NaOCL	Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm –super short pulse mode)	– Wavelength: 2940 nm – Frequency: 15 Hz – Pulse energy: 20 mJ – Power: 0,3 W – Pulse duration: 50 µs	PIPS conical tip with 9mm long and 600 µm diameter (Fotona)	Fiber placed and activated 1mm short of the WL	-	NaOCL 5,25% - 5 ml	60 sec
	10 for EDTA							EDTA 17% - 5 ml	
	10 for NaOCL + EDTA							NaOCL 5,25% + EDTA 17% - 5ml each	
	10 for NaOCL	Biolase, Ivrine, CA	Er, Cr: YSGG (2780 nm)	– Wavelength: 2780 nm – Frequency: 50 Hz – Pulse energy: 25 mJ – Power: 1,25 W – Pulse duration: 60 µs – 24% air – Water spray is off	Radial firing conical tips: *RFT2 (21mm long - 275 µm diameter) for the apical third *RFT3 (17 mm long - 415 µm diameter) for the coronal and middle third	RFT2 was introduced into the canal parallel to the root canal wall 1mm short to the WL and then it was changed with RFT3. The tips were pulled out to the orifice at a speed of 1mm/s	5 cycles in total	NaOCL 5,25% - 5 ml	60 sec
	10 for EDTA							EDTA 17% - 5 ml	
	10 for NaOCL + EDTA							NaOC L 5,25% + EDTA 17% - 5ml each	

Licata et al. 2015 [28]	13 in group 1	Biolase, Ivrine, CA	Er, Cr: YSGG		Group 1 and 2	Group 3	Waterlase MD Endolase RFT, with a diameter of 200µm and a length of 25 mm	Tip placed in the coronal entrance and kept stationary		5,25% NaOCL and 17% EDTA	30 sec for group 1
	13 in group 2			Wavelength: Pulse energy: Power: Pulse duration: Pulse/sec: Peak power: Mode: Free- running emission Coaxial water spray:	2780 nm 75 mJ 0,75 W 140 µs 10 535 W - off	2780 nm 25 m J 0,25 W 140 µs 10 178 W - off					60 sec for group 2
	13 in group 3										60 sec for group 3
Aydin et al. 2020 [29]	10	Waterlase, Biolase, Irvine CA, USA	Er, Cr: YSGG	– Frequency: 20 Hz – Power: 0,25 W – 10% air – Water spray is off			RFT2 (Waterlase) fiber tip with a 4mm section	Tip inserted into the canal	-	2,5% NAOC L – 5ml	30 sec
Dele u et al. 2015 [30]	20 for group 4	AT Fidelis, Fotona, Ljubljna, Slovenia	Er: YAG (2940 nm)	– Wavelength: 2940 nm – Frequency: 20 Hz – Pulse energy: 60 mJ – Pulse duration: 50 µs – Efficiency of the fiber: 90% – Water and air spray is off			A plain flat 300 µm diameter and 14mm long fiber tip (Fotona)	Tip placed 5mm above the WL and held still	4 repetitio ns of 5sec laser activations with 5 sec intervals	2,5% NaOCL	4*5 sec of activation + 5 sec interval s
	20 for group 5	Er- PIPS	Er: YAG (2940 nm – PIPS mode)	– Wavelength: 2940 nm – Frequency: 20 Hz – Pulse energy: 40 mJ – Pulse duration: 50 µs – Efficiency of the fiber: 90% – Water and air spray is off			A conical fiber PIPS tip with 14 mm in length (Fotona)	Tip was introduced no further than 4mm in the canal and was held still	4 repetitio ns of 5sec laser activations with 5sec intervals		4*5 sec of activation + 5 sec interval s
	20 for group 6	Fox diode laser, A.R.C. laser Gmb H, Nürnberg, Germany	Diode (980nm)	– Wavelength: 980 nm – Output power: 7,5 w – Frequency: 25 Hz			A 200 µm plain fiber	Tip was introduced no further than 2mm from the WL and moved in up and down motion along the groove	18sec of laser activation		18 sec
Lloyd et al. 2014 [31]	7	Fidelis, Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm- PIPS mode)	– Wavelength: 2940 nm – Frequency: 15 Hz – Pulse energy: 20 mJ – Pulse duration: 50 µs – Efficiency of the fiber: 90% – Water and air spray set to off			A 600 µm diameter and 9 mm long endodontic fiber tip	Tip placed into the access cavity only	Three 30s cycles of continuo us flow	6% NaOC l – 10 ml	3*30 sec
									30s cycle	Water	30 sec
									30s cycle	17% EDTA – 4 ml	30 sec
									Three 30s cycles	Water	3*30 sec
Guidotti et al. 2014 [32]	12 for group A	Fidelis plus3™	Er: YAG (2940 nm)	– Wavelength: 2940 nm – Frequency: 20 Hz – Output power: 1 W – Pulse energy: 50 mJ – Fluence: 7,100 J/cm$_2$ – Water and air spray set to off			A 300 µm endodontic fiber Preciso™ (Fotona)		3 cycles of 5s each with resting times of 5s	2,5% NaOCL	3*5 sec +5 sec resting times
	12 for group B								3 cycles of 5s each for NaOCL then 3 other cycles of 5s each for EDTA	2,5% NaOCL, then 17% EDTA then a final flush of 2,5% NaOCL (1 min – without laser irradiation)	2*(3*5sec + 5sec resting times) + 1 min
	12 for group C								3 cycles of 5s each with resting times of 5s	17% EDTA then a final ush of 2,5% NaOCL (1 min – without laser irradiation)	3*5sec +5sec resting times + 1 min

