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Disinfection efficacy of Laser induced photoacoustic streaming on primary root canals infected with Enterococcus faecalis: An ex-vivo study.

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Abstract

Aim of the study was to check the effectiveness of endodontic disinfection and smear layer removal by laser induced photoacoustic irrigation through a microbiologic and scanning electron microscopic evaluation of primary root canals. Twenty two primary anterior teeth extracted for therapeutic reasons were collected and chemically and mechanically prepared, sterilized and inoculated with E.Fecalis for 4 weeks. The specimens were randomly allocated into two groups. Group-I- conventional needle irrigation & Group-II- laser induced photoacoustic irrigation using Er:YAG laser. E.Fecalis colonies in both the groups were counted preoperatively and postoperatively. Representative samples from each group were processed and analysed to examine the smear layer removal by scanning electron microscopy (SEM). Statistical analysis was done using paired 't' sample tests, independent sample t test & Mann whitney U test.Group II had significantly better disinfection efficacy compared to group I ($p < 0.05$). The reduction in E Faecalis colony forming units post Er:YAG laser application was observed to be 99.99% when compared to 98% reduction after conventional needle irrigation with 2.5% Sodium hypochlorite. Laser induced photoacoustic irrigation had better disinfection efficacy compared to conventional needle irrigation using 2.5% NaOCl.

Keywords: Decontamination; endodontics; irrigation; Er:YAG laser.

Introduction

Eradication of microorganisms from an infected root canal before obturation is the primary emphasis of any endodontic treatment and is considered as the best predictor for the long-term success of such an intervention.[1] A plethora of clinical approaches have been evaluated for disinfection and control of the root canals laden by bacterial biofilms during endodontic treatment.[2,3] These bacteria in root canals have been thought to be in charge of failure of endodontic treatment.[4] Location, harbouring, and multiplication of bacteria within root canals are the factors most cited for making disinfection of this anatomical structure a clinical problem. These bacteria can colonize and survive in dentinal tubules, lateral canal ramifications, canal isthmuses, and other irregularities of the root canal which not only pose a hurdle for the clinician but also render the mechanical instrumentation ineffective.[1,4]Even the root canal systems found in primary teeth frequently contain many ramifications, deltas between canals, accessory foramina in the apical & furcal areas alongwith ever persistent root resorption makes thorough debridement quite challenging and virtually impossible by instrumentation alone. It thus entails excessive dependence on root canal irrigants to remove debris and necrotic tissue.[5]

Different techniques have been proposed to improve the efficacy of irrigating solutions, including changes in concentration, temperature, surfactant, as well as agitation.[6] Despite the fact that traditional chemomechanical cleansing measures have shown acceptable results, several literature reports have suggested that laser systems used in conjunction can be of valuable addition to reduce microbial loads in areas where traditional methods fail to succeed.[2,7,8]

Introduction of lasers has opened new vistas in the field of endodontic treatment. However the high power lasers used initially resulted in high amount of dose dependent heat generation leading to undesirable effects such as charring and thermal injury to the periodontal ligament, resulting in root resorption, ankylosis or periradicular necrosis .

To overcome these limitations, low level lasers were introduced involving activation of dye (photosensitizer) which in turn exerted a lethal effect on bacteria. But limited diffusion of the photosensitizer into intracanal irregularities, dentinal tubules and into the biofilm with restricted production of reactive oxygen species interfered with the efficacy of photodynamic therapy in root canal disinfection.[2] Recently introduced Laser Activated irrigation (LAI) by means of an erbium laser (2,780 nanometres and 2,940 nm) has proven to be more effective in removing dentinal debris and the smear layer, by a technique called laser induced photoacoustic irrigation compared with passive ultrasonic irrigation or hand irrigation. The use of laser energy has also been shown to enhance the decontaminating action of sodium hypochlorite (NaOCl) whilst sticking to the same technique.[9]

E. faecalis is the pathogenic microbe responsible for failure of endodontic treatment both in permanent as well as primary teeth. The effective removal of smear layer and bacterial colonies of *E. faecalis* through laser activated irrigation techniques has been proved effective in permanent teeth.[1, 9] But the effectiveness of the same technique is yet to be proven in root canals of primary teeth which pose a bigger challenge due to their anatomical complexities. Hence the present research was undertaken to comprehensively evaluate and compare the amount of disinfection achieved & removal of smear layer in root canals of primary teeth before and after using laser induced photoacoustic irrigation (PIPS) and conventional

needle irrigation through a microbiologic and SEM evaluation.

