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The Efficiency of the Er: YAG Laser and Photon-Induced Photoacoustic Streaming (PIPS) as an Activation Method in Endodontic Irrigation: A Literature Review

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Abstract

In the field of endodontics, lasers have been used for a long time for the optimization of Endodontic irrigation in particular. The laser-activated irrigation (LAI) technique is based on the photomechanical effects of the lasers at low settings. They create specific cavitation phenomena and acoustic streaming in intracanal fluids. More recently, a new technique with a Er:YAG laser has been used with sub-ablative energy (20 mJ, 15 Hz) and ultra-short pulses (50 µs). This leads to intracanal cavitation and shockwaves as a result of photoacoustic and photomechanical effects. This phenomenon is called photon-induced photoacoustic streaming (PIPS). PIPS and Laser activated irrigation are described in the literature as a revolutionary and powerful method to activate the irrigant; however, systematic literature on this topic is missing. This review compares the literature on Er:YAG LAI and PIPS on endodontic irrigation with other irrigation methods. An article search was performed on the PubMed database using a series of keywords related to endodontic irrigation, including Er:YAG LAI and PIPS; 59 articles were selected for the review according to the inclusion and exclusion criteria. No *in vivo* study was found. The Er:YAG LAI and PIPS outperformed other methods in 33 of the 59 articles. There was a great variety in the study designs including bacterial incubation time, laser parameters, irrigation protocols, and irrigating solution used. The evidence suggests that the Er:YAG LAI and PIPS are promising in canal disinfection as well as debris and smear layer removal. However, the large variety in the study methods makes the results less significant. Further studies are needed to better evaluate the efficiency of these techniques, especially *in vivo* studies.

Keywords: Endodontic irrigation; Er:YAG laser; Laser activated irrigation; Photon-induced photoacoustic streaming.

Introduction

Endodontics preserves pathologic teeth and restores their functions. A successful endodontic treatment should accomplish the triad of endodontics: shaping, cleaning, and filling of the root canal systems in three dimensions. Although a combination of those three factors needs to be achieved, it is widely accepted that root canal disinfection is central to the outcome of root canal treatment.[1,2] This procedure involves removing pulp tissue, bacteria, and related irritants from the root canal systems as well as the smear layer produced during the shaping step.[3]

Many irrigants, devices, and methods have been introduced for efficient disinfection. Sodium hypochlorite (NaOCl) is considered the gold standard for root canal irrigation thanks to its wide-spectrum, nonspecific antimicrobial efficacy, and great tissue-dissolving capacity.[4] NaOCl remains the best primary irrigant versus other traditional antimicrobial agents such as hydrogen peroxide, iodine, and chlorhexidine (CHX) as well as novel agents like MTAD (a mixture of doxycycline, citric acid, and detergent) and QMix (a mixture of ethylenediaminetetraacetic acid (EDTA), CHX, and detergent).[5]

While powerful as an efficient irrigant, NaOCl cannot accomplish another important aim of root canal irrigation—dissolving inorganic debris and removing and preventing the formation of the smear layer on the root canal walls during instrumentation.[6] In addition, root canal calcifications restricting mechanical preparation are common. Therefore, the use of chelator solutions

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such as EDTA[7] and citric acid[8,9] at the end of mechanical instrumentation is recommended. The demineralizing agents allow the removal of the smear layer, which consists of dentine debris, pulpal residues, bacteria, and endotoxins. This offers better cleaning and achieves a closer interface between obturation materials and root canal walls.[10,11] While the antiseptic effect of chelators is very limited, an alternating irrigating protocol of NaOCl and EDTA may be more efficient than NaOCl alone.[12] A final rinse with a disinfecting solution after the smear layer removal is often required because EDTA and citric acid can reduce the antimicrobial properties of NaOCl.[13,14]

Classically, the irrigation solutions are dispensed from a handheld syringe utilizing a gauge needle with various vent designs. However, this conventional method has several disadvantages due to the complexity of the root canal system and the limitations of the syringe/needle irrigation technique.[15] Many disinfection methods have been developed to presumably acquire better intracanal cleaning. Different irrigant agitation techniques have been introduced, including pumping a gutta percha master cone in a fluid-filled canal, activating irrigants with ultrasonic metal tips or sonic nonmetal tips, and energizing irrigants with lasers.

Several laser wavelengths have been investigated for root canal cleaning and disinfecting: erbium:yttrium aluminum garnet (Er:YAG), 2940 nm; erbium, chromium:yttrium scandium gallium garnet (Er,Cr:YSGG), 2780 nm; neodymium:yttrium aluminium garnet (Nd:YAG), 1064 nm; diode, 635 to 980 nm; potassium titanyl phosphate (KTP), 532 nm; carbon dioxide (CO_2), 9600 and 10 600 nm. The wavelengths of erbium lasers (Er:YAG, Er,Cr:YSGG) are well absorbed in water and hydroxyapatite; thus, they may have value in agitating irrigation solutions.[16]

The laser-activated irrigation (LAI) technique is based on the creation of cavitation phenomena and acoustic streaming in intracanal fluids related to the photomechanical effects of the lasers at low settings.[17] The strong absorption of the Erbium laser energy in water and NaOCl causes vaporization and formation of vapour bubbles. These large elliptical bubbles implode after 100 to 200 μs, inducing the secondary cavitation effect. More recently, a new technique with a Er:YAG laser has been used with sub-ablative energy (20 mJ, 15 Hz) and ultra-short pulses (50 μs). This leads to intracanal cavitation and shockwaves as a result of photoacoustic and photomechanical effects.[18] This phenomenon is called photon-induced photoacoustic streaming (PIPS). In contrast to LAI in which the tips are positioned 5 mm from the apex, PIPS specific tips are kept at the entrance of the root canal, reducing the need for large file instrumentation.[19,20] The aim of this study was to review previously published studies and evaluate the effectiveness of Er:YAG LAI with PIPS versus other currently used methods and techniques in endodontic irrigation.

Literature Search Methodology

Data Sources and the Search Strategy

The review was performed as determined by the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) protocols.[21] The structured research question was developed by using the population, intervention, comparison, and outcome (PICO) framework: Do PIPS and Er:YAG LAI: (I) perform better (O) than other irrigation methods (C) in *in vitro* experiments based on extracted tooth samples (P)? A comprehensive literature search was conducted on PubMed (MEDLINE), Ovid (MEDLINE), Web of Science (all databases), SCOPUS, and the Cochrane library from 2008 to 2018. The search strategy used to explore the database was as follows: (((((((Photon induced photoacoustic streaming) OR Photon initiated photoacoustic streaming) OR Laser activated irrigation) OR Er: YAG laser) OR Sodium hypochlorite irrigation) OR Irrigation solution) OR Photoacoustic streaming) OR Hypochlorite sodium Er YAG). Articles that resulted from the above search strategy were first screened based on the title and abstract. In the second screening, whole articles were read, and articles were excluded based on predetermined exclusion criteria.

