Efficacy of photon induced photoacoustic streaming (PIPS) on root canals infected with Enterococcus faecalis: A pilot study

Drs. David E. Jaramillo, Raydolfo M. Aprecio, Nikola Angelov, Enrico DiVito, and Thomas V. McClammy explore whether the Er:YAG laser tip is an efficient tool in elimination of Enterococcus faecalis from infected root canals

Abstract

Eradication of microorganisms from an infected root canal before obturation is a primary focus of endodontic treatment, as well as the best predictor for the long-term success of the endodontic therapy. The purpose of this *in vitro* laboratory study was to evaluate the efficacy of a new tapered and stripped Er:YAG laser tip using PIPS (Photon Induced Photoacoustic Streaming) in root canals infected with *Enterococcus faecalis* (ATCC4082). **Methods:** Twenty-four freshly extracted single-rooted human teeth were collected and inoculated with *E. faecalis*. After 4 weeks, the teeth were divided in four groups, two experimental, one positive, and one negative group. Laser treatment was performed for a period of 20 seconds with 6% sodium hypochlorite in groups 1 and 2, while PBS was used for group 3 and 4. Furthermore, dentin debris was produced, and colony-forming units were determined. **Results:** The combinations of 20 seconds laser activated irrigation with Er:YAG Laser and 6% sodium hypochlorite showed 100% inhibition using PIPS of growth of *E. faecalis* compared to 50% inhibition with the combination of Er:YAG Laser and PBS. **Conclusions:** The PIPS technology is efficient tool in elimination of *Enterococcus faecalis* from infected root canals.

Educational aims and objectives

The purpose of this article is to:
Explore whether additional use of lasers can be a valuable addition in removing bacterial load in areas where traditional methods may fail to succeed.

Expected outcomes

Correctly answering the questions on page 33, worth 2 hours of CE, will demonstrate that you can:

- Identify clinical approaches that have been evaluated for disinfection and control of the root canal biofilm during endodontic treatment.
- Realize the efficacy of a new Er:YAG laser tip called PIPS (Photon Induced Photoacoustic Streaming) in root canals infected with Enteroccocus faecalis.
- Realize that despite the fact that traditional chemo-mechanical cleansing measures have shown acceptable results in endodontic outcomes, the additional use of lasers can be a valuable addition in removing bacterial load in areas where traditional methods may fail to succeed.

Introduction

Many clinical approaches have been evaluated for disinfection and control of the root canal biofilm during endodontic treatment[1,2]. The presence of bacteria in root canals has been considered to be responsible for endodontic treatment failure[1,3]. Eradication of microorganisms from an infected root canal before obturation is a primary focus of endodontic treatment[3,4] as well as the best predictor for the long-term success of the endodontic therapy[5]. Location, harboring, and multiplication of bacteria within root canals are the factors most cited for making disinfection of this anatomical structure a clinical problem. Bacteria can colonize and survive in dentinal tubules, lateral canal ramifications, canal isthmuses, and other irregularities in the root canal[1,3], thus making the mechanical instrumentation approach limited in its effectiveness, unless supplemented with antimicrobial solutions that help in reducing the bacterial load[6,7]. *Enterococcus faecalis*, a gram-positive facultative anaerobe is able to resist and adapt to the harshest environmental conditions; this explains its presence and survival in endodontic infections and periradicular lesions[8]. Traditional therapeutic solutions such as sodium hypochlorite and chlorhexidine (either gel or liquid) or combinations of different irrigation vehicles have been shown to be effective in eliminating or reducing the presence of *E. faecalis* from root canals and dentinal tubules[9]. Different techniques have been proposed to improve the efficacy of irrigation solutions, including changes of concentration, temperature, surfactant, and agitation[10]. Despite the fact that traditional chemomechanical cleansing measures have shown acceptable results in endodontic outcomes, several literature reports have suggested that the additional use of lasers can be a valuable addition in removing bacterial load in areas where traditional methods may fail to succeed[1,11,12].