2.Methodology:

The present in vitro study was carried out in department of Pedodontics and preventive dentistry after the ethical clearance from Research Development and Sustenance Committee. Based on the information available from the previous studies Type I error was set at 5% while Type II error (1−β) was set at 20% with expected mean of (μA = 6.75 and μB = 8.00) and sample size of 11 per group was determined.[13,] [15]Twenty two primary anterior teeth extracted for therapeutic reasons were collected for the purpose of this study. [14] Teeth were cleaned of debris and were stored in 0.9% saline solution as preservative until use. Only teeth with two third root length remaining were included in the study. While primary teeth with root canal blockage or canal calcifications were excluded from the study.

Preparation of teeth specimens

Access opening was done in all the teeth & instrumentation was completed to size 30 K- file. Samples were irrigated using 10 mL of 2.5% NaOCl during canal preparation and canal patency was maintained.[14] The pulp chamber was flooded with NaOCl and replenished with 1 ml irrigant after each instrument. The teeth were sterilized by autoclaving for 30 minutes at 121ºC with 15 lbs pressure.[1]

Teeth were then randomly divided into two groups.

Group 1	Conventional Needle Irrigation
Group 2	Laser induced photoacoustic Irrigation

Bacteria and culture conditions: The sterilized tooth specimens were inoculated with *E. faecalis* in Brain Heart Infusion broth. Specimens were kept at 37°C to allow bacterial growth. The medium was replaced once in a week for 4 consecutive weeks. After 4 weeks of inoculation, the teeth were removed from the bacterial culture. Each tooth was wiped with 2.5% NaOCl to disinfect the outside of the tooth before further treatment.[1]

Bacterial counts before the experimental intervention:

Bacterial colonies were counted before the experimental intervention using paper point method. Canals were rinsed with sterile ringer solution and paper points were inserted into canal terminus and left for 60 seconds to soak up the contents of the canals. The wet paper points were then dropped into sterile ringer solution. Serial dilutions were performed and plated on blood agar and incubated at 37 ºC for 48 hours. The bacterial colonies were then counted.[9]

Experimental procedure

Group 1: Conventional Needle Irrigation[11]

The conventional needle irrigation protocol after canal preparation involved irrigation with conventional needle. The syringe was placed at a distance of 1 mm short of the working length. This was followed by irrigation of the canals with 2.5% NaOCl using a flow rate of 1ml per 10 seconds and was left in root canal for 20 seconds. This procedure was repeated for two more times.

Group 2: Laser induced photoacoustic Irrigation[11]

A 2,940-nm wavelength Er:YAG laser tip was placed only into coronal access of the open pulp chamber. 2.5% NaOCl was agitated using laser energy for 20 seconds. Additional NaOCl was used only when there is a depletion of the irrigant in the canal. This procedure was repeated for two more times.[11]The total volume of sodium hypochlorite was same as for the conventional needle irrigation group.

Bacterial counts after the experimental intervention:

For evaluation of bacterial counts, the samples from the root canals were obtained in a similar fashion as obtained preoperatively and plated on blood agar plates, incubated at 37ºC and bacterial colonies were counted using a digital colony count meter.

Evaluation of the removal of smear layer

The representative samples were dried for 24 hours at 21°C and then sectioned longitudinally and then split into two halves. The samples were dried for an additional 24 hours at 21°C and later sputter-coated with gold and examined by using a scanning electron microscope. The entire root canal area was examined.[9]

Statistical analysis: Statistical analysis was carried out using statistical package for social sciences (SPSS) version 20. Results were subjected to statistical analysis using Mann Whitney U test and independent t test for inter group comparisons (p value <0.05) and Paired t test was used for intra group comparisons. (p value <0.05).