Screening and Selection of the Studies

The titles identified by the searches were screened first. If the title indicated possible inclusion, then the abstract was evaluated. In case of any doubt, the full text of the article was read. Following the evaluation of the abstracts, the articles considered eligible for the review were identified, and all of the full-text articles were assessed. Two independent reviewers assessed all the citations. Studies were selected for inclusion if they fulfilled all of the following criteria: a study about endodontic irrigation, a study about the LAI technique, and a study about PIPS. The exclusion criteria of this review were as follows:

1. Study on a plastic bloc simulating the root canal system;
2. Study on non-human teeth;
3. Study on an Er:YAG laser without using PIPS or LAI techniques;
4. Study on Er,Cr:YSGG, diode and neodymium lasers without comparison with an Er:YAG laser;
5. Article not in English.

Data Extraction

Data extraction for the included studies used a data extraction form designed to summarize each study. Data were extracted by one reviewer directly from the full text, and a second reviewer independently verified the extracted data. The following variables were recorded: author information, year of publication, type of teeth, type of laser, number of teeth employed, master apical file, type of laser tip, laser settings, activation time, and concentration of irrigant solutions. Each study was analyzed in terms of similarities so that a meta-analysis could be performed. However, a meta-analysis was not

indicated because of the considerable methodological heterogeneity. Rather, a descriptive analysis of the results of the individual studies was undertaken.

Quality Assessment (Risk of Bias)

The quality of each selected study was evaluated based on the following criteria:

(1) Was the calculation of an adequate sample size performed before starting the experiments?

(2) Were the teeth preparations performed by one operator?

(3) Was the teeth preparation procedure standardized?

(4) Were the teeth randomly divided into groups?

(5) Was the irrigating procedure performed by one operator?

(6) Were the same irrigants used in the control groups and test groups?

(7) Was the experience of the operator on the PIPS and LAI techniques reported?

(8) Were the analyses performed by evaluators blinded to the groups?

After collecting these items, the studies were classified with a high, moderate, or low risk of bias. Studies that failed to report five or more of the above items were classified as high risk, studies that failed to report three or four items were classified as moderate risk, and studies that failed to report two items or less were classified as low risk.

Results

The PubMed database yielded 343 citations in the first screening (title and abstract reviewing) process; 218 articles were selected according to the exclusion criteria, and 125 articles were subjected to full text review for eligibility assessment with 66 additional articles excluded during that process. The final result was the inclusion of 59 articles for this review (Figure 1).

Outcomes and Interests

The 59 articles included in this review were published between 2008 and 2018. Teeth used in the studies are extracted monoradicular human teeth that are generally decoronated to have the same working length. They are instrumented by different file systems and are particularly prepared for each study, including creating artificial intracanal irregularities, incubating microorganisms, cutting in half, and so on. They received different irrigation protocols before being analyzed by optical, confocal, or electronic microscopy. To evaluate the results of these articles, we established the analysis criteria for this review as follows:

1. Root canal disinfection and debris removal
2. Smear layer removal
3. Root canal medication and sealer removal
4. Resin sealer bond strength
5. Dentinal tubule penetration
6. Apical extrusion

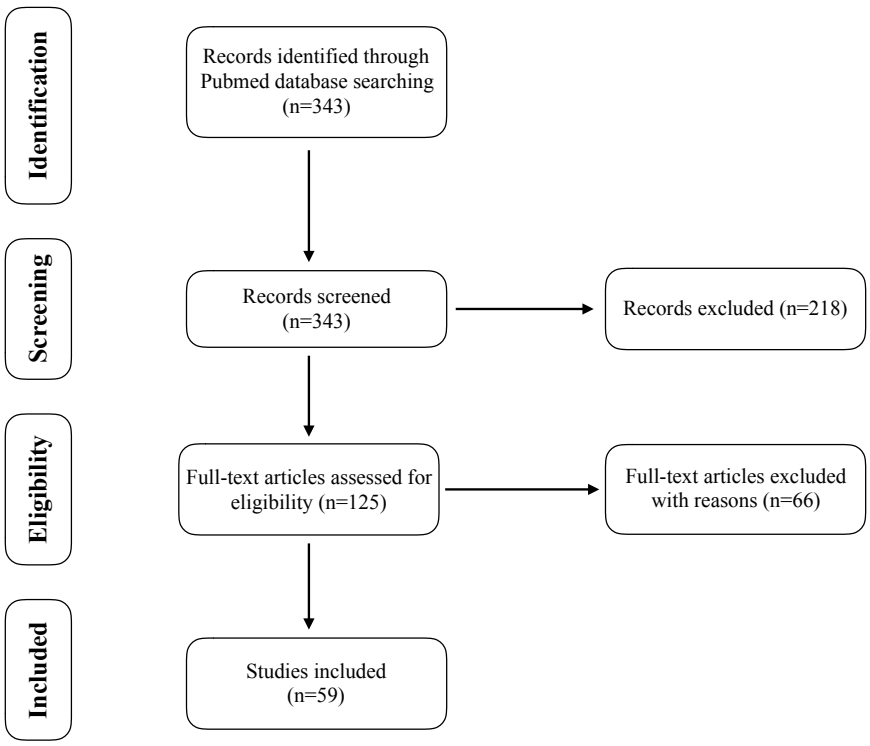

Figure 1. Articles Selection Flow Chart.

Risk of Bias

All 59 included studies were assessed for the risk of bias (Table 1), and only 4 (7%) showed a low risk of bias. Ten (17%) had a high risk. Most studies, 45 (76%), had a medium risk.

Debris Removal

There were 11 studies that discussed debris removal. Four of them concluded that LAI was more effective than other methods in removing dentine debris from root canals. All four studies found that the PIPS and LAI techniques are significantly more efficient to eliminate debris than conventional syringe irrigation (CSI) alone. Three studies also concluded that the PIPS and LAI techniques remove significantly more apical dentinal debris than passive ultrasonic irrigation (PUI).[22-24] Arslan et al[24] showed that PIPS is significantly more efficient than EndoActivator.

