ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
March2024 • Vol. 33

Efficiency of different irrigation protocols on disinfection of root canal (An In-vitro study)

Amr Emad Mohamed El Hady[1], Maram Farouk Obeid[2], Tariq Yehia[3]

Aim: The study's objective was to compare passive ultrasonic activation (PUI) and photon-induced photoacoustic streaming (PIPS) laser-activated irrigation methods for disinfecting the root canal.
Materials and methods: E. faecalis and Candida albicans were injected into Forty-five single-rooted upper anterior human teeth to create a biofilm, then randomly divided into four groups that differ in the activation method (PIPS group, PUI group, and a control group that employs syringe irrigation without activation). A confocal scanning electron microscope (CLSM) was then used to evaluate and compare groups in terms of bacterial reduction.
Results: The various test groups differed statistically significantly from one another ($p<0.001$). The mean value of PIPS was (57.34±3.70), while the lowest value was found in the PUI group (41.40±6.38). Comparisons between pairs showed that the PIPS group had a value that was significantly higher when compared to other groups ($p<0.001$).
Conclusions: The biofilm elimination capacity of sodium hypochlorite irrigant was improved by activating it with an Er:YAG laser using the PIPS approach, showing the best biofilm eradication in the apical third. However, None of the root canal disinfection techniques achieved full eradication.

Keywords: PIPS, PUI, CSLM and E faecalis.

1. B.D.S Faculty of Dentistry – Cairo University.
2. Associate Professor of Endodontics, Faculty of Dentistry, Ain Shams University.
3. Lecturer of Endodontics, Faculty of Dentistry, Ain Shams University.
Corresponding author: Amr Emad Mohamed El Hady, email: amr.emad@bue.edu.eg

Introduction

Endodontic therapy is quite complex in its procedures, yet its aim remains mostly the same; eliminating pulp infection and preventing future microbial invasion.[1] The antibacterial range of disinfection techniques is constrained, as is their capacity to infiltrate into the dentinal tubules. Another obstacle in the way of achieving ultimate root canal disinfection is the complexity of the root canal system morphology thus creating the need for activating the irrigants to enhance activation delivery.[2] Throughout the last two decades, many activation methods were employed and continuously evaluated. Passive ultrasonic irrigation (PUI) in particular was the method that gained much popularity because it is as effective as it is cheap and affordable, and this made the PUI a recurrent group in most comparative studie.s[3] The removal of pulpal tissue remains and dentine debris with PUI is more successful than with syringe needle irrigation, but it still has certain limitations.[4] PUI enhances irrigant flow velocity and subsequently its volume, however, it is unable to get through the apical vapor lock, hence the pursuit of new activation techniques.[5] recent activation method the Photon Induced Photoacoustic Streaming (PIPS) technique. PIPS employs Er:YAG laser and its interaction with the aqueous irrigant solutions, thus it is different from previous modes of laser-activated irrigation (LAI).[6] Instead of using photothermal phenomena, this method primarily makes use of photoacoustic and photomechanical interactions. PIPS activation has an intriguing concept, especially the fact that it allows the activation tip to be only placed in pulp chamber thus allowing for promising results with minimal root canal enlargement. This study uses a CLSM to compare the efficiency of the different aforementioned activation approaches. The alternative hypothesis states there is no appreciable difference between the two activation methods in the bacterial abate.

Materials and methods

Specimens' selection:

Forty-five extracted human single-rooted maxillary anterior were used. The selected teeth have intact roots, mature apices, and no evidence of defects or visible cracks. To ensure that teeth had a single canal and no internal calcifications, abnormalities, or other anomalies, they were examined using an ordinary radiograph.