The use of Photo Dynamic Therapy (PDT) added to conventional endodontic treatment leads to a further major reduction of microbial load, proving that PDT is an efficient treatment to kill multi-drug resistant microorganisms[13]. Studies have shown that combined treatments, such as the use of sodium hypochlorite, citric acid, and diode laser energy together have a synergistic effect, increasing treatment efficacy and leading to significantly better decontamination of the root canal[14]. Er:YAG laser is effective in removing debris and smear layer from root canal walls[15]. Standardized instrumentation, followed by a final Er:YAG laser irradiation in wet canals with EDTA irrigation can result in more cleaning of the root canal walls and a higher quantity of open tubules in comparison with the traditional irrigation method[16]. Noetzel et al, evaluated the efficacy of the $CaOH_2$, Er:YAG laser and gaseous ozone in root canal disinfection either alone or combined with mechanical instrumentation and different antimicrobial solutions in canals inoculated with *E. faecalis*[3]. Within the limitation of their study, the authors concluded that Er:YAG laser irradiation does not provide satisfactory reduction of *E. faecalis*-infected root canals when compared to $CaOH_2$ and gaseous ozone, both of which demonstrated higher efficacy. They also reported that the use of antimicrobial solutions such as 1% sodium hypochlorite and 0.2% chlorhexidine showed increased antibacterial efficacy[3]. In addition, Yavari et al, also suggested that even though 1% sodium hypochlorite solution had a more significant antibacterial effect on the microbiological flora present in infected root canals, the use of laser (Er,Cr:YSGG) also showed noticeable antimicrobial effects on the *E. faecalis*[12]. Conversely, De Moor et al, showed that

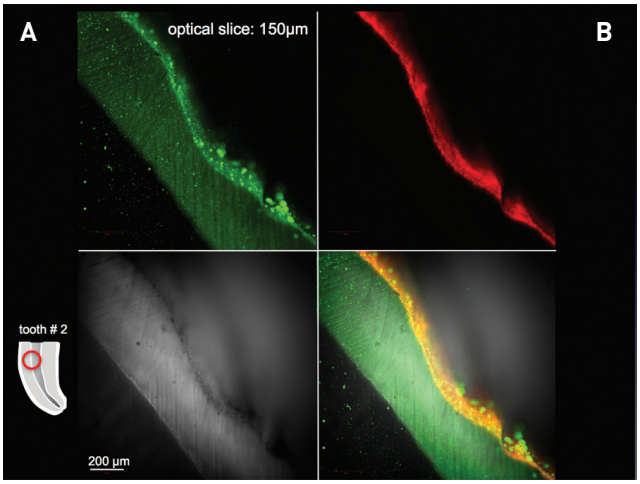


Figure 1: Bacterial growth before laser activated irrigation. BacLight staining, bright green indicates live bacteria (A), bright red indicates autofluorescence (B)

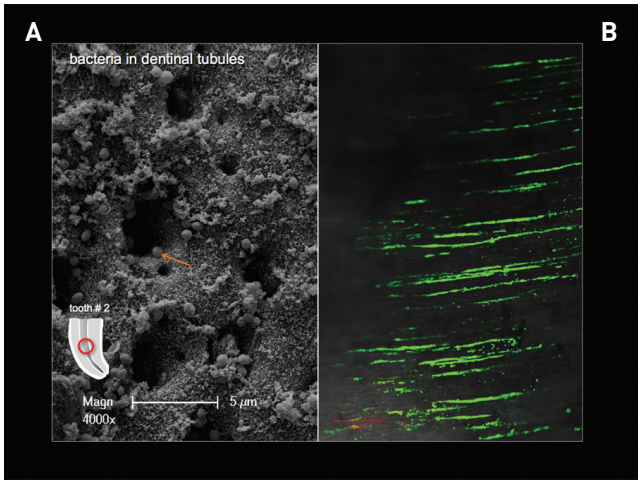


Figure 2: SEM (A) showing bacterial growth before PIPS treatment and confocal image (B) showing live bacterial growth into dentin tubules