The authors of the other seven studies found no significantly greater debridement efficacy of PIPS versus Er: YAG LAI or other techniques. They concluded that there is no difference between the LAI technique and/or PIPS groups and PUI,[25] EndoVac,[26] ultrasonically-activated irrigation (UAI),[27–29] manual-activated irrigation (MAI),[29] or CSI.[26,27] Two articles reported that LAI with an Er: YAG laser eliminated significantly more debris than PIPS (Table 2).[25,30]

Canal Disinfection

Eighteen studies addressed canal disinfection. Two-thirds of them (11/18) concluded that PIPS had high bacterial killing efficacy, which was significantly more effective than CSI.[31-40] This was also more effective than several other activation techniques including PUI,[31,35] Nd:YAG irradiation,[39] EndoActivator, and XP-endo Finisher file.[37] One study found that PIPS coupled with 6% NaOCl inhibited 100% of *E. faecalis* but did not compare it to other techniques.[41]

Seven other studies showed no significant improvement in bacterial eradication following the activation by PIPS. The results were tested and compared to CSI,[42-44] diode laser irradiation,[45] PUI,[44] UAI,[46] and Er,Cr:YSGG LAI.[47] One study varied the laser settings and NaOCl concentration and concluded that the decontamination was only efficient with 5% NaOCl and no irrigation protocol other than PIPS (Table 2).[48]

Smear Layer Removal

Seventeen studies were included in the analysis of smear layer removal. Seven remarked on better smear layer removal following Er:YAG laser treatment. The authors noticed that Er:YAG lasers were significantly more effective than Nd:YAG LAI and MAI,[49] PUI,[50,51] diode lasers,[52] and CSI.[30,50,52,53] They also had better results than ANP (EndoVac), Nd:YAG LAI, and self-adjusting file (SAF) [30,52] but this improvement was not statistically significant. Irrigation with NaOCl and EDTA activated by lasers was significantly better versus NaOCl activated alone, EDTA activated alone, and unactivated EDTA alone.[54]

The remaining studies showed no superiority of the Er:YAG laser in removing smear layers from root canals versus other techniques including the EndoActivator,[55,56] EndoVac,[26,56,57] LAI (Nd:YAG, Er,Cr:YSGG),[58] and CSI.[26,43,59] Despite this, three studies still found that PIPS was better than CSI.[55,57,58] The rest only tested PIPS or LAI and did not compare them with other techniques (Table 3).[19,20,60]

Root Canal Medication and Sealer Removal

All four studies involving this subject remarked that the use of Er:YAG as PIPS or LAI improved the results. LAI or PIPS produced similar results to PUI in two studies[61,62] and better ones in two other studies.[63,64] They also performed better than EndoActivator,[61,63] XP-endo Finisher file, and CanalBrush.[62] They gave better results than CSI in all four studies (Table 4).

Root Canal Sealer Bond Strength

We included seven studies here. Four of them remarked on higher bond strength of resin sealers following PIPS. PIPS was reported to give significantly better results compared to CSI[65-68] and UAI.[67] It performed as effectively as PUI in two studies[65,69] but better in one study.[66] In the latter, PIPS outperformed LAI (Er:YAG, Nd:YAG, diode lasers), PUI, and EndoVac. Nevertheless, the three other studies did not remark on the improved penetration caused by PIPS versus CSI,[26,70] MAI,[71] and EndoVac (Table 5).[26]

Irrigant Penetration in Dentinal Tubules

Only one study[69] was found on irrigant penetration into dentinal tubules. The authors concluded that PIPS led to a significantly improved penetration area versus PUI, EndoActivator, and CSI (Table 6).

Apical Extrusion

Three out of five studies concluded that PIPS and Er:YAG LAI produce more extrusion than all other irrigation methods except for CSI in one study.[72] Irrigation systems included EndoVac, EndoActivator, XP-endo Finisher file, and CSI.[72–74] The two remaining studies concluded that laser activation produced statistically similar amounts of extrusion to UAI and CSI and more than the side-vented needle (Table 7).[24,75]

Discussion

PIPS and LAI techniques are described in the literature as a revolutionary and powerful method to activate the irrigant. These tools use the photothermal effect of direct irradiation as well as the photoacoustic effect. The main advantage is to overcome unwanted effects on the carbonization of the root canal dentin. PIPS studies tend to replace the LAI technique. In fact, 17 of the 55 articles

Table 1. Quality of Assessment of Reviewed Articles

	Sample size calculation	Single operator in teeth preparation	Teeth preparation standardization	Teeth randomization	Single operator in teeth irrigation	Same irrigants for control and test groups	Operator experience	Blinding of the evaluator	Classification
George et al, 2008 (75)	N	N	Y	Y	N	Y	N	N	High
George et al, 2008 (60)	N	N	N	N	N	Y	N	N	High
de Groot et al, 2009 (22)	N	N	Y	N	N	Y	N	Y	High
De Moor et al,2010 (23)	N	N	Y	Y	N	Y	N	N	High
DiVito et al, 2011 (20)	N	N	Y	Y	N	N	N	N	High
Peters et al, 2011 (31)	N	N	Y	N	N	Y	N	N	High
DiVito et al, 2012 (19)	N	N	Y	Y	N	N	N	Y	High
Jaramillo et al, 2012 (41)	N	N	Y	N	N	Y	N	N	High
Pedullà et al, 2012 (42)	N	N	Y	Y	N	Y	N	N	High
Sahar-Helft et al, 2013 (50)	N	N	Y	Y	N	Y	N	N	High
Zhu et al, 2013 (43)	N	N	Y	N	N	Y	N	Y	High
Al Shahrani et al, 2014 (33)	N	N	Y	N	N	Y	N	N	High
Arslan et al, 2014 (24)	N	N	Y	N	N	Y	N	Y	High
Guidotti et al, 2014 (54)	N	N	Y	N	N	N	N	Y	High
Mathew et al, 2014 (45)	N	N	Y	Y	N	Y	N	N	High
Olivi et al, 2014 (34)	N	N	Y	Y	N	Y	N	N	High
Sathe et al, 2014 (49)	N	N	Y	N	N	Y	N	N	High
Akcay et al, 2015 (65)	N	N	Y	Y	N	Y	N	N	High
Akyuz Ekim et al, 2015 (52)	N	N	N	N	N	Y	N	Y	High
Akyuz Ekim et al, 2015 (66)	N	N	Y	N	N	Y	N	N	High
Arslan et al, 2015 (63)	N	N	Y	Y	N	Y	N	N	High
Arslan et al, 2015 (67)	N	N	Y	Y	N	Y	N	N	High
Arslan et al, 2015 (81)	N	N	Y	Y	N	Y	N	N	High
Deleu et al, 2015 (25)	N	N	N	N	N	Y	N	Y	High
Li et al, 2015 (61)	N	N	Y	N	N	Y	N	N	High
Neelakantan et al, 2015 (35)	N	N	Y	Y	Y	Y	Y	N	Moderate
Sahar-Helft et al, 2015 (50)	N	N	Y	Y	N	Y	N	N	High
Yost et al, 2015 (72)	N	N	Y	N	N	Y	N	N	High
Balić et al, 2016 (44)	N	N	Y	Y	N	Y	N	N	High