Root canal preparation:

Access cavity was prepared in all teeth. Using the MANI K-file #10 (Tochigi-Ken, Matsutain Seisakusho Co., Japan), the root canals' patency was determined. The Protaper Next rotary system was then used to instrument the canals starting with X1 (#17/4), X2 (#25/6), X3 (#30/7), X4 (#40/6) and finally X5 (50/6) employing the 300 RPM and 2.5 N.Cm torque of the X-Smart Endo Motor from Dentsply Sirona (USA), for all files.

Sterilization of samples:

The apical foramina of Forty-five teeth were then sealed with a light-cured resin composite. Samples were then inserted into sterilization bags and sterilized by autoclave for 15 minutes at 121°c using a Hygenius autoclave (FONA Dentals.r.o., Bratislava, Slovak Republic).

Bacterial infusion:

20 microns of *E. faecalis* (ATCC 29212) suspension and 20 microns of candida albicans were teeth were submerged in brain heart infusion broth medium (BHI) (Sigma-Aldrich, St. Louis, Missouri, USA) after teeth were used to infect the root canals using a micropipette. The flasks with the infected teeth were aerobically incubated for three weeks at 37°C with gentle daily shaking using an "Advanced Heratherm Protocol" in

a microbiological incubator (Thermo Fisher Scientific, Massachusetts, USA). Paper point sampling using size #50 paper points (Meta Biomed Co., Ltd., Chungbuk, South Korea) was done for the infected teeth. In a test tube containing a BHI broth medium solution, paper points were then immersed. The solution was then diluted to 1:10 concentration and spread over an agar plate using a glass rod and incubated for 24 hours at 37º C using Heratherm™ Advanced Protocol Microbiological Incubator. The colony-forming unit (CFU) was counted to ensure bacterial inoculation and growth.

Samples grouping and testing

According to the chosen irrigation method, the affected teeth were randomly divided into 4 equal groups (n=15 each):

PIPS Group: 5.25% Sodium hypochlorite (NaOCl) activated with PIPS tip using Er:YAG laser of 2940 nanometer (nm) wavelength (LightWalker®, Fotona d.o.o., Ljubljana, Slovenia) was used with quartz cylindrically tapered PIPS® tip 400/14. The operating conditions for the laser were 20 milli-joul (mJ) per pulse, 15 Hz, and a pulse duration of 50 s. The laser unit's air and water spray features were both set to "off". Then the canal and pulp chamber were bathed in 2.5% NaOCl. During the activation procedure, the PIPS tip was merely inserted into the coronal entrance aperture of the pulp cavity and left immobile without moving downward into the root canal (The laser activation protocol was as follows: the whole activation cycle was repeated three times for an entire period of 90 sec, comprising on and off each thirty sec. The amount of NaOCl solution used during each 30-sec exposure was 4 ml, 3 ml, and 3 ml respectively, hence the total NaOCl irrigation volume used was 10 mL.[7]

PUI Group: 5.25% NaOCl activated with passive ultrasonic activation (Poldent, Warsaw, Poland), 10 ml of NaOCl entirety was used to irrigate the root canals. The irrigation/activation protocol was as follows using a passive ultrasonic tip continuous (3 minutes) with NAOCL irrigant inserted 1 mm less than the predetermined working length. 10 ml of NaOCl were used in total and the total passive ultrasonic activation time was continuous for 3 min.

Control Group (CSI): The same volume of 5.25% NaOCl used in the experimental groups was delivered unactivated, using conventional syringe irrigation.