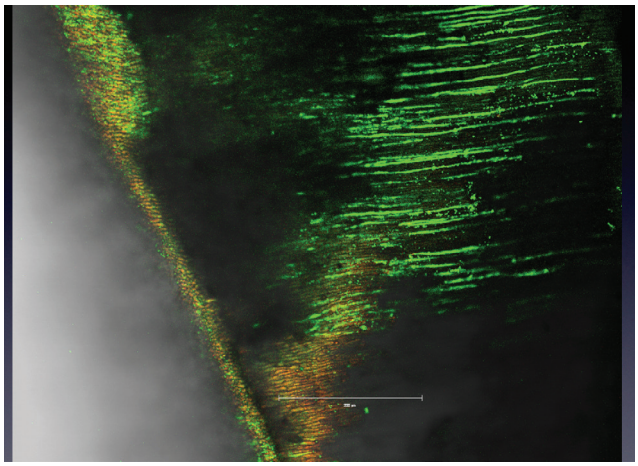


Figure 3: Close up of confocal image showing infiltration of bacteria deep into the dentin tubules

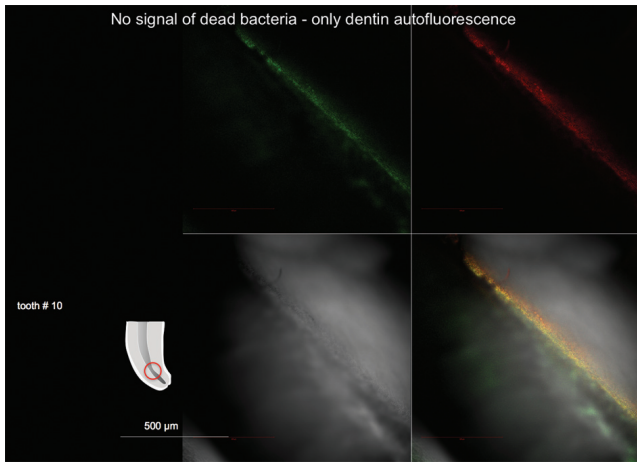


Figure 4: Confocal imaging after 20 seconds of laser activated irrigation with hypochlorites and PIPS. No signal of dead bacteria-only dentin autofluorescence

laser-activated irrigations with Er:YAG laser used for 20 seconds is comparable to the passive ultrasonic irrigation (PUI) with the intermittent flush technique[10]. Lastly, Yasuda et al, concluded that the Er:YAG laser showed high bactericidal effect in straight and curved root canals when compared to the Nd:YAG laser[17]. PIPS (Photon Induced Photoacoustic Streaming) is utilizing extremely low energy levels of laser light to generate a photoacoustic shockwave to stream irrigants throughout the entire root canal system[16]. The purpose of this in vitro laboratory study was to evaluate the efficacy of a new Er:YAG laser tip using PIPS in root canals infected with *Enteroccocus faecalis* (ATCC4082).

Materials and methods

Preparation of teeth specimens

A total of 24 freshly extracted single-rooted human teeth were collected and placed in phosphate buffered saline (PBS) solution until use. Radiographs were taken of all the teeth to rule out the possibility of root canal blockage, previous root canal treatment or canal calcifications. Access openings were prepared with a fissure carbide bur (Brasseler). A No. 10 Flexofile (Dentsply) was placed inside the root canal until it was seen at the apical foramen; 1 mm was subtracted from this measurement to establish the working length (WL). A ProTaper® Rotary File system (Dentsply) was used for root canal preparation. The root canal of each tooth was enlarged up to F1 to keep the canal enlargement as small as possible. One milliliter of 6% sodium hypochlorite was used for irrigation between each instrument use. After the canal preparation was completed, 2 ml of 17% EDTA was used as final irrigation to remove debris and smear layer and leave the dentinal tubules open to facilitate bacterial penetration. The teeth were transferred to a flask with deionized water for sterilization by autoclaving for 30 minutes at 121°C with 15 lb. pressure.