Chen g et al. 2017 [33]	5 groups with different apical terminal working width (#15, #20, #25, #30, #40) of 20 canal s each = 100	Fotona	Er: YAG (2940 nm- PIPS mode)	– Wavelength: 2940 nm – Frequency: 15Hz – Output power: 0,3 W – Pulse energy: 20 mJ – Mode: SSP	A 300 µm diameter PIPS tip (Fotona)	The optical tip was placed and activated at 1mm below the orifice of the canals	Irradiation of NaOCL then Sodium thiosulfate and NS with 15sec intervals	NaOCL 5,25% - 5ml followed by Sodium thiosulfate -5 ml and NS	20 sec for NaOCL
	4 groups with different output powers and irradiation times of 20 canals each = 80			– An output power of 0,3 W with a frequency of 15 Hz for 40 sec and 60 sec – An output power of 0,5 W with a frequency of 25 Hz for 20 sec – An output power of 1W with a frequency of 50 Hz for 20 sec					40 or 60 or 20 sec
Mancini et al. 2018 [34]	15	AT fidelis, Fotona, Ljubljana, Slovenia	Er: YAG (2940 nm)	– Wavelength: 2940 nm – Frequency: 20 Hz – Pulse energy: 60 mJ – Pulse duration: 50 µs – Efficiency of the fiber: 90% – Water and air spray is off	A 300 µm diameter and 14 mm long PIPS tip (Fotona)	Tip inserted 5mm above the WL and held still during 5s of laser activation	4 repetitio ns of the 5s cycle with 5s intervals	5,25% NaOCL	4*5sec of activation+ 5 sec intervals
Seet et al. 2012 [35]	-	Waterlase, Biolase Technology, Irvine, CA, USA	Er, Cr: YSGG	– Output power: 0;25 w – Frequency: 20 Hz – Water spray is off – Air spray 10%	A radial firing tip (17 mm, 52°)	Tip was inserted 4mm into the canal and was withdrawn coronally during energizing of the irrigant	4 repetitio ns of the 5s cycle with 5s intervals	4% NaOCL	60 sec
Cretella et al. 2017 [36]	24	Fox (Sweden & Martina, Padova, Italy)	Diode laser (810 m)	– Wavelength: 810 nm – Output power: 8 W – Radiant energy: 75 J – Radiant power: 2.5 W	*An optical fiber of 200 µm in scope was used for the first 2 cycles *A fiber of 300 µm was used in the third cycle	*In the first 2 cycles the tip was inserted 1 mm to the WL and helicoidal movements from apical to cervical were performed manually *The third cycle, the tip was used to irradiate the middle and the coronal thirds of the canal	3 cycles of 30 sec each	5,25% NaOCL	90 sec in total
Bahr ololoomi et al. 2017 [37]	30	Fidelis Plus, Fotona, Slovenia	Er: YAG (2940 nm- short pulse mode)	– Wavelength: 2940 nm – Frequency: 10 Hz – Power: 1 W – Energy: 100 mJ – Pulse duration: 250 µs (short pulse mode)	A 20 mm length and 300-micron diagonal fiber tip	Laser irradiation started from the coronal part that was 10 mm away from the radiographic apex or 9 mm from the WL	2 spans of 10 sec each with an interval of 2sec	5,25% NaOCL	20 sec

Table 5: Characteristics of use for Passive Ultra-sonic Irrigation (PUI).

Study	Sample size	Device	Settings	File/Taper size	Distance from WL	Irrigant + volume	Time
Cheng et al. 2017 [20]	15	UDS-L; Guilin Wood-Pecker Medical Instrument Co, Guangxi, China	Powe r: 5W	Standard ultrasonic needle (#25 K-type NII- TI file, 32.5 mm length)	Tip moved back and forth at a range of about 1-6mm short of the WL at a speed of 1mm/s	5,25% NaOCL – 5ml	1min
Aldeen et al. 2018 [26]	15	Satelec, Aceton group, Norwich, UK	Powe r: 25%	K/21 mm file (Irri-safe; Satelec, Aceton group, Norwich, UK)	The file was inserted 1 mm coronal to the WL	5,25% NaOCL – 6ml	3 cycles of activation of 20 sec each (Total activating time of 1 min)
Aydin et al. 2020 [29]	10	Piezo electric Ultra-sonic unit (EMS, Nyon, Switzerland)	-	Stainless steel file numbered 15 (Varios U file; Nakanishi Inc., Tochigi, Japan)	File placed 1 mm short of the WL and activated by short vertical movements	2,5% NaOCL – 5ml	30 sec
Deleu et al. 2015 [30]	20	Suprasson Pmax Newtron, Satelec	Powe r: 50%	Non-cutting #20 file (Irrisafe, Satelec Aceton, Merignac, France)	Tip kept steady 1mm short of the WL	2,5% NaOCL	20 sec
Mancini et al. 2018 [34]	15	MiniEndo II; Sybron Endo, West Collins, Orange, CA	Powe r set at 5	No 15 K-file (Dentsply Maillefer)	File placed at 1mm short of the WL	5,25% NaOCL	1 min

Table 6: Characteristics of use for Sonic Activated Irrigation (SAI).

Stud y	Sample size	Device	Settings	File/Taper size	Distance from WL	Irrigant + volume	Time
Man cini et al. 2018 [34]	15	EndoActivator system (Dentsply Tusla Dental Specialties, Tulsa, OK)	-	15/.02 point	2mm from the WL	5,25% NaOCL – 5 ml	1 min
Seet et al. 2012 [35]	-	EndoActivator®	A maximum power of 166 Hz	#30/.04 polymer tip		4% NaOCL – 5ml	1 min

Table 7: Outcomes.