Evaluation of antimicrobial efficiency using CLSM

IsoMe ™ (Buehler Ltd., Lake Bluff, IL, USA) precision sectioning saw was used to slice teeth roots into specimens measuring 4 mm by 2 mm and 1 mm thick, taken from the apical third. The fluorescence LIVE/DEAD BacLight Microbial Survival dye was used to prepare and colour each specimen as follows: The specimen was placed in an Eppendorf tube containing 1 ml of deionized water. Ten microliters of Acridine Orange (AO) stain (100µg/ml) (Sigma-Aldrich, Missouri, USA) and 10 microliters of Propidium Iodine (PI) stain (100µg/ml) were added respectively using a micropipette. Fluorescent markers enabled the targeting of certain cells or even specific extracellular matrix components. The use of specific stains (e.g. live/dead stains) allowed differentiation of live and dead bacteria. That was demonstrated by green fluorescent signals representing live bacterial cells, and red fluorescent signals representing dead bacterial cells[8,9]. The solution was then mixed using Thermo Scientific™ LP Vortex Mixer for 30s. The specimen was then left at room temperature (~25ºC) for 15 minutes and kept in darkness. After 15 min the specimen was gently washed with 1 mm deionized water. This procedure was repeated 3 times. Finally, the specimen was placed on a microscope glass slide and scanned by the CLSM (Zeiss LSM 710, CarlZeiss, Göttingen, Germany) with a 40X oil immersion lens. The Propidium Iodide

stain is excited at a wavelength of 458 nm for excitation and emission, and the wavelength of 514 nm for the excitation of the Acridine Orange stain. The interaction of Propidium Iodide stain with dead bacteria results in the re-emission of red fluorescent light, at the same time, the interaction of Acridine Orange stain with the live bacteria results in the re-emission of green fluorescent light. The red fluorescence (dead cells) and green fluorescence (living cells) were displayed simultaneously using dual-channel imaging. Using ZEN 2012 (blue edition) software from CarlZeiss, Göttingen, Germany, the CLSM pictures were quantified at two to three random locations. By monitoring the light's fluorescent intensity, it was possible to determine the fluorescence from both live and dead bacterial cells. The percentage of dead cells out of all the cells was represented by the volume ratio of red fluorescence to green-and-red fluorescence in the photographs. The following equation was used:

$$percent = \frac{intensity\ of\ red}{intensity\ of\ red + intensity\ of\ green} \times 100$$

Statistical analysis

By examining the data pattern using the Kolmogorov-Smirnov and Shapiro-Wilk tests, numerical data were analysed for normality and displayed as mean and standard deviation (SD) values. Data had a parametric distribution, thus one-way ANOVA and Tukey's post hoc analysis were used to analyse them. For all tests, the confidence level was set at p less or equal to 0.05. The IBM SPSS Statistics Version 26 (® IBM Corporation, NY, USA) for Windows was used for the statistical analysis.

Results

Table (1) and Figure (1) show the mean and SD values of bacterial percentage elimination for various groups.

Between all the groups, there was a significant statistical difference ($p<0.001$). The PIPS value was the highest (57.34±3.70), followed by XP finisher (42.02±6.40), then the PUI group (41.40±6.38), while the lowest value was found in CSI (21.66±5.13). According to pairwise analyses, the PIPS group had a value that was significantly greater than that of the other groups ($p<0.001$). The pairwise analyses also demonstrated that CSI had much lower values than the other categories ($p<0.001$).

Table (1): Average biofilm eradication (%) for different groups

Biofilm eradication (%) (mean±SD)			p-value
CSI	PUI	PIPS	
$21.66±5.13^{C}$	$41.40±6.38^{B}$	$57.34±3.70^{A}$	<0.001*

Means with different superscript letters are statistically significantly different*; significant ($p \leq 0.05$)

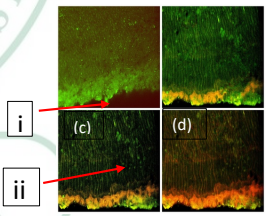

Figure (1): CLSM images representing biofilm eradication in conventional syringe irrigation (CSI) Group (a), passive ultrasonic irrigation (b), Er:YAG laser activationutilizing PIPS tip Group (c,d). i.Root canal lumen, ii.Dentinal tubules containing live bacteria (green) and dead bacteria (red)