Study design

The teeth (n = 24) were divided in 4 groups: 2 experimental (groups A and B), 1 positive control (group C) and 1 negative control group (group D). The first experimental group (group A) consisted of 8 teeth that were all evaluated by Confocal Microscopy Analysis (LSM 5 Pascal Inverted, Carl Zeiss MicroImaging, Inc.). Four of these teeth (teeth 1 to 4; group A1) were previously treated with Er:YAG laser (Fidelis AT, Fotona, Ljubljana, Slovenia) and 6% sodium hypochlorite (6% NaOCl) for 20 seconds; while the remaining 4 teeth (teeth 5 to 8; group A2) were treated with saline instead of 6% NaOCl. All samples (teeth 1 to 8) were stored in PBS solution until the evaluation with the confocal microscopy. The second experimental group (group B; teeth number 9-16) was evaluated by the use of Scanning Electronic Microscope-SEM (XL 30 S, FEG, FEI Company). The first half of group B (teeth 9 to 12; group B1) was treated with Er:YAG laser and NaOCl 6%, while the second half of this group (teeth 13 to 16; group B2) was treated

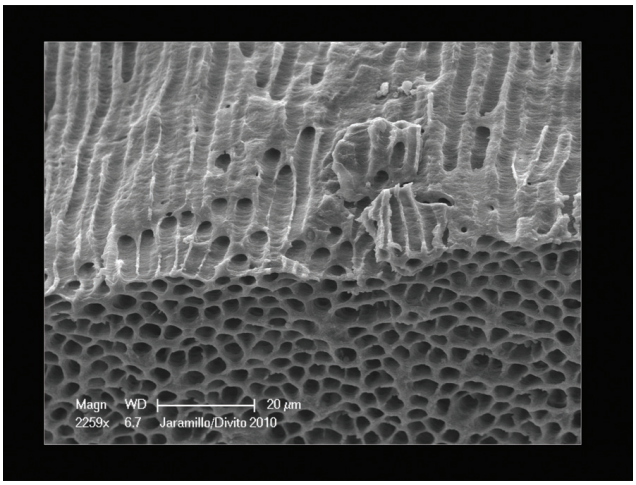


Figure 5: SEM image showing the absence of bacteria in tubules as seen on previous confocal image (Figure 4)

with Er:YAG laser and PBS. All samples (teeth 9 to 15) were stored in 4% formalin until the evaluation with the SEM. The positive control group (teeth 17 to 20; group C) included 4 teeth that were all inoculated with *E. faecalis* (ATCC 4082, Manassas, Virginia). Two teeth (17 and 18; group C1) from this group were stored in PBS and later evaluated using confocal microscopy, while the remaining 2 teeth (teeth 19 and 20; group C2) were stored in 4% formalin and then evaluated using SEM. Finally, the negative control group (teeth 21 to 24; group D) were all coated with clear nail polish to prevent bacterial penetration into the root canal. From this group, the first two teeth (teeth 21 and 22; group D1) were examined using confocal microscopy and teeth 23 and 24 (group D2) using SEM.

Bacteria and culture conditions

The sterilized tooth specimens were inoculated with *E. faecalis* (ATCC) in Brain Heart Infusion (BHI; Becton Dickinson) broth. Specimens were kept at 37°C to allow bacterial growth. The medium was replaced once a week for 4 consecutive weeks. After 4 weeks of inoculation, the teeth were removed from the bacterial culture. The root canal openings were covered with Cavit™ (3M™ ESPE™). Each tooth was wiped with 6% sodium hypochlorite to disinfect the outside of the tooth before further treatment. Cavit was removed, and experimental laser treatment by was completed.