Table 1. Continued

	Sample size calculation	Single operator in teeth preparation	Teeth preparation standardization	Teeth randomization	Single operator in teeth irrigation	Same irrigants for control and test groups	Operator experience	Blinding of the evaluator	Classification
Akcay et al, 2016 (69)	N	N	Y	Y	N	Y	N	N	High
Arslan et al, 2016 (55)	N	N	Y	Y	N	Y	N	N	High
Ayranci et al, 2016 (51)	N	N	Y	Y	N	Y	N	N	High
Azim et al, 2016 (37)	N	N	Y	N	N	Y	Y	N	High
Cheng et al, 2016 (38)	N	N	N	N	N	Y	N	N	High
Jaramillo et al, 2016 (36)	N	N	Y	Y	N	Y	N	N	High
Keles et al, 2016 (30)	N	N	Y	Y	N	Y	N	N	High
Lloyd et al, 2016 (64)	N	N	Y	Y	N	Y	N	Y	Moderate
Miletic et al, 2016 (70)	N	N	Y	N	N	Y	N	N	High
Nasher et al, 2016 (59)	N	N	N	N	N	Y	N	Y	High
Vangala et al, 2016 (71)	N	N	N	Y	N	N	N	N	High
Akcay et al, 2017 (68)	N	N	Y	N	N	Y	N	N	High
Cheng et al, 2017 (40)	N	N	Y	Y	N	Y	N	N	High
Cheng et al, 2017 (46)	N	N	Y	Y	N	Y	N	N	High
Gokturk et al, 2017 (62)	N	N	Y	Y	N	Y	N	Y	Moderate
Golob et al, 2017 (48)	N	N	Y	N	N	N	N	N	High
Kamaci et al, 2017 (27)	N	N	Y	Y	N	Y	N	Y	Moderate
Kasić et al, 2017 (47)	N	N	Y	Y	N	Y	N	N	High
Mancini et al, 2017 (56)	N	N	Y	Y	N	Y	N	Y	Moderate
Ozses Ozkaya et al, 2017 (39)	N	N	Y	Y	N	Y	N	Y	Moderate
Suman et al, 2017 (57)	N	N	Y	Y	N	Y	N	Y	Moderate
Turkel et al, 2017 (26)	N	N	Y	Y	N	Y	N	Y	Moderate
Verstraeten et al, 2017 (28)	N	N	Y	Y	N	Y	N	N	High
Arslan et al, 2018 (74)	Y	N	Y	Y	N	Y	N	N	Moderate
Azim et al, 2018 (73)	N	N	Y	N	N	Y	Y	N	High
Gorus et al, 2018 (53)	N	N	Y	Y	N	Y	N	N	High
Ozbay et al, 2018 (58)	N	N	Y	N	N	Y	N	N	High
Passalidou et al, 2018 (29)	N	N	Y	Y	N	Y	N	N	High

Y, reported in the article; N, not reported in the article.

Table 2. Descriptive Data Related to Disinfection and Debris Removal Effect

Author/Year	Study Objects	Number of Teeth	MAF	Laser Tip	Laser Settings (Pulse Energy, Repetition Rate, Power, Pulse Widths)	Activation Time	NaOCl	Main Findings
de Groot[22] (2009)	LAI, PUI, CI	60	35/0.06	280 µm, 30 mm	100 mJ, n/a, n/a, n/a	50 s	2%	LAI removed more debris than CI
De Moor[23] (2010)	LAI, PUI, CI	100	40/0.06	200 µm, n/a	75 mJ, 20 Hz, n/a, n/a	20 s	2.5%	LAI removed more debris than CI
Peters[31] (2011)	PIPS, PUI, CI	70	20/0.07	400 µm, 21 mm	50 mJ, 10 Hz, n/a, n/a	30 s	6%	PIPS killed *E. faecalis* most effectively
Jaramillo[41] (2012)	PIPS	24	20/0.07	400 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	20 s	6%	PIPS inhibited 100% the development of *E. faecalis*
Pedullà[42] (2012)	PIPS, CI	148	25/0.06	400 µm, 12 mm	20 mJ, 15 Hz, n/a, 50 µs	30 s	5%	No difference in bacterial reduction between groups
Sahar-Helft[50] (2013)	LAI (Er:YAG), CI	60	30/0.09	0.4 µm, n/a	500 mJ, 12 Hz, n/a, n/a	4x15 s CHX, 4x15 s EDTA	2% CHX 17% EDTA	LAI killed more *E. faecalis* than CI
Zhu[43] (2013)	PIPS, CI	48	40/0.06	400 µm, 12 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	60 s	3%	No difference in bacterial reduction between groups
Al Shahrani[33] (2014)	PIPS, CI	60	25/0.08	600 µm, 9 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	90 s	6%	PIPS killed more *E. faecalis* than CI
Arslan[24] (2014)	PIPS, SI, UAI, CI	48	40/0.06	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	60 s	1%	PIPS removed debris most effectively
Mathew[45] (2014)	PIPS, SI, diode laser, CI	130	20/0.07	200 µm, n/a	75 mJ, 20 Hz, n/a, n/a	60 s NaOCl + 60 s EDTA	5% NaOCl 17% EDTA	Diode laser killed *E. faecalis* most effectively
Olivi[34] (2014)	PIPS, CI	26	25/0.06	600 µm, 9 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	60 s	5%	PIPS inhibited 100% the development of *E. faecalis*, unlike CI
Deleu[25] (2015)	PIPS, LAI, PUI, CI, MAI, diode laser	25	30/0.06	PIPS, LAI: 300 µm, 14 mm	PIPS: 40 mJ, 20 Hz, n/a, 50 µs LAI: 60 mJ, 20 Hz, n/a, 50 µs	20 s	2.5%	LAI performed similar to PUI and more effectively than the rest in debris removal
Neelakantan[35] (2015)	PIPS, UAI, diode laser, CI	280	25/0.06	400 µm, 21 mm	50 mJ, 10 Hz, n/a, 50 ms	30 s, 60 s, 90 s	3%, 6%	PIPS performed similarly to the diode laser, both killed more *E. faecalis* than the rest
Balić[44] (2016)	PIPS, SI, CI	91	30/0.07	600 µm, n/a	20 mJ, 15 Hz, n/a, 50 µs	60 s	2.5%, Qmix	No difference in bacterial reduction between groups
Lloyd[64] (2016)	PIPS, CI	14	30/0.06	600 µm, 9 mm	20 mJ, 15 Hz, 0.3 W, n/a	240 s	6%	PIPS removed more debris than CI
Jaramillo[36] (2016)	PIPS, CI	48	30/0.09	n/a	n/a	3x30 s	Buffered 0.5%	PIPS killed more *E. faecalis* than CI
Keleş[30] (2016)	PIPS, Er:YAG, Nd:YAG	42	45/ n/a	300 µm, 14 mm	50 mJ, 20 Hz, 1 W, 50 µs	60 s	5%	Er:YAG LAI removed debris most effectively
Azim[37] (2016)	PIPS, XP Finisher, SI, CI	18	25/0.04	n/a	20 mJ, 15 Hz, n/a, n/a	3x30 s	6%	PIPS killed *E. faecalis* most effectively
Cheng[38] (2016)	PIPS, CSI	155	n/a	300 µm, n/a	20 mJ, 15 Hz, 0.3/0.5/1 W, 50 µs	20/30 s	5.25%	PIPS killed more *E. faecalis* than CI
Keles[30] (2016)	PIPS, LAI (Er:YAG, Nd:YAG), PUI, SAF, CI	90	45/n/a	PIPS, Er:YAG: 300 µm, 14 mm Nd:YAG: 320 µm, n/a	PIPS: 45 mJ, 20 Hz, 0.9 W, 50 µs LAI: 50 mJ, 20 Hz, 1 W, 50 µs	PIPS, LAI: 3x10 s PUI, SAF: 60 s	5%	All other methods removed more debris than CI. The Er:YAG LAI group had the least amount of residual debris

