Original Article

Efficacy of Laser Photoacoustic Streaming in Paediatric Root Canal Disinfection - An *Ex-Vivo* Study

Abstract

Background: Various techniques have been employed to enhance the root canal disinfection rate using sodium hypochlorite (NaOCl) as an irrigant. Photon initiated photo-acoustic streaming using lasers is a newer method of endodontic disinfection. **Aim:** The aim of this study is to evaluate and compare the efficacy of photo-initiated photoacoustic streaming and conventional irrigation technique using 2.5% NaOCl for root canal disinfection in primary teeth. **Setting and Design:** Laboratory setting and *ex-vivo* design. **Materials and Methods:** Twenty extracted primary teeth were selected in which *Enterococcus faecalis* incubation was done in the root canals and bacterial counts were obtained before the intervention. The teeth were randomly allocated into two groups. Group I samples were irrigated with conventional syringe method using 2.5% NaOCl and Group II samples were irrigated using photon-initiated photo-acoustic streaming method with erbium-doped yttrium aluminum garnet laser (wavelength - 2940 nm). Postintervention samples were obtained, and bacterial colony count was done. Wilcoxon Signed- Ranks Test and Mann–Whitney test were applied to test the intragroup and intergroup differences in the bacterial counts. **Results:** Postintervention results showed no bacterial growth in the canals irrigated using Laser ($P = 0.004$) whereas, fewer bacterial colonies were observed in NaOCl group ($P = 0.005$). There was statistically significantly higher reduction in *E. faecalis* counts in Laser Activated irrigation (LAI) group compared to NaOCl group ($P < 0.05$). **Conclusion:** Total elimination of *E. faecalis* counts was obtained by the use of laser activated NaOCl irrigation in the infected root canals, hence, it can be considered as an effective method for pediatric endodontic disinfection.

Keywords: *Disinfection, erbium-doped yttrium aluminum garnet, laser, primary teeth, root canal*

Chandrashekar Murugesh Yavagal, Viplavi Chavan Patil, Puja Chandrashekar Yavagal[1], Kiran Kumar N[2], Madhu Hariharan[3], Sachin B Mangalekar[4]

Department of Pedodontics and Preventive Dentistry, Maratha Mandal Dental College, Belgaum, [1]Department of Public Health Dentistry, Bapuji Dental College and Hospital, Davangere, Karnataka, [2]Department of Conservative Dentistry and Endodontics, Government Dental College and Research Centre, Bangalore,Karnataka, [3]Department of Conservative Dentistry and Endodontics, Amrita School of Dentistry, Ponekkara, Kochi, Kerala, [4]Department of Periodontology, Bharati Vidyapeeth Dental College, Wanlesswadi, Sangli, Maharashtra, India

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Address for correspondence:
*Dr. Puja Chandrashekar Yavagal,
Department of Public Health Dentistry, Bapuji Dental College and Hospital, Davangere - 577 004, Karnataka, India.
E-mail: pujacyavagal@gmail.com*

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Introduction

The success of any endodontic treatment depends mainly on the debridement of pulpal remnants as well as the elimination of microorganisms and their toxins from the root canal systems. However, current endodontic techniques are seen to be deficient in their goal to completely disinfect the root canals of primary teeth consistently.[1] This shortfall in case of primary teeth may be due to their biological cycle, complex anatomy of root canals and apical delta difficulties in instrumentation, progressive physiological resorption as well as lack of cooperation from the child.[2]

To overcome these lacunae different agitation techniques for root canal irrigation have been tested; such as, solution agitation with hand files, gutta percha cones, plastic instruments, and sonic/ultrasonic devices to enhance the disinfection rates of conventional irrigants like sodium hypochlorite (NaOCl). However, all of these passive strategies have only met with modest success. NaOCl is the most popular irrigating solution used in endodontic practice and is considered a "Gold standard" owing to its efficiency of removing organic constituents and remarkable tissue dissolving ability.[3] The concentration of NaOCl recommended for endodontic use varies in the range of 0.5%–5.25%. Studies have proven that NaOCl is an effective irrigant in eradicating *Enterococcus faecalis* as well.[4] In order to overcome the shortcomings and improvise the existing disinfection methods, research and innovation are paving its way and the use of laser photonic energy seems to be a step in this direction.