Discussion

The eventual cleaning of the root canal cavity is the major objective of endodontic therapy. This primarily hinges on effectively getting rid of the bacterial biofilm. Many studies had emphasized the fact that one of the main causes of endodontic therapy failure is the presence of germs within the root canal network following the intervention.[10-12] Thus, the aim of the present study was to evaluate the efficacy of sodium hypochlorite activation in human-extracted single-rooted teeth using 3 different types of activation: Er:YAG laser (2940 nm) using PIPS, PUI, and biofilm eradication. Although an in-vivo situation would be

preferred, a comparative plan for in vitro research was selected to ensure control of variables and uniformity of consequences. For this study, single-canal, single-rooted teeth were selected because the oval cross-section highlights the importance of irrigation since the cleaning effect of the mechanical shaping is limited by the cross-sectional with a rounded scheme of most endodontic files, leaving vast swaths of the root canal space's abnormalities and the canal walls' untreated surfaces are left intact.[13-16] Research had displayed that these unscathed areas may extend as much as 35 percent of the entire wall canal.[17] Since E. faecalis was discovered to be the most prevalent and at times the only solitary bacterium isolated from cavities of teeth with persisting apical periodontitis, the E. faecalis strain was utilised for infecting the root canals.[18-19] The inherent antimicrobial resistance and the acquired adaptation to changing environments help E. faecalis to survive the difficult environment in endodontically treated teeth.[20] Additionally, the E. faecalis strain can develop in a biofilm condition, which is another survival tactic.[21] This strain demonstrates the traits of the biofilm type of growth, including greater virulence, stronger adhesion, and higher tolerance to antimicrobial treatments.[22-23]

Teeth apices were sealed with flowable composite to imitate in-vivo conditions regarding gas entrapment in the root canal and periodontal ligament and enabling the root canal area to serve as an irrigant tank during the irrigation/activation process.[24] Following the instrumentation protocol, 17% EDTA was used to remove the smear layer that can hinder bacterial colonization into the dentinal tubules.[25] The 3-week incubation protocol followed was necessary to allow for biofilm aging and was utilized in many previous studies.[26] Because 5.25% NaOCl is the most widely used irrigant for root canal therapy, it was chosen for this procedure.[27] It is used in concentrations ranging between 0.5% and 5.25% and it provides adequate antibacterial properties and the capability to liquefy dentin's organic components, viable and necrotic pulp tissues, and biofilm.[28] In the current study, the volume of NaOCl irrigant was standardized to be 10 ml instead of standardizing the time and rate of irrigation, as the time of irrigation and irrigant activation differs from one group to another due to different irradiation protocols.

The decision to evaluate the efficiency of activation methods according to their performance in the apical third comes from the fact that it is the most challenging region to disinfect. This is the region that has the highest incidence of canal ramifications, accessory canals, and lateral branches. It is also where the highest percentage of microorganisms reside and the region that poses a dilemma to disinfect due to the "vapor lock", or apical air entrapment, that occurs with traditional syringe irrigation ".[5] These challenges that mandate irrigation activation make the comparison more logical and more relevant clinically.[29]

Utilising CLSM, which enables non-destructive exploration of these environments and the aqueous organisation at the cellular gage, an assessment of biofilm elimination was conducted.[30]

According to our results, the null hypothesis had to be rejected. All activation methods enhanced cleaning. PIPS activation was found to be significantly more efficient than PUI. However PIPS technique was used to represent laser activation because it employs 20 milli-Joul of sub-ablative energy at 15 Hertz (0.3 W), with impulses of only 50 microseconds 92 of duration to avoid undesired thermal side effects.[31,32] More importantly, Er:YAG laser, targets water molecules as its chromophore, thus producing primary and secondary cavitation effects, which is described as the formation

of vapor-containing bubbles inside a fluid.[33] The superior results with PIPS might be attributed to the photomechanical and photothermal effects associated with Er:YAG laser. Pressure shockwaves are created as a result of this procedure, and they are distinguished by rapid fluctuations in pressure and large amplitude, thus generating powerful acoustic streaming of fluids inside the canal. The Er:YAG laser activation protocol was 30 seconds on, then 30 seconds off. The "resting" or off phase, during laser activation, enabled for greater expulsion of the reactive versions of NaOCl as indicated by Macedo et al.[34] When bubbles are forced to collapse, implosions are created, which strike surfaces and result in shear stresses, surface distortion, and ablation of surface substance. These waves of shock may potentially tear bacterium cell walls and destroy biofilms of bacteria in the root system microenvironment.