Results

Antibacterial effect: Paired sample t test revealed that there was statistically significant decrease in postoperative bacterial counts in both the groups (table 1). Independent sample t test showed that there was no statistically significant difference between preoperative values between both the groups. (p = 0.305). Mann Whitney U test (table 2) revealed that there was statistically significant difference between postoperative values between both the groups. (p value<0.001). The mean reduction in bacterial colonies was 98% with 2.5% NaOCl, whereas the mean reduction in post laser treatment was observed to be 99.99%. This difference was highly statistically significant (*P* <0.001).

Scanning electron microscopic observation: Specimen from group I revealed presence of bacteria, undisturbed smear layer and occluded dentinal tubules. (Figure 1) and specimen from group II showed absence of bacteria, absence of smear layer, and open dentinal tubules. (Figure 2)

Discussion

The present study is a modest attempt to evaluate the disinfection efficacy of laser induced photoacoustic irrigation using Er:YAG laser when used in conjunction with 2.5% NaOCl in primary root canals contaminated with E.fecalis. One of the principle objective of root canal treatment is maximum elimination of bacteria from root canal system & to achieve sterile canals before obturation. This is accomplished through appropriate mechanical debridement followed by optimal irrigation.[3]Different irrigating solutions are used in an attempt to reduce the E.fecalis levels from the primary root canals.[10] However, no irrigant till date has been able to completely eliminate all the microorganisms from infected root canals. Long-term treatment failures and resistance to endodontic therapy are often observed when there is a hindrance to the complete depth of irrigant penetration. Moreover, microorganisms in the deeper layers of dentin are not affected due to the insufficient depth of penetration of the irrigant.[12]

Of all the technological advances that have been tried to overcome the shortcomings of conventional technique, laser-assisted endodontic disinfection has gained maximal acceptance. It has been proved that laser energy can eliminate microorganisms existing in the complex anatomical areas such as accessory canals, apical branches, isthmuses, and lateral canals. The present study thus focused on the evaluation of the efficacy of an Er:YAG laser when used as an adjunct to conventional needle irrigation. Enterococcus faecalis was chosen as the test organism in this study because it is the species most often associated with persistent endodontic infections and it can also be found in

primary root canal infections. This Gram-positive, facultative anaerobe coccus has been used by many other investigators, probably due to its ease of growth and laboratory manipulation.[12]In this study, 98% of disinfection was achieved by 2.5% NaOCl. This could be justified by the findings of Berutti et al. who had

demonstrated that chemical disinfectants like NaOCl penetrate no more than 130–300 μm of dentin.[12]

The resultant reduction of 99.99% bacterial colonies following laser induced photoaccoustic irrigation validate the outcomes of previous interventions. [10] The superior results obtained with regards to canal disinfection was due to very low energy levels and the high peak power produced by Er:YAG laser which generated photoacoustic shockwaves allowing three dimensional streaming of irrigant within the canal. Because the volume of the liquid in the root canal is small, this effect amplifies and improves the removal of bacteria, smear layer and residual tissue tags, which has been confirmed with the results of other studies.[8] The effectiveness of this laser technique also might be due to the increased consumption of available chlorine ions during the resting interval that occurred after the activation of the irrigant by means of an Er:YAG laser.

The placing of the tip close to the apex and its subsequent backward movement during the activation process is related to the risk of apical perforation, ledging and surface thermal damage which is negated by the use of this laser tip. Since the tip is placed only in the coronal portion pulpal chamber and left stationary it allows photoacoustic waves to spread into the openings of each canal requiring minimal enalargment of canal and no thermal damage as seen with those techniques requiring placement into the canal system.[10]One of the challenges posed by clinicians during endodontic disinfection is formation of smear layer during instrumentation. As smear layer consists of not only dentinal debris as in the coronal smear layer, but also the remnants of odontoblastic processes, pulp tissue and bacteria, which may pose a hindrance to outcome of treatment. Thus removal of smear layer is mandatory before obturation. In this aforementioned study, we noted that there was complete removal of smear layer and bacteria as well as open dentinal tubules in coronal, middle and apical sections of root in group 2 compared to group 1 wherein we observed only partial removal of bacteria and clogged dentinal tubules in group 1.This was in accordance to previous studies conducted by Zhu et al., De vito et al & da Silva et al.,who showed better results with laser compared to traditional needle irrigation.[9,12,13]It is claimed that smear layer was not removed by thermal vaporization, but probably by photomechanical streaming of the liquids, which were laser activated in the coronal part of the tooth.[14]