Table 2. Continued

Author/Year	Study Objects	Number of Teeth	MAF	Laser Tip	Laser Settings (Pulse Energy, Repetition Rate, Power, Pulse Widths)	Activation Time	NaOCl	Main Findings
Ozses Ozkaya[3] (2017)	PIPS, Nd:YAG, CI	110	50/n/a	PIPS: 400 µm, 14 mm Nd:YAG: 200 µm, n/a	PIPS: 35 mJ, 15 Hz, n/a, 50 µs Nd:YAG: n/a, 15 Hz, 1.5 W, n/a	PIPS: 20 s LAI: 4x5 s	1%	PIPS killed more *E. faecalis* than Nd:YAG when using saline, but similarly when using NaOCl
Cheng[40] (2017)	PIPS, UAI, CI	115	40/0.04	300 µm, n/a	20 mJ, 25 Hz, 0.5 W, 50 µs	PIPS: 30 s, UI: 60 s	5.25%	PIPS killed more *E. faecalis* than CI, but similarly to UAI
Kasić[47] (2017)	PIPS, LAI (Er,Cr:YSGG), Nd:YAG laser	30	30/0.09	PIPS: 400 µm, 14 mm LAI, Nd:YAG: 200 µm, n/a	PIPS: 20 mJ, 15 Hz, 0.3 W, 50 µs LAI: n/a, 15 Hz, 1.25 W, 150 µs Nd:YAG: n/a, 15 Hz, 1.5 W, 100 µs	PIPS: 40 s LAI: n/a	0.9% saline	Er,Cr:YSGG killed *E. faecalis* most effectively, followed by PIPS
Cheng[46] (2017)	PIPS, CI	355	15/0.04 20/0.04 25/0.04 30/0.04 40/0.04	300 µm, n/a	20 mJ, 15/25/50 Hz, 0.3/0.5/1 W, 50 µs	20 s, 40 s, 60 s	5.25%	PIPS improved disinfection efficiency of NaOCl at each apical terminal working width
Turkel[26] (2017)	PIPS, ANP, CI	142	40/0.06	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	30 s	5%	PIPS removed debris similarly to ANP, CI
Kamaci[27] (2017)	PIPS, LAI (diode laser), UAI, CI	75	50/0.05	PIPS: 300 nm, n/a LAI: 200 µm, n/a	PIPS: 20 mJ, 15 Hz, 0.3 W, n/a LAI: n/a, n/a, 2 W, n/a	20 s	2.5%	UAI removed more debris than CI. No difference between other groups
Verstraeten[28] (2017)	PIPS, LAI (Er:YAG), UAI	69	30/0.07	PIPS: 400 µm, 14 mm, LAI: 300 µm, 14 mm	PIPS: 20 mJ, 20 Hz, n/a, 50 µs LAI: 20 mJ, 20 Hz, n/a, 50 µs	3x20 s	2.5%	No difference in debris removal between groups
Golob[48] (2017)	PIPS	86	30/0.06	600 µm, 9 mm	10/20 mJ, n/a, 0.15/0.3 W, n/a	30 s NaOCl + 30 s EDTA + 2x30 s NaOCl + 30 s water	1%, 3%, 5% NaOCl 17% EDTA	Decontamination was only efficient when using 5% NaOCl
Passalidou[29] (2018)	LAI (Er:YAG), UAI, MAI, CI	50	25/0.08	400/600 µm, n/a	20 mJ, 20 Hz, n/a, 50 µs	3x20 s	2.5%	LAI removed more debris than CI, but similarly to UAI, MAI

CI, conventional irrigation; LAI, laser-activated irrigation; MAF, master apical file; MAI, manual activated irrigation; n/a, not available; ANP, apical negative pressure; PIPS, photon-induced photoacoustic streaming; PUI, passive ultrasonic irrigation; SAF, self-adjusting file; SI, sonic irrigation; UAI, ultrasonically activated irrigation.