Recently, lasers have been explored as a means to enhance endodontic treatment outcomes in various ways of which latest strategies involve the active enhancement

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of irrigation solutions by transfer of pulsed laser energy. Specifically, the impact of pulsed erbium-doped yttrium aluminum garnet (Er: YAG) laser through photoacoustic streaming has shown to be effective in "Near total" root canal debridement.[5] Photoacoustic streaming has already been talked about as the next "Big breakthrough" in adult endodontics but there is a gross paucity of the literature to claim the same in pediatric endodontics.[6]

Unlike permanent teeth, deciduous roots are difficult to treat because of their physiological cycle of resorption, anatomical complexities, and rhizolysis; hence, posing a big challenge to successful endodontic treatment. There are studies which have been conducted on primary teeth using direct laser irradiation and photodynamic therapy but, they have their own limitations such as staining of tooth structure by photosensitizing dyes, breaking of laser tips in an attempt to go close to the canal apex unlike photoacoustic protocols where the tip is only placed in the coronal pulp chamber.[7] Thus, the aim of this study was to evaluate the efficacy of root canal disinfection of primary teeth with photon initiated photoacoustic streaming and conventional irrigation technique using 2.5% NaOCl.

Materials and Methods

A sample size of 20 teeth with ten in each group was calculated using standard deviations from previous reference studies. Fixing Type I error at 0.05, power of the study was chosen at 80%.

Extracted primary teeth were collected from the Department of Pedodontics and Preventive Dentistry of the college where the study was conducted with prior consent from the parents of children who underwent extraction Teeth with at least 2/3 of root length (single and multi-rooted) and teeth extracted for therapeutic reasons (over retained primary teeth and teeth causing ectopic eruption) were included in the study. Primary teeth with radicular resorption of more than half of the total root length and obliterated root canals were excluded from the study.

Preparation of teeth specimen

Scaling was done to remove any calculi from the teeth followed by access opening and working length determination**.** Samples were then irrigated using 2.5% NaOCl (VENSONS INDIA) during canal preparation and canal patency was maintained. The pulp chamber was flooded with NaOCl and replenished with 2.5% NaOCl irrigant after instrumentation with each file. The teeth were then transferred to a flask with deionized water for sterilization by autoclaving for 30 min at 121°C with 15 lb. pressure. The specimens were then tested for adequate sterilization using the spore test.[8]

Bacterial inoculation in the root canals

The sterile tooth specimens were inoculated with *E. faecalis* American Type Culture Collection 29,212 that was cultured in Brain–Heart Infusion broth (Difco, Detroit, MI) at 37°C for 18 h in an atmosphere of 5% CO_2 using inoculating tips. The organisms were harvested by centrifugation at 10,000 ×*g* for 5 min and then suspended in saline and adjusted to 3×10^6 cells/ml using a spectrophotometer. Specimens were kept immersed in broth at 37°C to allow bacterial growth and the medium was replaced once a week for 4 consecutive weeks. Thereafter, teeth were removed from the bacterial culture and root apices were covered with Cavit (temporary restorative material). Each tooth end was wiped with 2.5% NaOCl to disinfect the outside of the tooth before further treatment.[8] Cavit was removed and experimental irrigation procedure was completed for the two groups.

Bacterial colony count before the experimental intervention

Root canals were filled with sterile ringer's solution that acted as a medium for carrying the bacteria from canal to agar plate. Paper points were inserted into the canal terminus and left for 60 s to soak up the contents in the canals. The paper points were used to inoculate the bacteria onto blood agar plates which were then incubated at 37°C in a CO_2 chamber for 48 h. The bacterial colonies were counted in colony-forming units (CFU)/mm^3 using a digital colony counter.[8]

Teeth specimens were randomly allocated to two interventional groups based on random numbers generated using the online software [Table 1].