Experimental procedures

The infected canal spaces from sample 1 through 16 were sampled using paper point and then exposed to laser irradiation with an Er:YAG laser (Fidelis) with a wavelength of 2940 nm for 20 seconds in pulse mode (50 microsecond pulse mode) at 0.3 watts (15 HZ, 20 millijules). Er:YAG laser was used to irradiate the root canals after traditional instrumentation. A newly designed quartz tip was used (PIPS, 400 micron 14 mm long). The tip was tapered and had 3 mm of the polyamide sheath stripped back from its end. The co-axial air and water spray feature of the handpiece was set to off. The tip was placed in the coronal portion of the access opening only, remaining stationary and not advanced into the root canal, accompanied by constant irrigation with 6% sodium hypochlorite. After laser treatment, a Hedström file (Maillefer, Switzerland) was used to produce dentin shavings inside all the root canals. The shavings inside the root canals were collected using a sterile paper point and placed in a 2 ml microtest tube containing 1.5 ml Brain Heart Infusion (BHI) broth for colony forming unit (CFU) counts. The tube with paper point was vortexed for 30 seconds and prepared in 1:10 serial dilutions for plating. Each sample was placed in a tube with 2ml of BHI and incubated for 72 consecutive hours. Bacterial growth was determined via visual assessment by two examiners who evaluated turbidity of each sample. Absence of turbidity indicated no bacterial growth, while turbidity was indication of viable bacteria. All non-turbid test samples were vortexed and plated with 25 ml in BHI plates. Two positive and two negative control specimens were also plated on BHI. All plates were incubated for 24 hours and again evaluated for bacterial growth by the same examiners. The presence of white pinpoint colonies indicated growth of *E. faecalis* on agar plates, and microscopic evaluation with gram stain confirmed the presence of gram-positive bacteria. Subsequently, the roots were sliced longitudinally with a diamond disk (Brasseler). The sliced roots were placed in 17% EDTA for 30 seconds to remove sectioning debris. The laser-treated teeth from group A (samples 1-8) were stored in PBS for Confocal Microscopy Analysis (Carl Zeiss MicroImaging). To determine the viability of the cells, a BacLight™ staining kit (Invitrogen™) was used. Live cells were shown as bright green dots, while dead bacteria were stained red. The laser-treated teeth from group 2 (samples 9-16) were stored in 4% formalin solution for fixation for SEM microscopy.

Statistical analysis

Significant differences in bacterial colony forming units were determined using a Friedman's two-way Analysis of Variance by Ranks for comparisons between the three laser treatment groups (before laser treatment, after laser treatment, and dental shavings after laser treatment). The Kruskal-Wallis procedure was implemented to test for differences in the bacterial colony forming units between the four solution groups at each laser treatment level: laser with sodium hypochlorite 6%, and laser with PBS for confocal microscopy, laser with sodium hypochlorite 6% (4% formalin for SEM), laser with PBS (4% formalin for SEM), Appropriate post-hoc comparisons with adjustments for multiple testing were performed at the completion of the Friedman and Kruskal-Wallis procedures if warranted. All hypotheses were two-sided and tested at an alpha level of 0.05. The statistical analyses were conducted with SAS v. 9.1.3 (SAS Institute, Cary, NC).

Results

Statistically significant differences in colony forming units per milliliter were found between the laser treatment groups in two of the four groups; Teeth 1-4, laser with sodium hypochlorite (confocal group) (Friedman test, $p < 0.001$), teeth 9-12, laser with sodium hypochlorite (SEM group) (Friedman test, $p = 0.018$), teeth 13-16, laser with PBS (SEM group) (Friedman test, $p = 0.039$), and bacterial growth in the "before laser treatment" was significantly higher than the after laser treatment in teeth 5-8 ($p = 0.034$) and "after laser treatment dental shavings" teeth ($p = 0.034$) for 9-12 sodium hypochlorite (SEM group). Similar pattern of significance was detected in the PBS (SEM group) group of higher "before laser treatment" bacterial colony forming units when compared to "after laser treatment" ($p = 0.013$) and weaker evidence when compared to "after laser treatment dental shavings" groups. No statistically significant difference in bacterial colony forming units per milliliter was found for teeth in group 5-8 (Table 1).