Table 3. Descriptive Data Related to Smear Layer Removal Effect

Author	Study objects	Ni. of teeth	MAF	Laser tip	Laser settings (pulse energy, repetition rate, power, pulse widths)	Activation time	EDTA	Main findings
George[60] (2008)	LAI (Er:YAG, Er,Cr:YSGG)	150	50/0.05	Er:YAG, Er,Cr:YSGG: 400 µm, n/a	Er:YAG: 200 mJ, 20 Hz, 4 W, n/a Er,Cr:YSGG: 62.5 mJ, 20 Hz, 1.25 W, n/a	50 s	15% EDTAC	Conical fibers performed better than plain fibers, no difference between 2 laser systems
DiVito[20] (2011)	PIPS	50	K-file #30 30/0.06	400 µm, 14 mm	20 mJ, 10 Hz, 0.2 W, 50 µs	20 s, 40 s	17%	PIPS removed more smear layer with EDTA than with saline and saline alone
DiVito[19] (2012)	PIPS	80	30/0.06	400 µm, 12 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	30 s	17%	PIPS removed more smear layer with EDTA than with saline and saline alone
Zhu[43] (2013)	PIPS, CI	48	40/0.06	400 µm, 12 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	60 s	PIPS: 3% NaOCl, CI: 3% NaOCl +17% EDTA	PIPS removed the smear layer similarly to CI
Sathe[49] (2014)	PIPS, LAI (Nd:YAG), MAI	30	30/0.09	PIPS: 400 µm, 12 mm LAI: 200 µm,n/a	PIPS: 40 mJ, 20 Hz, n/a, 50 µs LAI: n/a, 15 Hz, 1.5 W, 50 µs	PIPS: n/a LAI: 3x5 s NaOCl, 3x5 s EDTA	17% EDTA 5.25% NaOCl	PIPS removed more smear layer than LAI, MAI
Guidotti[54] (2014)	LAI, CI	48	30/0.09	300 µm, n/a	50 mJ, 20 Hz, 1 W, n/a	3x5 s NaOCl; 3x5 s NaOCl + 3x5 s EDTA; 3x5 s EDTA	2.5% NaOCl 17% EDTA	The LAI with NaOCl+EDTA group gave better results than NaOCl alone activated, EDTA alone activated and EDTA alone non-activated groups
Akyuz Ekim[52] (2015)	PIPS, LAI (diode laser, Nd:YAG, Er:YAG), ANP, PUI, CI	80	40/0.06	PIPS, LAI: 300 µm, 17 mm	LAI: diode laser (n/a, 15 Hz, 1.5 W, n/a), Nd:YAG (100 mJ, 15 Hz, 1.5 W, n/a), Er:YAG (50 mJ, 10 Hz, 0.5 W, n/a) PIPS: 20 mJ, 15 Hz, 0.3 W, 50 µs	20 s	17%	PIPS removed more smear layer than CI and the diode laser, similarly to the rest
Sahar-Helft[50] (2015)	LAI (Er:YAG), PUI, CI	60	30/0.09	400 µm, 17 mm	50 mJ, 10 Hz, 0.5 W, n/a	60 s	17%	LAI removed more smear layer than PUI and CI
Arslan[55] (2016)	PIPS, LAI (Er:YAG), SI, CI	64	40/0.06	LAI: 300 µm, n/a PIPS: 400 µm, n/a	LAI: 50 mJ, 20 Hz, 1 W, n/a PIPS: 20 mJ, 15 Hz, 0.3 W, n/a	3x20 s	QMix	PIPS performed similarly to SI, both were better than CI

Table 3. Continued

Author	Study objects	Ni. of teeth	MAF	Laser tip	Laser settings (pulse energy, repetition rate, power, pulse widths)	Activation time	EDTA	Main findings
Ayranci[51] (2016)	LAI (Er:YAG), PUI	48	40/0.06	300 µm, 14 mm	n/a, n/a, n/a, 50 ms	60 s	17%	LAI removed more smear layer than PUI
Nasher[59] (2016)	PIPS, CI	64	40/n/a	600 µm, 9 mm	20 mJ, 50 Hz, 0.3 W, 50 µs	2x30 s	20%	PIPS performed similarly to CI
Keles[30] (2016)	PIPS, LAI (Er:YAG, Nd:YAG), PUI, SAF, CI	90	45/n/a	PIPS, Er:YAG: 300 µm, 14 mm Nd:YAG: 320 µm, n/a	PIPS: 45 mJ, 20 Hz, 0.9 W, 50 µs LAI: 50 mJ, 20 Hz, 1 W, 50 µs	PIPS, LAI: 3x10 s NaOCl + 3x10 s EDTA PUI, SAF: 60 s EDTA + 60 s NaOCl	5% NaOCl 17% EDTA	All other methods were more effective than CI. The Er:YAG LAI group had the least amount of residual smear layer
Suman[57] (2017)	LAI (Er:YAG), SI, ANP, CI	40	40/0.06	300 µm, n/a	50 mJ, 10 Hz, 0.5 W, n/a	LAI: 3x15 s EDTA + 3x15 s NaOCl SI: 60 s EDTA + 60 s NaOCl	5.25% NaOCl 17% EDTA	ANP was significantly more effective than all other groups in the apical third.
Turkel[26] (2017)	PIPS, ANP, CI	142	40/0.06	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	30 s NaOCl + 30 s EDTA	5% NaOCl 17% EDTA	PIPS performed similarly to ANP and CI
Mancini[56] (2017)	LAI (Er:YAG), PUI, ANP, SI	80	40/0.06	300 µm, 14 mm	60 mJ, 20 Hz, n/a, 50 µs	LAI: 4x5 s NaOCl PUI, SI: 60 s NaOCl	5.25% NaOCl	ANP was the most effective at 1 mm from apex; SI was more effective than PUI, LAI at 3, 5, and 8 mm from apex
Gorus[53] (2018)	LAI (Er:YAG), CI	60	30/0.09	n/a	20/40 mJ, 15 Hz, 0.3/0.6 W, n/a	LAI: 2x3 s NaOCl SI: 60 s NaOCl	5% NaOCl	LAI removed more smear layer than CI; no difference between parameters
Ozbay[58] (2018)	PIPS, LAI (Nd:YAG, Er,Cr:YSGG), CI	96	40/0.06	PIPS: 300 µm, n/a Nd:YAG: 320 µm, n/a Er,Cr:YSGG: 300 µm, n/a	PIPS: 20 mJ, 15 Hz, 0.3 W, n/a Nd:YAG: 60 mJ, 15 Hz, 1.5 W, n/a Er,Cr:YSGG: n/a, 20 Hz, 1.5 W, 140 ms	8x5 s distilled water 4x5 s NaOCl + 4x5 s EDTA	2.5% NaOCl 17% EDTA	Lasers were more effective than CI; no difference between laser systems

CI, conventional irrigation; LAI, laser-activated irrigation; MAF, master apical file; n/a, not available; MAI, manual activated irrigation; ANP, apical negative pressure; PIPS, photon-induced photoacoustic streaming; PUI, passive ultrasonic irrigation; SAF, self-adjusting file; SI, sonic irrigation; UAI, ultrasonically activated irrigation.