Conclusion

Within the constraints of this research, it may be said that:

None of the investigated approaches for cleaning root canals completely eliminated the E. faecalis biofilm. Activation of Sodium hypochlorite irrigant by means of Er:YAG laser utilizing PIPS technique increased its biofilm eradication capability, showing the best biofilm eradication in the apical third.

References

1. *Kakehashi S, Stanley HR, Fitzgerald RJ.* The effects of surgical exposures of dental pulps in germ free and conventional laboratory rats. J Southern Calif Dent Assoc 1966:34:449-451.
2. *Peters OA, Laib A, Göhring TN, Barbakow F.* Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod 2001;27:1–6.
3. *Van der Sluis LW, Versluis M, Wu MK, Wesselink PR.* Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007;40:415-426.
4. *Metzler RS, Montgomery S.* The effectiveness of ultrasonics and calcium hydroxide for the debridement of human mandibular molars. J Endod 1989;15:73-78.
5. *Ahmad M, Ford TR, Crum LA.* Ultrasonic debridement of root canals: an insight into the mechanisms involved. Journal of Endodontics. 1987 Mar 1;13(3):93-101.
6. *Peters OA, Bardsley S, Fong J, Pandher G, DiVito E.* Disinfection of root canals with photon-initiated photoacoustic streaming. Journal of endodontics. 2011 Jul 1;37(7):1008-12.
7. *Nasher R, Franzen R, Gutknecht N.* The effectiveness of the Erbium: Yttrium aluminum garnet PIPS technique in comparison to different chemical solutions in removing the endodontic smear layer—an in vitro profilometric study. Lasers in medical science. 2016; 31(9):1871-82.
8. *Ma J, Wang Z, Shen Y, Haapasalo M.* A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. Journal of endodontics. 2011; 37(10):1380-5.
9. *Zapata RO, Bramante CM, de Moraes IG, Bernardineli N, Gasparoto TH, Graeff MS, Campanelli AP, Garcia RB.* Confocal laser scanning microscopy is appropriate to detect viability of Enterococcus faecalis in infected dentin. Journal of endodontics. 2008; 34(10):1198-201.
10. *Alves FR, Andrade-Junior CV, Marceliano-Alves MF, Pérez AR, Rôças IN, Versiani MA, Sousa-Neto MD, Provenzano JC, Siqueira JF.* Adjunctive steps for disinfection of the mandibular molar root canal system: a correlative bacteriologic, micro–computed tomography, and cryopulverization approach. Journal of endodontics. 2016; 42(11):1667-72.
11. *Socransky SS, Haffajee AD.* Dental biofilms: difficult therapeutic targets. Periodontology 2000. 2002; 28(1):12-55.
12. *Costerton JW.* Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. Trends in microbiology. 2001; 9(2):50-2.
13. *Peters OA, Schönenberger K, Laib A.* Effects of four Ni–Ti preparation techniques on root canal geometry assessed by micro computed tomography. International endodontic journal. 2001; 34(3):221-30.
14. *De-Deus G, Belladonna FG, Silva EJ, Marins JR, Souza EM, Perez R, Lopes RT, Versiani MA, Paciornik S, Neves AD.* Micro-CT evaluation of non-instrumented canal areas with different enlargements performed by NiTi systems. Brazilian dental journal. 2015; 26(6):624-9.