Study	Intervention groups	Comparison groups	Control	Outcomes
Lagema nn et al. 2014 [13]	Activation of 15% EDTAC with Diode laser (940 nm – pulsed mode)	CNI with 1% NaOCL and a final flush with 15% EDTAC and 1% NaOCL	CNI with distilled water	The best smear layer removal was seen in the laser group with EDTAC
Korkut et al. 2018 [14]	Activation of 5% NaOCL with Er: YAG (2940 nm – PIPS mode)	CNI using 5% NaOCL	Growth control group with no treatment	– Antibacterial efficacy: CNI: 3,82 < PIPS: 5,24 (statically significant reduction was achieved in the PIPS activated irrigation group) – Smear layer removal efficacy: scores: CNI: 4±0,0 < PIPS: 1,2±0,4 (PIPS activated irrigation group resulted in more cleaning of the root canals and a higher quantity of open tubules)
Suer et al. 2020 [15]	Activation of 2,5% NaOCL with Er, Cr: YSGG	CNI using 5% NaOCL	Growth control group with no treatment	– Bacterial reduction: 100% at both 5% NaOCL and 2,5% NaOCL+ 0,75 W LASER – Smear layer removal: partial removal of smear layer in the laser groups compared to thick, dense and homogeneous smear layer remaining in the NaOCL group
Tokuc et al. 2019 [16]	Activation of 5% NaOCL with Er, Cr: YSGG (2780 nm)	CNI using 5% NaOCL	Growth control group with no treatment	– Bacterial reduction: Maximal bacterial elimination was observed in the LAI group (Er, Cr: YSGG+NaOCL)
Dai et al. 2018 [17]	Activation of 5,25% NaOCL with diode laser (810 nm – continuous mode)	CNI using 5,25% NaOCL	Growth control group with no treatment	– Bacteriological evaluation: - the diode-NaOCL group showed nearly 100% disinfection in each part of the canal with a significant difference compared to the other groups - the bacteria were eliminated more effectively in the middle and coronal parts compared to the apical parts – SEM examination: the diode-NaOCL group presented the best disinfection outcome: the smear layer was cleaned; the tubules were opened and almost no bacteria existed on the root canal system – CLM analysis: the diode-NaOCL group showed little green fluorescence (viable bacteria) and mostly red fluorescence (non-viable bacteria) and no green fluorescence in the deeper dentinal tubules; which is the best result compare to the other groups who showed greener fluorescence
Merigo et al. 2021 [18]	Activation of 2,5% NaOCL with 2780 nm Er, Cr: YSGG	CNI using 2,5% NaOCL	-	– Group 5: the apical third showed several debris and smear layer while in the center an irradiated area was present with cracks and melting dentin of the radicular wall. The middle third showed a darker part with smear layer and open dentinal tubules and a light part without smear layer and debris and with all the dentinal tubules opened; no track of visible biofilm was observed. – Group 6: bacteria were not removed and destroyed and biofilm is present on the root canal walls. – Group 5 showed better results than group 6
Cheng et al. 2016 [19]	Activation of 5,25% NaOCL with 2940 nm Er: YAG	CNI using 5,25% NaOCL	Growth control group with no treatment	• Bacterial reduction: it reached 100% in all Er: YAG 2940 nm groups both at the canal walls and at 100 and 200 µm inside the dentinal tubules Only the groups treated with 0,5 and 1,0 W for 30 sec exhibited no bacterial growth at 300, 400 and 500 µm inside the dentinal tubules. • SEM: – Canal walls observation showed no bacteria in the Er: YAG 2940 nm groups and almost no bacteria in the CNI group – Dentinal Tubules observation showed few bacterial cells left in the CNI group and even fewer in the Er: YAG 2940 nm groups – The 0,3W for 20s group showed a disinfection up to 300 µm depth – The 0,3W for 30s and the 0,5/1,0 W for 20s groups showed a disinfection up to around 300 and 400 µm depth – The 0,5/1,0 W for 30s groups showed a disinfection to more than 500 µm depth
Cheng et al. 2017 [20]	Activation of 5,25% NaOCL with 2940 nm Er: YAG	*CNI using 5,25% NaOCL *US using 5,25% NaOCL	Growth control group with no treatment	• Bacterial reduction in treatment groups in descending order: Er: YAG + NaOCL (98.8%), US + NaOCL (98.6%) > NaOCL (94.0%) • SEM evaluation: the Er: YAG + NaOCL group showed the cleanest and most smooth root canal wall compared to US + NaOCL and NaOCL
Wang et al. 2018 [21]	Activation of 5,25% NaOCL with 2940 nm Er: YAG and 2780 nm Er, Cr: YSGG	CNI using 5,25% NaOCL	Growth control group with no treatment	– More Bacteria were dead in each experimental group after 3 min of treatment rather than after 1 min of treatment – The bactericidal effects of laser-activated irrigations were more effective than the CNI group – The Er, Cr: YSGG + NaOCL (73– 85%) and Er: YAG + NaOCL (76–89%) lasers were the most effective antibacterial protocol at both exposure times, and no significant difference was found between the two groups