Conclusion

Disinfection efficacy of Laser induced photoacoustic streaming on primary root canals infected with Enterococcus faecalis was better compared to conventional needle irrigation using 2.5% NaOCl. Thus, it could be considered as a promising adjunctive method for debriding root canals in a minimally invasive manner in deciduous root canals.

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Legends Tables and figure

Table-1: Intragroup Comparison of E Faecalis Counts (Colony Forming units x200) before and After Intervention in Group I and Group II

	Time	N	Mean	SD	Std Error	t Value	p	Reduction	% Reduction
Group I	Pre	11	289.73	6.798	2.050	136.286	<0.001*	287.91	98
	Post	11	1.82	0.602	0.182				
Group II	Pre	11	293.91	5.049	1.522	195.549	<0.001*	293.73	99.99
	Post	11	0.18	0.405	0.122				

*p>0.01 –highly significant

Table-2: Intergroup Comparison of E Faecalis Counts (mean Colony forming units x200) Before and After Intervention in Group I and Group II

Group	Pre-test mean	Post-test Mean	Mean Reduction in counts
I	289.73± 6.798	1.82± 0,60	288.91±5.196
II	293.91±5.04	0.18±0.04	292.73± 4.634
			Test value = 102 (p<0.001)*

*p>0.01 –highly significant

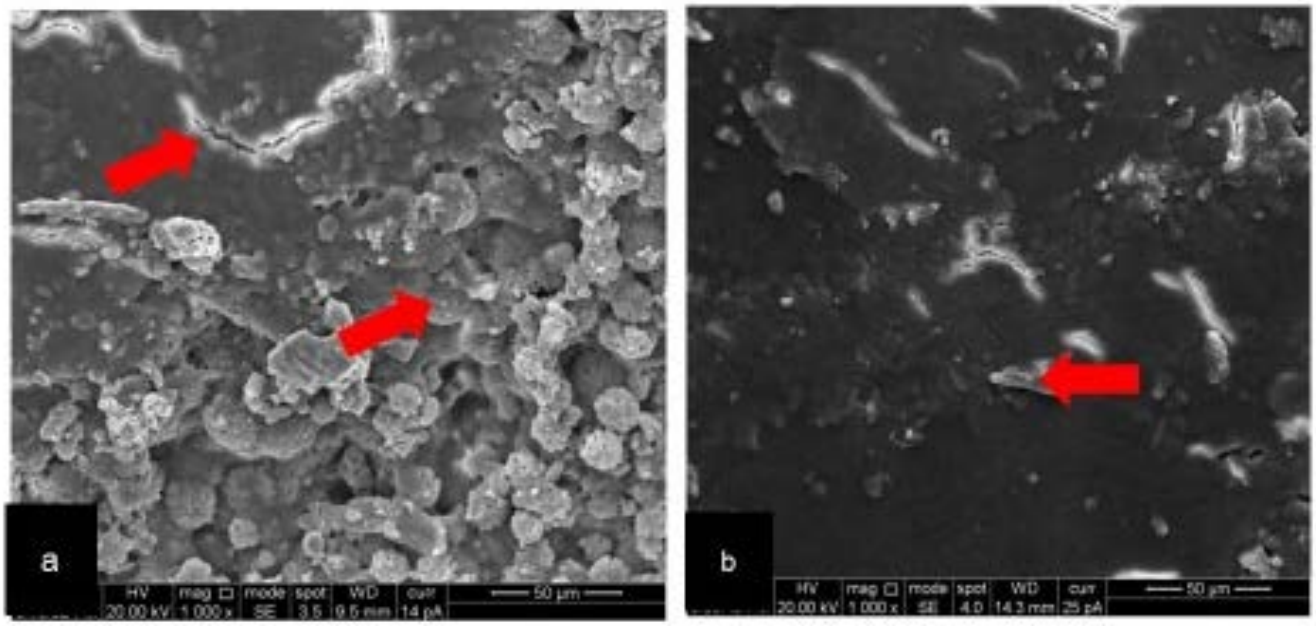

Figure 1. Micrograph representative of specimen from group I-conventional needle irrigation (a.2000x) and (b.1000x) showing presence of bacteria, undisturbed smear layer and occluded dentinal tubules

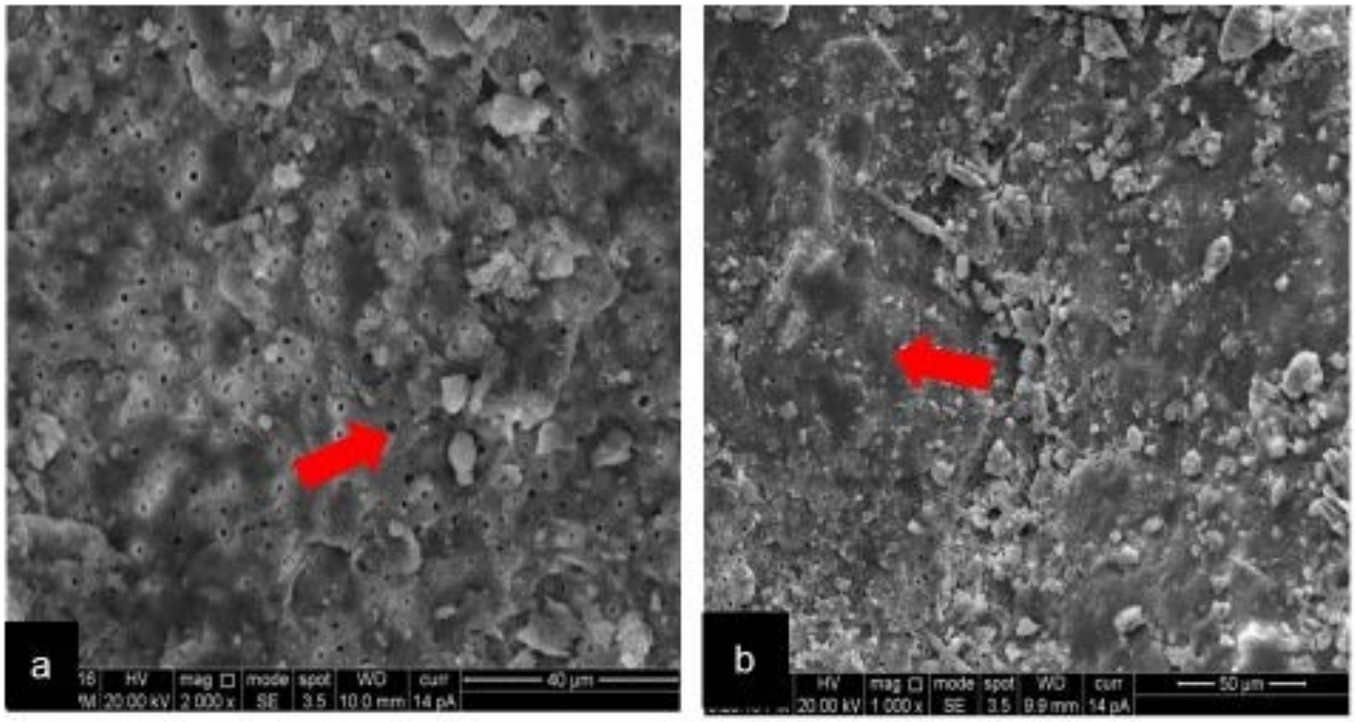

Figure 2 : Micrograph representative of specimen from group II-laser induced photoacoustic irrigation (a.2000x) and (b. 1000x) showing absence of bacteria ,absence of smear layer, and open dentinal tubules.