Two of the three laser treatment groups demonstrated a statistically significant difference in bacterial colony forming units per milliliter: "before laser treatment" (Kruskal-Wallis, $p = 0.042$) and "after laser treatment dental shavings" (Kruskal-Wallis, $p = 0.011$). Significantly less colony forming units per milliliter were observed in the "sodium hypochlorite-PBS" group (1-4) when compared to the "sodium hypochlorite–4% formalin" ($p=0.026$) and "PBS" (5-9) groups ($p=0.019$). No statistically significant difference in bacterial growth was detected between the solutions for the "after laser treatment" group teeth 1-8, (Kruskal-Wallis, $p = 0.181$; Tables 2 and 3).

Table 1

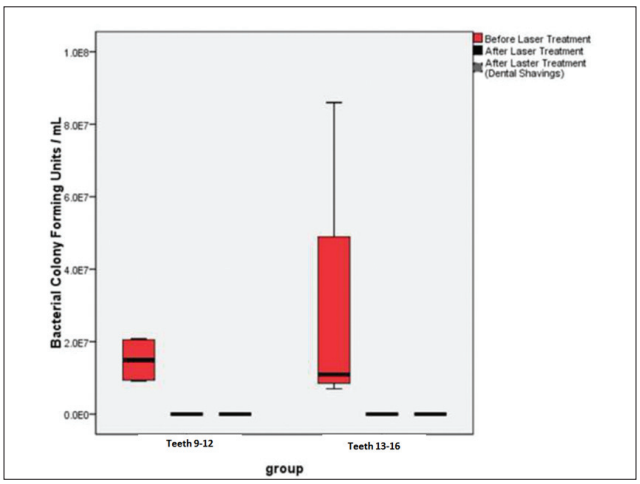
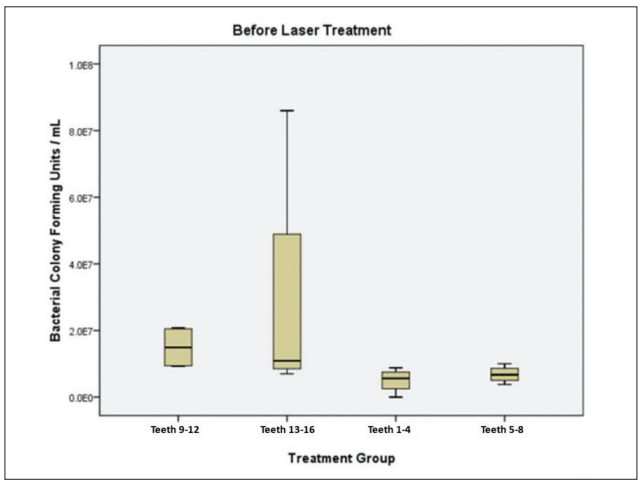


Table 2



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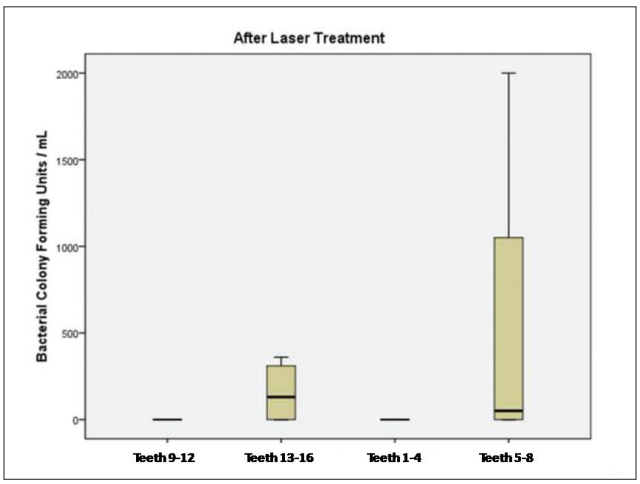