Table 4. Descriptive Data Related to Calcium Hydroxide Removal Effect

Author	Study Objects	No. of Teeth	MAF	Laser Tip	Laser Settings (Pulse Energy, Repetition Rate, Power, Pulse Widths)	Activation Time	Irrigant	Main Findings
Arslan[63] (2015)	PIPS, UAI, SI, CI	48	40/0.06	300 µm, 14 mm	30 mJ, 30 Hz, 0.9 W, n/a	PIIPS, UAI: 3x20 s SI: 60 s	17% EDTA	PIPS performed most effectively
Li[61] (2015)	PIPS, UAI, SI, CI	24	25/0.08	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, n/a	3x20 s	3% NaOCl	PIPS and PUI performed similarly, better than the rest
Lloyd[64] (2016)	PIPS, PUI, CI	30	25/0.06	600 µm, 9 mm	20 mJ, 15 Hz, n/a, n/a	3x30 s NaOCl + 30 s EDTA	8.25% NaOCl 17% EDTA	PIPS performed most effectively
Gokturk[62] (2017)	LAI (Er:YAG), PUI, SI, XP Finisher, CanalBrush, CI (beveled needle, double side-vented needle)	105	40/0.06	ISO 30, 28 mm	100 mJ, 10 Hz, 1 W, n/a	LAI, PUI, XP Finisher, CanalBrush: 60 s SI: 120 s	2.5% NaOCl	LAI and PUI performed similarly, better than the rest

CI, conventional irrigation; LAI, laser activated irrigation; MAF, master apical file; n/a, not available; ANP, apical negative pressure; PIPS, photon-induced photoacoustic streaming; PUI, passive ultrasonic irrigation; SI, sonic irrigation; UAI, ultrasonically activated irrigation.

published since 2011 (year of the first PIPS article) cover the LAI technique.

This review shows heterogeneity in the protocols tested: laser fiber parameters, laser parameters, irrigant concentration, activation time, and the number of activation cycles. There are also differences in sample preparation: the type of the teeth, the state of the teeth (freshly extracted or coming from a collection), the teeth preparation protocol, incubation methods, bacterial counting methods, and analysis methods. Other factors such as the experience of the operators and the differences in irrigant use between test and control groups can also affect the results.

We found that 11 of 18 articles concluded that the Er:YAG laser benefited the activation procedure as judged by disinfection outcomes. However, there is little evidence in terms of evidence-based medicine because these studies were *in vitro* or *ex vivo* studies. The teeth were only infected with *E. faecalis*. The clinical reality is a poly-microbial infection, even including fungi. To date, no experimental *in vitro* model has reproduced endodontic biofilms.[76] Its elimination via the PIPS or LAI technique has never been explored. In addition, some studies[19,20,41,48,60] only examined the LAI technique or PIPS without comparison to other techniques. The absence of *in vivo* studies or clinical trials reduces the scientific power of the Er:YAG laser for the activation of irrigating solutions. Additional clinical research studies, especially *in vivo* studies, are required to prove the scientific efficacy of these techniques and establish a sufficient level of proof.

Another factor to be considered is the irrigating solutions. Three major irrigants were used: NaOCl, EDTA, and QMix. There is a large variety in the concentration of NaOCl: 1%, 2%, 2.5%, 3%, 5%, 5.25%, and 6%. The 2.5% NaOCl was found to be better due to its higher efficiency and lower cytotoxicity.[77] However, 6% NaOCl was shown to be the most effective disinfecting solution against a 3-week-old *E. faecalis* biofilm.[78] The articles used various incubation times to evaluate the effectiveness of *E. faecalis* disinfection protocols: 2 weeks, 3 weeks, and 4 weeks. Other authors found that the *E. faecalis* biofilm was not completely established until 3 weeks.[76]

We only found consensus in the results on the removal of root canal medication and sealer—all studies remarked that the use of the Er:YAG laser improved the results versus other removal methods. Many factors such as the variation in sample conditions, irrigating protocols, and irrigating solutions hamper direct comparison of the results of the studies. The lack of concrete scientific proof makes it difficult to draw broad conclusions about the effectiveness of PIPS and LAI.

Nevertheless, we conclude that irrigation is a key element of endodontic success. The PIPS technique is a time-saving tool for clinicians. An important advantage of this technique is that the irrigant is propelled throughout the entire root canal system. Thus, we should discuss the results to establish a complete operating protocol and prognosis for treated teeth.

There are no clear recommendations in the literature about irrigation or activation times.[35] The duration of the application should be as short as possible but with maximum efficiency. There is no consensus on application time. We noted a wide variety of activation times from 20 seconds to 240 seconds; none of the articles reviewed worked to create a reproducible protocol.

A complete clinical protocol for the final rinse was first suggested in 2012.[79] The protocol is three cycles of 30-second activation by PIPS with NaOCl followed

Table 5. Descriptive Data Related to Resin Sealer Bond Strength

Author	Study Objects	No. of Teeth	MAF	Laser Tip	Laser Settings (Pulse Energy, Repetition Rate, Power, Pulse Widths)	Activation Time	Concentration of Irrigant Solutions	Main Findings
Akcay[65] (2015)	PIPS, PUI, SI, CI	72	40/0.06	300 µm, 14 mm	30 mJ, 30 Hz, 0.9 W, n/a	All 30 s EDTA + 30 s NaOCl	2.5% NaOCl 17% EDTA	PIPS and PUI gave similar results, better than the rest
Akyuz Ekim[66] (2015)	PIPS, LAI (diode laser, Nd:YAG, Er:YAG), ANP, PUI, CI	32	40/0.06	PIIPS, LAI: 300 µm, n/a	LAI: diode laser (n/a, n/a, 1.2 W, n/a), Nd:YAG (100 mJ, 15 Hz, 1.5 W, n/a), Er:YAG (50 mJ, 10 Hz, 0.5 W, 1000 µs), PIPS: 20 mJ, 15 Hz, 0.3 W, 50 µs	PIPS, LAI, PUI: 4x5 s NaOCl + 4x5 s EDTA, ANP: 20 s NaOCl + 20 s EDTA	2.5% NaOCl 17% EDTA	PIPS gave the best result
Arslan[67] (2015)	PIPS, UAI, CI	72	40/0.06	400 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, n/a	PIPS: 60 s NaOCl, 60 s EDTA UI: 60 s NaOCl	5% NaOCl 17% EDTA	PIPS with distilled water gave the best result; negative effect of NaOCl and EDTA on bond strength of RelyX U200
Akcay[69] (2016)	PIPS, PUI, CI	156	40/0.06	300 µm, 14 mm	30 mJ, 30 Hz, 0.9 W, 100 µs	All 60 s NaOCl	5% NaOCl	PIPS and PUI gave similar results, better than CI
Miletic[70] (2016)	PIPS, CI	40	30/0.09	400 µm, 14 mm	20 mJ, 15 Hz, n/a, 50 µs	60 s	17% EDTA	PIPS gave similar result to CI
Vangala[71] (2016)	PIPS, MAI, CI	30	30/0.09	n/a	40 mJ, 20 Hz, n/a, n/a	PIPS: 40 s EDTA MAI: 60 s EDTA	17% EDTA	PIPS gave similar results to MAI, both were better than CI
Turkel[26] (2017)	PIPS, ANP, CI	142	40/0.06	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	30 s NaOCl + 30 s EDTA	5% NaOCl 17% EDTA	PIPS gave similar results to the rest