Zhu et al. 2013 [22]	Activation of 3% NaOCL with 2940 nm Er: YAG	CNI using 3% NaOCL CNI using 17% EDTA CNI using 3%NaOCL+17% EDTA	0,9% Normal saline irrigation	• Antibacterial effect: no significant difference between NaOCL group, PIPS group and NaOCL+EDTA who all showed better results than pre-treated samples, EDTA and NS groups • Smear layer removal: all the groups showed an incomplete decontamination – The PIPS group and NaOCL+EDTA had the best score compared to the NaOCL and the other groups in the coronal and the middle third. – In the apical third there were no significant difference between all the groups; – The difference between PIPS and NaOCL+EDTA is that PIPS groups showed a decreased decontamination between the coronal, the middle and the apical third of the canals, meanwhile NaOCL+EDTA showed no difference in the decontamination of the coronal and middle third of the canal, while a decreased decontamination was demonstrated in the apical third
Pedullà et al. 2012 [23]	Activation of 5% NaOCL with 2940 nm Er: YAG	CNI using 5% NaOCL	Growth control group with no treatment	Group with 2940 nm Er: YAG and NaOCL (99,8%) showed the greatest percentage of bacterial reduction among the other groups with no significant difference compared to NaOCL group (97,1%)
Olivi et al. 2014 [24]	Activation of 5% NaOCL with 2940 nm Er: YAG	CNI using 5% NaOCL	Growth control group with no treatment	• SEM: PIPS+NaOCL group showed no bacteria nor smear layer, which is a better result than NaOCL group • Immediate bacterial count: in PIPS+NaOCL group (10/10) there were no detectable growth which is a better result than NaOCL group (6/10) • Bacterial count after 48h: disinfection was maintained better in PIPS+NaOCL group compared to NaOCL group
Arslan et al. 2013 [25]	Activation of 15%% EDTA with 808nm diode laser at different agitation times	CNI using 15% EDTA	Growth control group with no EDTA irrigation	– In the middle third: 20 sec of agitation of 15% EDTA showed the best results – In the apical third: 20 sec of agitation of 15% EDTA showed the best results – Decontamination in the middle third was better than the apical third
Aldeen et al. 2018 [26]	Activation of 5,25% NaOCL with 2940 nm Er: YAG	*CI using 5,25% NaOCL *PUI using 5,25% NaOCL	-	– The LAI removed significantly more dentine debris than PUI and CNI both in the coronal and apical third (LAI presented the highest values for score 0) – There is no significant difference in removing the dentinal debris in the coronal and apical third in the same experimental irrigation group
Wang et al. 2017 [27]	Activation of 5,25% NaOCL or 17% EDTA or 5,25% NaOCL + 17% EDTA with 2940 nm Er: YAG or 2780 nm Er, Cr: YSGG	CNI using: – 5,25% NaOCL – 17% EDTA – 5,25% NaOCL + 17%EDTA	Growth control group with no treatment	– LAI (NaOCL+EDTA) showed the very best result among the groups for the entire root canal wall – In a descending order: LAI (NaOCL+EDTA), LAI (EDTA) > LAI (NaOCL), NaOCL+EDTA > EDTA > NaOCL – No difference was observed between the two types of lasers, except for the morphological differences of the root canal surfaces (rough for Er: YAG and scaly for Er, Cr: YSGG)
Licata et al. 2015 [28]	Activation of 5,25% NaOCL and 17% EDTA with 2780 nm Er, Cr: YSGG at different parameters	CNI using 5,25% NaOCL and 17% EDTA		The highest bactericidal effect was observed at LAI with 75 mJ for 60 sec (100%) followed by LAI with 75 mJ at 30 sec and CNI who had the same result (92,3%) and the least bactericidal effect was observed in LAI with 25 mJ for 60 sec (46,1%)
Aydin et al. 2020 [29]	Activation of 2,5% NaOCL with Er, Cr: YSGG	*SNI (Standard needle irrigation) using 2,5% NaOCL *PUI using 2,5% % NaOCL	Growth control group with no treatment	LAI (99,9658%) and PUI (99,9616%) were both successful on root canal disinfection but there was no significant difference between them and SNI (99,7000%)
Deleu et al. 2015 [30]	Activation of 2,5% NaOCL with Er, Cr: YSGG (2940 nm) at different settings and with diode laser (980 nm)	*CNI using 2,5% NaOCL *PUI using 2,5% % NaOCL	-	– CNI removed less debris than all the other groups – LAI with the flat fiber tip removed more debris than the LAI with diode laser and PIPS tip – LAI with the flat faber tip and PUI had no significant difference in removing the debris
Lloyd et al. 2014 [31]	Activation of 6% NaOCL or 17% EDTA or water with Er: YAG (2940 nm- PIPS mode)	CNI using 6% NaOCL + 17%EDTA	Same samples before treatment	There were significant differences between SNI and PIPS: PIPS had an increase in debris removal x2,6 greater than for SNI
Guidotti et al. 2014 [32]	Activation of 2,5% NaOCL and 17% EDTA with Er: YAG (2940 nm)	CNI using 17% EDTA		– The Er: YAG fiber double irradiation (NaOCL+EDTA) showed to be the most effective in removing smear layer in the coronal, middle and apical third of the canal – Apical third: B > C > D > A – Middle third: B > C > D > A – Coronal third: B > D > C > A

Cheng et al. 2017 [33]	Activation of 5,25% NaOCL with YAG (2940 nm) at different apical terminal working width and different laser parameters and irradiation times	CNI using 5,25% NaOCL at different apical terminal working width	Untreated group	– The LAI showed a higher disinfection efficacy compared to CNI at each ATWW – The bacterial reduction percentage increased as the ATWW increased (from #15 to #40) in both CNI and LAI groups – The disinfection efficacy of LAI increased with irradiation time – The disinfection efficacy of LAI increased with the output power of the laser – Increasing the output power showed better results than increasing the irradiation time
Mancini et al. 2018 [34]	Activation of 5,25% NaOCL with Er: YAG (2940 nm)	*CNI using 5,25% NaOCL and 17% EDTA *PUI using 5,25% NaOCL *EA sonic activation of 5,25% NaOCL	Untreated group	The Endo Activator was significantly more efficient than LAI, PUI, CNI in removing the smear layer at 1, 3, 5 and 8mm from the apex
Seet et al. 2012 [35]	Activation of 4% NaOCL with Er, Cr: YSGG	*CNI using 4% NaOCL *AE using 4% NaOCL	Group treated with saline water	Sonic and laser activation of 4% NaOCL resulted in greater bacterial reduction compared with syringe irrigation, but LAI showed the overall greatest reduction
Cretella et al. 2017 [36]	Activation of 5,25% NaOCL with diode laser (810 nm)	CNI using 5,25% NaOCL	Group treated with saline water	Both the groups treated with 5,25% NaOCL and groups treated with NaOCL and Diode showed a complete decontamination of the root canal which mean that Diode laser was not more effective than NaOCL in reducing bacteria
Bahrolol oomi et al. 2017 [37]	Activation of 5,25% NaOCL with Er: YAG (2940 nm)	CNI using 5,25% NaOCL		There was no significant difference between colony counts in the CNI and the LAI groups, but the number of colonies in the LAI group was lower than CNI group

Table 8: Methodological quality assessment with JBI critical appraisal checklist adapted for in-vitro studies.