In summary, the combinations of 20 seconds irradiation with Er:YAG laser and 6% sodium hypochlorite completely inhibits the growth of *Enterococcus faecalis* when compared with the combination of Er:YAG laser and phosphate buffered saline. The dental shavings of teeth treated with Er:YAG laser and sodium hypochlorite showed 100% elimination of *Enterococcus faecalis* compared to 50% with the combination of Er:YAG laser and phosphate buffered saline. All untreated (baseline) teeth showed growth of *Enterococcus faecalis* (Figures 1, 2, and 3).

The SEM analysis, as well as the confocal microscopy analysis further confirms our findings. The SEM representative sample (Figure 5) clearly shows that there is no bacteria present on the canal wall of specimens treated with PIPS and sodium hypochlorite, when compared to the PBS control groups. Furthermore, the confocal microscopy analysis demonstrated absence of live bacteria on the canal wall, and even within the dentinal tubules in the treatment group compared to the controls (Figure 4).

Discussion

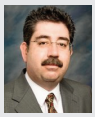
Persistence of bacteria following endodontic therapy has been identified as a major contributor to endodontic failures. Clinicians and researchers have investigated several ways to eliminate bacteria from the root canal system, including mechanical and chemical techniques[9,10]. Lasers have been used to aid in bacterial decontamination of root canal systems, with varying degrees of success. *E. faecalis* is a well-studied microorganism in the endodontic literature due to its resistance to treatment. It is therefore of remarkable interest to appreciate the results of the present study. Our in vitro pilot study clearly indicates that 20 seconds of laser activated irrigation with sodium hypochlorite using Er:YAG and PIPS technique with 50 microsecond pulse at 15HZ, with 0.3 Watts of power was effective in eliminating *E. faecalis* from in vitro infected root canal systems. This finding could be attributed to the photomechanical effect seen when light energy is pulsed in liquid[18-20]. Other studies have shown; however, that use of laser may not result in optimal bacterial reduction[3]. The possible reasons for differences in the efficacy of laser in endodontic therapy could be due to the different parameters used, including the delivery technique, the time of application within the canal, presence of aqueous solution that would affect the absorption of the laser beam and power of the laser and finally the density of energy delivered. All the previous literature cited were utilizing lasers as a thermal event. Our application was a photoacoustic subablative technique[21,22]. Our study shows that following decontamination and mechanical conventional use of sodium hypochlorite, the single application with PIPS for 20 seconds was sufficient to achieve zero growth of *E. faecalis* within the canal. Most likely, the amount of energy and the motion used during the emission of the laser beam was sufficient to penetrate and disrupt the biofilm created by the *E. faecalis*, and kill bacteria not by a thermal event, but rather via the photomechanical effects of the PIPS tapered and stripped tip. We also opine that the release of hydroxyl radicals as described during a photoacoustic event[22] also lead to the dentin tubule bacterial disinfection seen. The control groups used in this study validate the findings. The fact remains that *E. faecalis* is not the only microorganism involved in endodontic infections, so it may be beneficial to design future studies that will explore the effect of this treatment on other endodontic pathogens.

Our results are in accordance with previous studies that confirmed the efficacy of lasers in decontamination of the endodontic canals[16-20]. We speculate the low energy laser light generates a photoacoustic shockwave that pulses its way three dimensionally to all the internal aspects of the root canal system, effectively disrupting the biofilm. This may be of particular interest to the clinical practice of endodontics, allowing the clinician to use the PIPS method for effective disinfection of the entire root canal system, without unnecessary over-instrumentation, especially in the apical third of the canal.