Experimental procedure

Group 1: Conventional needle irrigation technique

The root canals were irrigated using 2.5% NaOCl by placing the needle into the middle one third of the root canal without any activation and with a resting time of 30 s between the two cycles.[1]

Group 2: Irrigation with laser-assisted photoacoustic streaming (LAI)

This group specimens were exposed to laser irradiation by an Er: YAG laser (Lite Touch, Israel) with a wavelength of 2940 nm, in 30 s exposure intervals. The laser was set to 50 μs pulse duration at 15 Hz pulse rate and 20 mJ of energy, thereby delivering a total of 0.3W of power.[6] The root canals were filled with 2.5% NaOCl and the laser tip (radial firing) was placed in the coronal pulp chamber of the access opening only. Laser irradiation was performed with the irrigant in the canal using the LAI protocol i.e., 30 s on and 30 s off, performed for three cycles (i.e., total of 90 s of activation).[6] For evaluation of disinfection after the intervention, the samples from the root canals were obtained in a similar manner as obtained preoperatively and placed on blood agar plates followed by incubation at 37°C and bacterial colony count using a digital colony count meter was performed. All the samples were labeled and coded appropriately and sent for microbial analysis. The microbiologist who performed the microbial analysis was blinded towards the intervention details.

Results

Statistical analysis was performed using the SPSS software version 24.0. A comparison between antimicrobial efficacies of NaOCl versus Laser activated photoacoustic streaming in pediatric endodontics was done using the Mann–Whitney test as the data did not follow normal distribution.

Comparison between conventional NaOCl and photoacoustic streaming showed significantly ($P < 0.05$) lesser CFU in the photoacoustic streaming group [Table 2, Figures 1 and 2].

Discussion

Successful endodontic treatment requires a combination of several factors, such as an accurate diagnosis, thorough cleaning, a predictable disinfection protocol followed by obturation of the pulp space with an adequate final restoration. However, it is imperative to remember that there exists certain inherent anatomical differences between primary and permanent teeth with respect to their size, internal and external morphology. [9] Total elimination of *E. faecalis* counts was obtained by the use of photon-initiated photoacoustic streaming in the infected root canals in the present *ex vivo* study. Similar results were observed in few studies where Er:YAG laser activation of NaOCl irrigant was more effective than conventional irrigation.[1,10] However, pulsed Er:YAG laser-activated irrigation did not completely remove bacteria from the apical root canal and infected dentinal tubules, and there were no significant differences in bacterial reduction between the LAI group and conventional irrigation with NaOCl as observed in few studies.[5,8,11]

Table 1: Interventional groups

Group types	Intervention
Group 1	Specimens undergoing irrigation with 2.5% sodium hypochlorite
Group 2	Specimens undergoing irrigation with laser-assisted photon-initiated photoacoustic streaming

Endodontic treatment in primary teeth is considered highly complicated as they exhibit bizarre internal geometry and features such as furcal connections and horizontal anastomoses which are uncommon in permanent teeth.[12] The recent imaging techniques have shown that some parts of the pulp canal space remain uninstrumented with the use of mechanical preparation alone, implying that irrigation and instrumentation complement each other in the complete debridement and disinfection of root canals.[13] In primary teeth root canal infections, the largest number of microorganisms can be found in the main root canal. However, a considerable portion of infection is located deeper, in the lateral canals, apical ramifications, and dentinal tubules. This places additional burden on the clinician to accomplish all the endodontic protocols within a time span that can cope up with the cooperation of young pediatric patients.[14] The microorganisms associated with endodontic infections comprise a complex of bacterial species both aerobic and anaerobic. It has been reported that the root canal microbiota recovered from asymptomatic teeth is different from those isolated from clinically symptomatic teeth.[15] The most prevalent species of bacteria in primary teeth root canals are *E. faecalis*, *Porphyromonas gingivalis,* and *Treponema denticola*. Although *E. faecalis* is occasionally found in the initial root canal infections of permanent teeth, it was found to be present in 63% of the necrotic primary teeth as well. Such necrotic teeth are commonly seen in early childhood caries, which is a form of dental caries largely prevalent in children.[16] Considering this, LAI (laser-assisted irrigation) efficacy was tested against *E. faecalis* organisms in the present study.