Study	JBI1	JBI2	JBI3	JBI4	JBI5	JBI6	JBI7	JBI8	JBI9	Score	Appraisal of methodological quality
Lagemann et al. 2014 [13]	0	0	1	0	0	1	1	1	1	5/9	Moderate
Korkut et al. 2018 [14]	1	1	1	0	1	1	1	1	1	8/9	High
Suer et al. 2020 [15]	1	0	1	1	0	1	1	1	1	7/9	High
Tokuc et al. 2019 [16]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Dai et al. 2018 [17]	1	0	1	0	1	1	1	1	1	7/9	High
Merigo et al. 2021 [18]	1	0	1	0	0	1	1	1	0	5/9	Moderate
Cheng et al. 2016 [19]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Cheng et al. 2017 [20]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Wang et al. 2018 [21]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Zhu et al. 2013 [22]	0	0	1	1	0	1	1	1	1	6/9	Moderate
Pedullà et al. 2012 [23]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Olivi et al. 2014 [24]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Arslan et al. 2013 [25]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Aldeen et al. 2018 [26]	1	0	1	0	1	1	1	1	1	7/9	High
Wang et al. 2017 [27]	1	0	1	0	1	1	1	1	1	7/9	High
Licata et al. 2015 [28]	1	0	1	0	1	1	1	1	1	7/9	High
Aydin et al. 2020 [29]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Deleu et al. 2015 [30]	0	0	1	0	1	1	1	1	1	6/9	Moderate
Lloyd et al. 2014 [31]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Guidotti et al. 2014 [32]	1	0	1	0	1	1	1	1	1	7/9	High
Cheng et al. 2017 [33]	1	0	1	0	0	1	1	1	1	6/9	Moderate
Mancini et al. 2018 [34]	1	0	1	0	1	1	1	1	1	7/9	High
Seet et al. 2012 [35]	0	0	1	0	0	0	1	1	0	3/9	Low
Cretella et al. 2017 [36]	1	0	1	0	0	1	1	1	0	5/9	Moderate
Bahrololoomi et al. 2017 [37]	1	0	1	0	0	1	1	1	1	6/9	Moderate

All the studies included used Human teeth, in 3 studies, they were primary ones [14,17,37]. While the rest of the studies used permanent teeth. Out of 25 study, 21 used single rooted teeth, while one study mentioned using mandibular premolars without specifying the number of roots [15] one study did not specify the type of teeth used [13] and 2 studies declared using mandibular molars as a sample but only one canal was assessed. Canal's length details were mentioned in 4 studies [13,14,18,34] and ranged from 10 +/-1 to 16 mm. 6 studies declared using Straight canals, 2 studies tolerated curvatures less than 5° [3] and less than 10° [29], while the rest of the studies did not mention any information about the canal's curvatures.

Many systems were adopted for preparation and instrumentation of the canals; 3 studies used manual instrumentation with K files [16,17,37], while the rest of the studies used various rotary instruments; 8 studies used Protaper files, 3 studies used Sybro Endo files, 2 studies used Mtwo files, while the rest of the studies used respectively either Profile vortex files, M3 Ni-Ti files, Profile GT files, BioRace files, Wave One primacy reciprocating files, and Nitiflex files. One study [30] did not specify the type of the system but did mention the manufacturer's name (Dentsply). Two studies prepared the canals using ISO021 round burs, and Gates Glidden drill. For the preparation's technique 7 studies used the crown down technique, two studies used the Step-back technique, yet the rest 16 studies did not mention how they prepared the canals. Apical sizes and tapers ranged from #15 [33] to #55 [16], however 2 studies did not mention it. 9 studies opted for a working length 1mm short of the apex foramen, 2 studies opted for 0,5mm short of the apex foramen, one study did not mention the distance between the WL and the foramen and one study normalized the WL to 9mm for all the canal, yet the rest 12 studies did not mention anything about the WL.

The irrigation solutions that were used while preparing the canals are sterile saline in 2 studies, NaOCL with a concentration that varies from 0,5% to 6% in 18 studies, EDTA with a concentration of 17% in one study, both NaOCL and EDTA in 2 studies; and 2 studies did not mention any information about the irrigant solution.

In what concerns the apical region, one study declared working on teeth with an open system [13], while 10 study did not give any information about the apex end; 14 studies sealed the apex with different substances: 7 studies used flowable composite, 2 studies used a bonding system, the rest of the studies used either boxing wax, or cyanoacrylate, or Cavit and coats of varnish, or temporary resin, or SuperEba.

Different methods were adopted to assess the antibacterial effect and the dentine debris removal efficiency; SEM was used in 6 study, CFU was used in 6 studies, both SEM and CFU were used in 8 studies, one study used CLSM and SEM, another study used SEM, CLSM and CFU, 2 studies used pictures taken by a digital camera, while one study used a 3D reconstitution of the canals for assessment. For the statistical analysis, 10 studies used the SPSS software, 2 studies used Kruskall-Wallis test, 3 studies used both Kruskall-Wallis and Mann-Whitney U test, one study used JMP10 test, another study used MedCal test and another study used the StatView test; yet 7 studies gave no information about the statistical procedure they opted for.

Irrigation

As it was mentioned earlier, the aim of this review is to assess the effectiveness of LAI compared to CNI, PUI, and to SAI. Among these 25 studies, 19 compared LAI and CNI, 3 compared LAI to both CNI and PUI, 2 compared LAI to CNI and SAI, and only one study compared all of the four irrigation protocols.

The irrigants in CNI groups were either NaOCL with a concentration that ranged from 1% to 6%, or EDTA 15% or 17%, or both. The same thing goes for LAI; yet in PUI and SAI, only NaOCL was used and with a concentration that was either 2,5% or 5,25%. As for the volume, it ranged from 1ml to 10 ml for NaOCL and from 4ml to 10 ml for EDTA.