CI, conventional irrigation; MAF, master apical file; MAI, manual activated irrigation; n/a, not available; ANP, apical negative pressure; PIPS, photon-induced photoacoustic streaming; PUI, passive ultrasonic irrigation; UAI, ultrasonically activated irrigation.

Table 6. Descriptive Data Related to Irrigant Penetration Into the Dentinal Tubule

Author	Study Objects	Number of Teeth	MAF	Laser Tip	Laser Settings (Pulse Energy, Repetition Rate, Power, Pulse Widths)	Activation Time	Irrigant	Main Findings
Akcay[69] (2017)	PIPS, LAI (Er:YAG), PUI, SI, CI	65	40/0.06	PIPS, LAI: 300 µm, 14 mm	PIPS: 30 mJ, 30 Hz, 0.9 W, n/a LAI: 50 mJ, 20 Hz, 1 W, n/a	60 s NaOCl	5% NaOCl	Lasers gave the best results

CI, conventional irrigation; LAI, laser-activated irrigation; MAF, master apical file; n/a, not available; PIPS, photon-induced photoacoustic streaming; PUI, passive ultrasonic irrigation; SI, sonic irrigation.

Table 7. Descriptive Data Related to Apical Extrusion Effect

Author	Study Objects	No. of Teeth	MAF	Laser Tip	Laser Settings (Pulse Energy, Repetition Rate, Power, Pulse Widths)	Activation Time	Irrigants	Main Findings
George[75] (2008)	LAI (Er:YAG, Er,Cr:YSGG), CI	16	50/0.05	400 µm, n/a	Er:YAG: 200 mJ, n/a, 4 W, 50 ms; Er,Cr:YSGG: 62.5 mJ, n/a, 1.25 W, 50 ms	5 s	Dye	The volume of extruded fluid was similar to conven- tional 25-G needles, but the fluid was distributed further from the apex
Arslan[81] (2015)	PIPS, UAI, CI	64	30/0.09	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs; 30 mJ, 30 Hz, 0.9 W, 50 µs	30 s	1% Na OCl	PIPS at both 0.3 W and 0.9 W resulted in similar solution extrusion to the conventional irrigation or ultrasonic irrigation
Yost[72] (2015)	PIPS, ANP, SI, CI	36	35/0.04 55/0.04	600 µm, 9 mm	20 mJ, 15 Hz, n/a, 50 µs 10 mJ, 15 Hz, n/a, 50 µs	PIPS: 3x30 s SI: 60 s	6% NaOCl	No difference was found between the 10 mJ and 20 mJ PIPS laser groups. EndoVac demonstrated significantly less potential for apical extrusion than PIPS and Max-i-Probe
Arslan[74] (2018)	PIPS, CI	60	25/0.06	300 µm, 14 mm	20 mJ, 15 Hz, 0.3 W, 50 µs	20 s	bidistilled water	PIPS activation was associated with significantly more extrusion debris in curved canals compared with no activation
Azim[73] (2018)	PIPS, ANP, XP Finisher, SI, CI	20	40/0.04	n/a	20 mJ, 15 Hz, n/a, n/a	3x30 s	3% NaOCl	PIPS extrudes more irrigant than other systems

CI, conventional irrigation; LAI, laser-activated irrigation; MAF, master apical file; n/a, not available; ANP, apical negative pressure; PIPS, photon-induced photoacoustic streaming; SI, sonic irrigation; UAI, ultrasonically activated irrigation.

by three rounds of 30-second off cycles. The next step is 30 seconds of irrigation using PIPS with water only. EDTA is then used with PIPS for 30 seconds, and finally, a 30-second cycle with PIPS and water is applied. One publication also tested this effectiveness and concluded that this protocol was the most effective compared to CI and PUI.[64]

Several laser parameters are also important. Meire et al reported that greater energy (40 mJ compared to 10 and 20 mJ) coupled with 30 Hz of frequency eliminates more root canal debris. There are more powerful photoacoustic shockwaves at higher powers.[80] We note that the energy used for the LAI technique can go up to 500 mJ[32] with 4 W of power.[60,75] However, no other publication has reported such parameters. Moreover, recently published articles showed that this technique used parameters close to those of PIPS. The use of PIPS at the 20 mJ/0.3 W setting was reported to be effective in removing the smear layer without any damage to the dentinal tissue.[19] Cheng et al also tested the Er:YAG laser at 0.3, 0.5, and 1.0 W for either 20 or 30 seconds. They concluded that the most efficient methods were activation with 0.5 and 1.0 W for 30 seconds.[38] Laser activation at 0.5 W for 30 seconds combined with NaOCl is the preferable option because of the lower emission power. In addition, PIPS used at either 10 or 20 mJ and 0.3 or 0.9 W produced a similar apical extrusion volume.[72,81] It would be interesting to test these parameters in clinical situations by exploring postoperative pain differences.[82]

Conclusion

In the field of endodontics, PIPS and Laser activated irrigation are described in the literature as a revolutionary and powerful method to activate the irrigant. The aim of this study was to review previously published studies and evaluate the effectiveness of Er:YAG LAI with PIPS versus other currently used methods and techniques in endodontic irrigation. There was a great variety in the study designs including bacterial incubation time, laser parameters, irrigation protocols, and irrigating solution used. The evidence suggests that Er:YAG LAI and PIPS are promising in canal disinfection as well as debris and smear layer removal. However, the large variety in the study methods makes the results less significant. Further studies are needed to better evaluate the efficiency of these techniques, especially in vivo studies.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare no conflict of interest.

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