Table 2: Intragroup and intergroup comparison of colony-forming units/ml of *Enterococcus faecalis* in the interventional groups

Samples	Group 1 Before intervention CFU (10^6)	Group 1 After intervention CFU (10^6)	Group 2 Before intervention CFU (10^6)	Group 2 After intervention CFU (10^6)	Mann-Whitney U-test (*P*)
1	50,000	No growth	36,000	No growth	0.031
2	36,000	No growth	50,000	No growth	
3	40,000	400	40,000	No growth	
4	36,000	200	30,000	No growth	
5	40,000	400	50,000	No growth	
6	30,000	No growth	36,000	No growth	
7	50,000	5000	50,000	No growth	
8	50,000	2000	50,000	No growth	
9	50000	No growth	50,000	No growth	
10	50,000	3000	40,000	No growth	
Median	45,000	400	45,000	0	
Wilcoxon signed-ranks test based on positive ranks	Z=−2.807 P=0.005		Z=−2.844 P=0.004		

CFU: Colony-forming unit

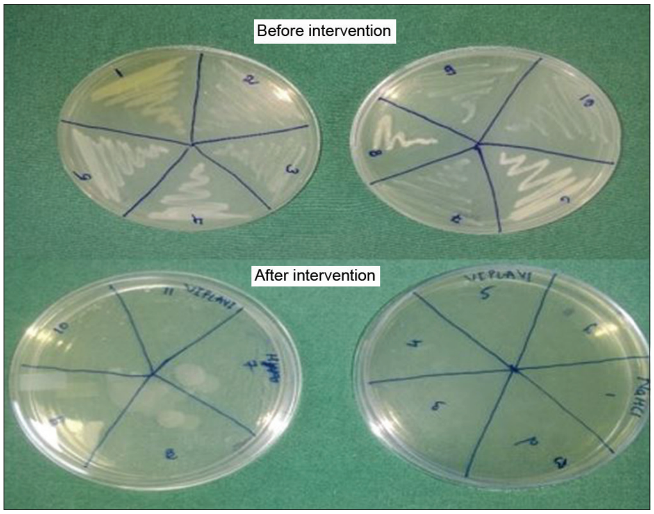

Figure 1: ***Enterococcus faecalis*** **colony-forming units/mm^3 on blood agar plates before and after conventional needle root canal irrigation using 2.5% sodium hypochlorite (Group 1)**

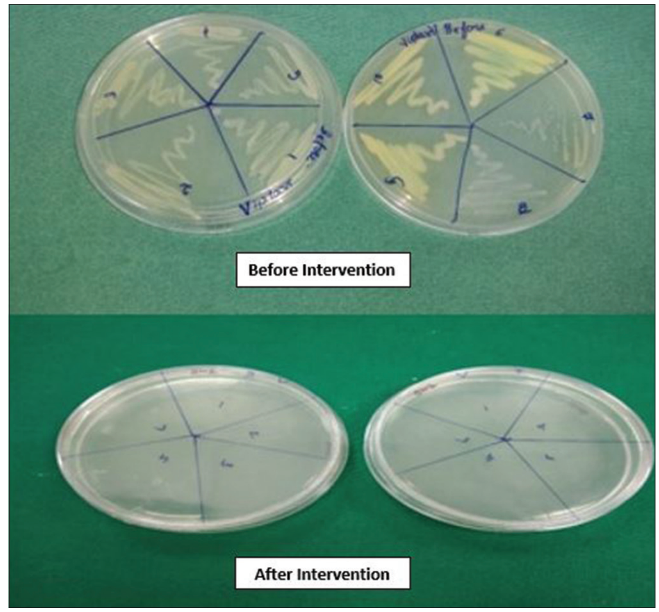

Figure 2: ***Enterococcus faecalis*** **colony-forming units/mm^3 on blood agar plates before and after photon-initiated photoacoustic streaming of root canals (Group 2)**