Regarding CNI, the sample sizes ranged from 6 to 32; irrigation was performed through needles with a gauge of 27-G in 8 studies, 30-G in 6 studies, 31-G in on study and not mentioned in 10 studies, their end type was side-vented in 9 studies but not defined in the rest 16 studies. The distance from the Working Length was not precised in 9 studies, while it was determined as 1mm short of the Working length in 9 studies, 2mm in 2 studies, at or as close to the working length. The time of activation ranged as well from 30 sec to 3 min; and was not precised in 5 studies.

In LAI groups, the sample sizes ranged from 10 to 90; and the active mediums that were assessed were either:

- Diode laser in 5 studies, with a wavelength of 808 nm, 810 nm in 2 studies, 940 nm and 980 nm, and in a pulsed mode in 2 studies, continuous mode in 1 study and a non-defined mode in 2 studies.
- Er, YSGG in 8 studies, with a wavelength of 2780 nm in 5 studies but not defined in 3 studies; the mode was either pulsed mode in 2 studies, a free running mode in one study and a non-defined mode in 5 studies.
- Er, YAG in 15 studies, with a wavelength of 2940 nm in all the studies, and PIPS mode in 8 studies, a free running mode in one study, a SSP in 2 studies, a pulse mode in 2 studies and a non-defined mode in 2 studies.

The disposition of the tip was either in the coronal reservoir in 8 studies, 1mm short of the WL in 7 studies, 2mm, 4mm or 5mm short of the WL in the rest of the studies, yet it was not defined in one study. In 9 studies the tip was not kept steady but was moving along the canal during the activation. The activation time was mostly done through cycles with resting time in between, and ranged from 10 sec to 2 min. In some studies, different types of laser active medium were used [21,30], different modes were used [28], different output power (30,33) and different activation time [28,33].

For PUI, the sample sizes ranged from 10 to 20, the device was defined in all the studies, the power settings as well, except in one study. The distance from the Working Length was defined at 1mm

Table 9: Secondary outcomes in terms of variables.

Study	Variable	Outcomes
Suer et al. 2020 [15]	Concentration of the irrigant solution	• CNI with NaOCL 5% have the same effect as LAI with 2,5% NaOCL and Er, Cr: YSGG
Cheng et al. 2016 [19]	-Depth inside the dental tubules -Time of activation -Output power of the laser device	• 100 and 200 µm inside the dentinal tubules showed better results than 300, 400 and 500 µm inside the dentinal tubules. • 0,5/1,0 W of power for 30s was better than 0,5/1,0 W for • 20s and 0,3W for 30s which were also better than 0,3W for 20s
Wang et al. 2018 [21]	-Time of activation -Active medium	• Activation for 3 mins was better than 1min • There was no significant difference between Er, YSGG and Er, YAG
Zhu et al. 2013 [22]	-Area of interest	• Coronal and middle third showed that LAI can be better than CNI • Apical third showed no difference between LAI and CNI efficiency
Olivi et al. 2014 [21]	-Moment of Assessment	• Both immediately after the activation and 48h later showed that LAI is better than CNI in terms of disinfection
rslan et al.2013 [25]	-Time of activation -Area of interest	• The middle third showed better outcomes than the apical third • Activation for 20 sec showed better results than 10, 30 and 40 sec
Wang et al. 2017 [27]	-Irrigant solution -Active medium	• In a descending order: LAI (NaOCL+EDTA), LAI (EDTA) > LAI (NaOCL), NaOCL+EDTA > EDTA > NaOCL • No difference was observed between the two types of lasers, except for the morphological differences of the root canal surfaces (rough for Er: YAG and scaly for Er, Cr: YSGG)
Licata et al. 2015 [28]	-Time of activation -Output power	• LAI with 75 mJ for 60 sec was better than LAI with 75 mJ for 30 sec and CNI who were also better than LAI with 25 mJ for 60 sec
Deleu et al. 2015 [30]	-Active medium -Tip of the Device	• LAI with the flat fiber tip (Er: YAG) resulted the same way as PUI and they both removed more debris than the LAI with diode laser and LAI with PIPS tip (Er: YAG)
Cheng et al. 2017 [33]	-Apical terminal working width (ATWW) -Output power -Time of activation	• The disinfection efficacy of LAI increased with the ATWW • The disinfection efficacy of LAI increased with irradiation time • The disinfection efficacy of LAI increased with the output power of the laser

short of the WL in all the studies except for one [19] where it was either at 1mm or 6mm short of the WL. The time of agitation was either 30 sec, 1min or 2 min.

In the 2 studies that treated SAI, the sample was not defined in one, but was of 15 in the other, and they both used the EndoActivator device. The distance from the Working Length was defined at 2mm short of the WL in one study and was not defined the another. And the activation time in both studies was of 1 min.

Risk of Bias within Studies

Table 9 demonstrates the evaluation of the inner methodological quality of the studies included.

None of the studies met all the criteria according to the JBI critical appraisal checklist that was adapted to in-vitro studies.

8 studies got a score that allowed them to be ranked as High methodological quality, 16 as moderate and one as low methodological quality.

Summary Measures

For a better visibility and organization, the analysis of the outcomes will be proceeded as follows:

Among the 16 studies that assessed the antibacterial effect of LAI, 13 compared LAI to CNI, and concluded that LAI showed better disinfection potential than CNI in 9 studies, the 4 studies left concluded that there's no significant difference between LAI and CNI in terms of disinfection of the root canal system. Meanwhile 2 studies compared LAI to both CNI and PUI, one resulted in similar disinfection efficiency between the three irrigation's protocols and the other demonstrated that LAI have better disinfection ability than PUI and CNI. Finally, the one study that compared LAI to CNI and SAI, concluded that LAI have shown the best results.