15. *Versiani MA, Leoni GB, Steier L, De-Deus G, Tassani S, Pécora JD, de Sousa-Neto MD.* Micro–computed tomography study of oval-shaped canals prepared with the Self-adjusting File, Reciproc, WaveOne, and Protaper Universal systems. Journal of Endodontics. 2013; 39(8):1060-6.
16. *Zuolo ML, Zaia AA, Belladonna FG, Silva EJ, Souza EM, Versiani MA, Lopes RT, De-Deus G.* Micro-CT assessment of the shaping ability of four root canal instrumentation systems in oval-shaped canals. International endodontic journal. 2018; 51(5):564-71.
17. *Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M.* Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. International endodontic journal. 2001; 34(6):429-34.
18. *Portenier I, Waltimo TM, Haapasalo M.* Enterococcus faecalis–the root canal survivor and 'star'in post-treatment disease. Endodontic topics. 2003; 6(1):135-59.
19. *Distel JW, Hatton JF, Gillespie MJ.* Biofilm formation in medicated root canals. Journal of endodontics. 2002; 28(10):689-93.
20. *Tronstad L, Sunde PT.* The evolving new understanding of endodontic infections. Endodontic Topics. 2003; 6(1):57-77.
21. *Love RM.* Enterococcus faecalis–a mechanism for its role in endodontic failure. International endodontic journal. 2001; 34(5):399-405.
22. *Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M.* Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. Journal of Endodontics. 2004; 30(11):778-81.
23. *Podbielski A, Spahr A, Haller B.* Additive antimicrobial activity of calcium hydroxide and chlorhexidine on common endodontic bacterial pathogens. Journal of endodontics. 2003; 29(5):340-5.
24. *Bago I, Plečko V, Gabrić Pandurić D, Schauperl Z, Baraba A, Anić I.* Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. International endodontic journal. 2013; 46(4):339-47.
25. *Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N, Bai Q, Tian Y, Yu Q.* Evaluation of the bactericidal effect of Nd: YAG, Er: YAG, Er, Cr: YSGG laser radiation, and antimicrobial photodynamic therapy (aPDT) in experimentally infected root canals. Lasers in surgery and medicine. 2012; 44(10):824-31.
26. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. International Endodontic Journal. 1982; 15(4):187-96.
27. *Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR.* Review of contemporary irrigant agitation techniques and devices. Journal of endodontics. 2009; 35(6):791-804.
28. Azim AA, Aksel H, Zhuang T, Mashtare T, Babu JP, Huang GT. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis. Journal of endodontics. 2016; 42(6):928-34.
29. *Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD.* Mechanism of action of sodium hypochlorite. Brazilian dental journal. 2002;13(2):113-7.
30. *Peterson BW, He Y, Ren Y, Zerdoum A, Libera MR, Sharma PK, Van Winkelhoff AJ, Neut D, Stoodley P, Van Der Mei HC, Busscher HJ.* Viscoelasticity of biofilms and their recalcitrance to mechanical and chemical challenges. FEMS microbiology reviews. 2015; 39(2):234- 45.
31. Herce-Ros N, Álvarez-Sagües A, Álvarez-Losa L, Nistal-Villan E, Amador U, Presa J, Azabal M. Antibacterial Ability of Sodium Hypochlorite Activated with PUI vs. XPF File against Bacteria Growth on Enterococcus faecalis Mature Biofilm. Dentistry Journal. 2021 Jun;9(6):67
32. *Blanken J, De Moor RJ, Meire M, Verdaasdonk R.* Laser induced explosive vapor and cavitation resulting in effective irrigation of the root canal. Part 1: a visualization study. Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery. 2009; 41(7):514-9.
33. *Doukas AG, Flotte TJ.* Physical characteristics and biological effects of laser-induced stress waves. Ultrasound in medicine & biology. 1996; 22(2):151-64.
34. *Macedo RG, Wesselink PR, Zaccheo F, Fanali D, Van Der Sluis LW.* Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH. International Endodontic Journal. 2010; 43(12):1108-15.