During the shaping phase of endodontic treatment, the smear layer produced may be compacted in the anastomosis, isthmus areas, and over tubular openings produced by the blades of endodontic instruments. The smear layer deposited in these areas consists of inorganic residues and bacterial biofilms that are unlikely to be removed by conventional root canal irrigation. To improve and upgrade the activity of the irrigants, various strategies are implemented; such as, heating the irrigating solution to enhance the NaOCl action that can be done outside the root canal system inside special containers or by heating the syringes or by using ultrasonic inserts. High temperatures increase NaOCl reaction rate, positively influencing its antibacterial action and its ability in dissolve organic residues.[17] Ultrasonic irrigation has been used wherein the vibration of the ultrasonic tip produces an acoustic stream that generates a shear stress sufficient to dislocate the debris of instrumented canals. This method has the advantage of increasing both the shear stress and the reaction rate, but there is an increased risk of extruding the irrigant beyond the apex.[18] Deficiencies in these above-mentioned methods of irrigation are observed pertaining to the time required as well as antimicrobial activity. In recent times, Laser-activated irrigation (LAI) has been introduced as an effective photoactivation method of irrigating root canals. The mechanism of action consists of generating a micro cavitation effect with subsequent implosion of irrigant bubbles due to the rapid absorption of laser energy using photoacoustic streaming. Photoactivation techniques with cavitation lead to better results than ultrasonic methods, if used for the same amount of time, with greater removal of debris, a longer lasting and increased reaction rate, as well as increased irrigant temperature.[19,20] The technique involves the use of photoacoustic streaming with sub-ablative power in a pulsating mode, which creates a series of extremely effective photoacoustic waves for the removal of the smear layer. The traditional laser applications necessitate conventional preparation for at least up to size 30 since the laser tip needs to reach the apical third of the root, and it also has a charring effect on the dentinal walls. However, the photoacoustic streaming tip does not need to reach the canal terminus and is placed only into the coronal reservoir of the root canal because Er: YAG wavelength has a very high affinity to hydroxyapatite and water. When Er: YAG laser irradiation is absorbed by water, the energy produces vapors that start to expand and form a void in front of the laser light. MMatsumoto, *et al* (2011) demonstrated that bubble increased in size and reached up to 1800 μ in 220 μs which aids in driving the solution to the inaccessible areas and suggesting that it is not always necessary to insert the laser tip up to the canal terminus because the cavitation bubbles also assist in cleaning the apical region.[21] Therefore, this technique allows for minimally invasive preparation of the root canals. The results of this study are also in accordance with the studies conducted by de Groot, *et al*.[22] (2009) and Macedo,*et al*.[23] (2010). where enhanced NaOCl reaction kinetics was seen with laser activation.[24] It utilizes a unique tapered and radial firing tip design that allows for lateral dispersion and propagation of the generated shock wave in liquids at sub ablative levels, via photoacoustic and photomechanical events. This avoids the possibility of thermal damage and allows for effective three-dimensional streaming when the specific parameters and protocols are used. Hence, in the present study, LAI was carried out using Er: YAG laser of 2940 nm wavelength wherein laser tip (Radial firing) was placed in the coronal pulp chamber. Subablative pulsed energy (20 mJ at 15 Hz, average power 0.3 W) was used in the study to produce an effective activation and streaming of fluids within the canal while reducing the thermal side effects of laser irradiation on the dentin walls.[8,25] With laser-activated NaOCl irrigation (LAI), complete eradication of *E. faecalis* was observed in the present study. The probable reason for the observed effect may be, lateral emission of photonic energy from the tip that creates three-dimensional streaming of irrigants and forceful shock waves within the root canals leading to lysing and mechanical damage of bacterial biofilm. With small volume of irrigant in the root canal, this shock-effect is amplified leading to removal of bacteria, smear layer and residual tissue tags.[8,25] Increased antibacterial activity may be caused by increased consumption of available chlorine ions during the resting interval that occurs after activation of the irrigant by means of laser.[23]

Conclusion

Total elimination of *E. faecalis* counts was obtained by the use of laser-activated NaOCl irrigation in the infected root canals; hence, it can be considered as a revolutionary attempt in achieving complete disinfection of the primary root canals, in spite of the morphological complexities.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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