On the other hand, 14 studies assessed the dentine debris removal within the canals, 11 compared LAI to CNI, and 10 revealed that LAI have better results than CNI, while only one study concluded that there's no significant difference between the two irrigation protocols. Furthermore, 2 studies assessed LAI to CNI and PUI, one study demonstrated that LAI have better efficiency than both PUI and CNI, although the other study deduced that there's no significant difference between LAI and PUI but they were both better than CNI. Eventually the one study that assessed LAI in comparison to CNI, PUI and SAI, conducted that the SAI have the best result among the 4 techniques.

Many studies gathered primary outcomes which are the assessment of the antibacterial effect and dentine debris removal, and additional secondary outcomes that assessed some variables that could have an impact over the efficiency of the irrigation's protocol; the table 10 below recite these variables and their outcomes:

Discussion

To sum up, this systematic review assessed the efficacy of laser assisted irrigation in the removal of bacteria and smear layer in the root canal system when compared to passive ultra-sonic irrigation, to sonic irrigation and to conventional needle irrigation.

In order to comprehensively answer the research question, many studies were excluded because of their internal or external

validity issues, leaving a number of 25 included in-vitro studies. These studies were fully reviewed despite their different level of evidence and they were carefully weighed before using its results and embracing its conclusions.

And so as to have a more representative conclusion, the results of the studies were divided into two parts, those who dealt with the dentine debris removal and those who treated the antibacterial effect.

Regarding the removal of smear layer and dentine debris, LAI showed better results when compared to both CNI and to PUI, whereas SAI was demonstrated to be more efficient than LAI according to a high-quality study [34].

Meanwhile, for the antibacterial effect, LAI showed better results compared to CNI and SAI [35], and had similar results to PUI.

These differences in the results may be construed according to several approaches; such as the bacteria inoculated, the quality of the samples and the canal's anatomy, the irrigants, the time, the devices used, the parameters, and the different identification techniques.

In what concerns the bacterial type that were inoculated in the canal system and was used to testify the effectiveness of the different activation devices, all the studies used Enterococcus faecalis, which is a gram-positive facultatively anaerobic cocci and is the most widely used test organism in endodontic studies [6]. Yet one study used also Porphyromonas gingivalis, Streptococcus salivarus and Prevotella intermedia. The use of Enterococcus faecalis as a bacterial sample could be justified with the fact that it can withstand harsh environmental conditions [38] making it a good criterion to assess a disinfection device or technique, also it has a great capacity to invade dentinal tubules [39] and being a facultative anaerobic, it has the ability to grow faster when cultured so that the analyses can be started after a couple of days of incubation [6].

The samples that were used were all human teeth with a generally straight root canal, because a straighter line access for the irrigation needle might allow more mechanically effective flow of the irrigant [40], in this matter finding suggests that increased root canal curvature impedes the flow of irrigant, thereby reducing its flushing ability and decreasing its mechanical efficacy [41]. Two included studies declared using curved canals, and according to a classification listed by Shneider [42], the study [34] that used canals with a curvature less than 5° would be also classified as straight canals, meanwhile the other study [29] that used canals with a curvature less than 10° would be classified as moderately curved canals; and this same study resulted in no significant difference between LAI, PUI and CNI. There another is an in-vitro study that assessed the effect of activation of the irrigants in simulated curved root canals and it declared that canal curvature negatively affects the cleaning efficacy of different irrigation methods, and the effect was most pronounced for the sonic techniques [43].

A spare major matter is the irrigant that have been used, only studies using NaOCL which is a chemical tissue dissolving agent or EDTA, which is a chemical chelating agent with no antibacterial effect were included; both these irrigant solutions are considered to be the most used and the most reliable among all [7]. In vitro trials could not prove that the application of LAI with saline or water could efficiently replace NaOCL. Specifically, De Meyer et al. who showed that LAI applied by a 2940 nm laser system with saline could not reduce the viable counts of a dual - species biofilm of Enterococcus faecalis and Streptococcus mutans more than LAI with NaOCL [44]. Based on this finding and on recent literature, it can be concluded that lasers have bactericidal effects. However, they still cannot replace sodium hypochlorite [7]. A finding that could lead us to the fact that the chemical aspect of the disinfection using a proper irrigant solution with effective disinfection and chelating properties is primordial to achieve a higher level of disinfection. Similarly, Kreisler et al. showed that laser irradiation alone with an 809 nm diode laser in vitro was no more effective than the simultaneous use of the laser with NaOCL [44]. They concluded that the potential application of this diode laser should not be a substitute for conventional treatment, but should be regarded as a possible adjunctive treatment [45].

Regarding the irrigants concentration, it was implied that the minimum antibacterial concentration of NaOCL is 0.5% [46] and 10% for EDTA [47], while in all the studies included in our review the NaOCL concentration rated between 1% and 6% and EDTA was either at 15% or 17%, which means that the irrigants concentration was never deficient, even though, a study declared that a range in which a concentration of NaOCL is higher than 2.5% has not been clinically proven to be more effective [48].

The minimal irrigation time for an optimal effectiveness was demonstrated to be 1min [49] for both irrigants, even though in [21] it was stated that irrigation for 3min resulted better than 1min. In the studies included, they all opted for an irrigation time of 1min or so, except for one study that used an irrigation time of 30sec [23].

In the other hand, another determinant that is so important to discuss is the laser active medium that was used, it's wavelength and pulsation mode; as a matter of fact, the studies included, used 3 active mediums which are: Diode laser with a wavelength of 808 nm, 810 nm, 940 nm and 980 nm. Er, YSGG with a wavelength of 2780. and Er, YAG with a wavelength of 2940 nm. Which matches the findings of this review [50] who identified the laser active mediums and wavelengths described for cleaning and disinfecting of the root canal system, and it cited:

- Erbium: yttrium aluminum garnet (Er: YAG) - 2940 nm
- Erbium, chromium: yttrium scandium galium garnet (Er, Cr: YSGG) - 2780 nm
- Neodimium: yttrium aluminum garnet (Nd: YAG) - 1064 nm
- Diode - 635 to 980 nm
- Potassium titanyl phosphate (KTP) - 532 nm
- Carbon dioxide (CO2) - 9600 and 10 600 nm

In what concerns the mode of activation, the studies included used either a pulsed or a continuous mode for Diode laser, a pulsed or a free running emission mode for Er, YSGG and a pulsed, a super short pulsed, a free running or a PIPS mode for Er, YAG. According to Roy George, who affirmed in his review that laser lights can be delivered to target tissue as a continuous wave in which the beam is emitted at one power level continuously as long as the foot switch is pressed, Gated-pulse mode where the laser is in an on and off mode at periods with an interval time of microseconds or free running pulse mode which a very large laser energy is emitted for an extremely short span, in microseconds followed by a relatively long time which the laser is off [51].

Yet, a review by Donald J. Coluzzi [52] conducted that the dental laser device can emit the light energy in two modalities as a function of time, constant or pulsed modes. The pulsed lasers can be further divided into two distinctive ways in which the energy is delivered to the target tissue. Thus, three different emission modes were described:

The first is the continuous wave, the second is termed gated-pulse mode, meaning that there are periodic alternations of the laser energy, much like a blinking light. This mode is achieved by the opening and closing of a mechanical shutter in front of the beam path of a continuous wave emission. One variation of this type of pulsing is the super-pulsed mode, which significantly shortens the pulse width to \50 milliseconds. And the third mode is termed free-running pulsed mode, sometimes referred to as ‘‘true pulsed.’’ This emission is done in a short time span, usually in microseconds, followed by a relatively long time in which the laser is off. For example, a free-running pulsed laser with a pulse duration of 100 microseconds with pulses delivered at 10 per second means that the energy at the surgical site is present for 1/1000 of a second and absent for the remaining 99.9% of that second. Free-running pulsed devices have a rapidly strobing flashlamp that pumps the active medium. With each pulse, high peak powers in hundreds or thousands of watts are generated, but because the pulse duration is short, the average power that the tissue experiences is small.

The important principle of any laser emission mode is that the light energy strikes the target tissue producing a thermal interaction [53]. If the laser is in a pulsed mode, the targeted tissue has time to cool before the next pulse of laser energy is emitted. In continuous wave mode, the operator must cease the laser emission manually so that thermal relaxation of the tissue may occur. In addition, a gentle air stream or an air current from the high-volume suction aids in keeping the area cooler. Similarly, when using hard- tissue lasers, a water spray helps to prevent micro-fracturing of the crystalline structures and reduces the possibility of carbonization. [52]

What’s more, Donald J. Coluzzi, in another study affirmed that the currently available continuous wave of the dioxide laser produces a dangerously excessive exposure time and heat; the very short free-running pulsed erbium lasers (Er: YAG and Er: YSGG) easily ablate layers of calcified tissue with minimal thermal effects. [53]

And the final point to talk trough is the assessment methods that have been used in order to evaluate the effect of the different irrigation methods and to elaborate the results; the main methods that were used to appraise the antibacterial effect were either CFU or CLSM, in fact Over 500 bacterial species have been isolated from endodontic infections using conventional culture-based and biochemical molecular methods [6]. Culture methods have been highly successful over the past years, it was demonstrated that there are major challenges in using culture-based diagnostics, especially in anaerobic infections [54]. Most anaerobes require specific transport media to maintain their viability, and delays in sample transportation have a significant impact on their survival. Meanwhile, facultative anaerobic and aerobic bacteria normally grow faster and analyses can be started after a couple of days. Hence, endodontic samples should always be cultured both aerobically and anaerobically [6].

Also quantifying biofilm formation on surfaces is challenging because traditional microbiological methods, such as total colony-forming units (CFUs), often rely on manual counting. These are laborious, resource intensive techniques, more susceptible to human error, in contrary of Confocal laser scanning microscopy (CLSM) which is a high-resolution technique that allows 3D visualization of biofilm architecture [55]. It is mostly used with a live/dead stain Bacterial viability kit.

No study included used a molecular method to detect and identify microbial genes or genomes from samples or cultures. Although the sensitivity of molecular methods is significantly higher than culture-based methods. The principle of action is as follows: Polymerase chain reaction (PCR) can be designed to copy a microbe-specific DNA strand from a mixture of multiple DNA molecules with assistance of short microbe-specific primers of a known DNA sequence.

Besides, the assessment of the removal of dentine debris and smear layer was mainly done with SEM or with pictures taken and magnified then observed and scored. SEM has been used worldwide in many disciplines. It can be regarded as an effective method in analysis of organic and inorganic materials on a nanometer to micrometer (μm) scale. SEM works at a high magnification reaching to 300,000x and even 1000000 in some modern models [56]. After the pictures were taken, blinded multiple reviewers analyzed and scored the results according to well-constructed scoring system in order to achieve reliable and objective results.

Limitations

This systematic review only assessed in-vitro studies, while clinical trials or in-vivo studies would be the closest to the clinical reality.

The canal curvature has a major influence either for the instrumentation of the canal or its disinfection; it is true that the use of straight root canals makes the work much easier, but it would massively mislead the results.

Also, the irrigation protocol was not completely standardized between all the studies, in term of concentration, time, depth of the needle inside the canal. The laser irradiation protocol as well, needs to be standardized in terms of the active medium, wavelength, output power, time, cycles and mode of emission.

Additionally, further studies need to compare LAI to PUI or SAI, in order to make clearer vision and to have definitive results

Conclusion

The purpose of this systematic review was to give a comprehensive and comparative analysis on different supplemental disinfection and dentine debris removal techniques used during root canal therapy and to assess the effectiveness of LAI when compared to CNI, PUI and SAI.

Therefore, the use of LAI significantly increased the irrigants potential of disinfection and hard tissue debris removal within the root canal system, especially when compared to CNI. Yet the current data could not give an absolute and conclusive judgment of the effectiveness of LAI when compared to PUI and to SAI; eventually further studies and trials need to be performed.

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