Conclusions

Within the limitation of this study, we can conclude that the combinations of 20 seconds irradiation with Er:YAG laser via a

photoacoustic delivery system and 6% sodium hypochlorite is very effective in inhibiting *Enterococcus faecalis* growth. The results are encouraging in terms of the possible application of the Er:YAG laser in endodontics. The PIPS technology can be used as an efficient additional tool in the decontamination of infected root canals during endodontic treatment. EP



David E. Jaramillo, DDS, completed dental school and an advanced program in endodontics in Mexico in 1990 and has been teaching since then. He has also successfully practiced endodontics for 15 years. In 2004, he accepted the faculty position at USC Endodontic advanced program. In 2005, he started working at the Center for Biofilms at USC, run by Dr. Bill Costerton where he got trained in the use of SEM (Scanning Electron Microscopy) and Confocal (Laser Scanning Microscopy). In 2006, he accepted a full-time position at Loma Linda University School of Dentistry where he is an associate professor and the clinical director of endodontics. Dr. Jaramillo also works at the Center for Dental Research LLUSD. He teaches at six different advanced endodontic programs in Mexico along with USC and Long Beach VA Hospital. He has lectured in the US, Mexico, Europe and South America and has been published in numerous journals. He has co-authored several papers with his colleagues from the Department of Electrical Engineering-Electrophysics, Viterbi School of Engineering University of Southern California on the use of new plasma technology for biofilm removal from root canal system, as well as a chapter in the 6th edition of *Ingle's Endodontics*. Dr. Jaramillo has donated scanning electronic microscopy images for illustration for a chapter entitled "The Microbiology of the Necrotic Pulp" for Textbook of *Endodontology* by Dr. Gunnar Bergenholtz- 2nd edition. Dr. Jaramillo has done extensive research in the field of biofilms and has two temporary patents filed through the university patent office.

Raydolfo M. Aprecio Sr., BS, OD, is a Research Associate with extensive research experience and expertise in microbiology. Before joining the Center for Dental Research, he was a Senior Research Assistant with the Department of Microbiology and Molecular Genetics, LLU School of Medicine for 25 years. He has worked on numerous research projects in the areas of endodontics and periodontics and helped numerous graduate students and residents from Departments of Endodontics, Pediatrics and Implant Dentistry for their thesis research projects. He has served as a mentor to dental and hygiene students for their table clinic research. Each year, his student table clinics have won awards during the annual Student and Alumni Convention, California Dental Association and national meetings. He was an original member of the research team that developed the MTA and MTAD.

Nikola Angelov, DDS, MS, PhD, is a professor and a Program Director of Undergraduate Periodontics at Loma Linda University School of Dentistry. Dr. Angelov was born in Skopje, Macedonia in 1968. He earned his DDS, MS and PhD degree from St. Cyril and Methodius University and has been teaching for the Department of Oral Pathology and Periodontology in Macedonia since 1993. In 2001, he received a postdoctoral fellowship at the National Institute of Health, National Institute of Dental and Craniofacial Research (NIH/NIDCR). At NIH/NIDCR, Dr. Angelov successfully developed a new model of oral mucosal wound healing and was also involved in collaborative clinical studies, clinical trial evaluations, as well as other studies. Dr. Angelov also holds a Certificate of Specialty in Periodontics and is a Diplomate of the American Board of Periodontology. In 2008, Dr. Angelov received the prestigious annual Educator of the Year Award from the American Academy of Periodontics for outstanding results in teaching in the area of Periodontics. Dr. Angelov has several other academic awards and is actively involved in teaching and research. His research area includes oral infection and immunity, mucosal wound healing, periodontal microbiology, as well as dental implants. Dr. Angelov has extensively been published in peer review articles, and has been an invited speaker to prominent professional and research meetings. He is a member of numerous professional societies in the USA and abroad. Dr. Angelov also serves as a reviewer on the Journal of Periodontology, an official journal of the American Academy of Periodontology, as well as a School of Dentistry representative to the Institutional Review Board, and the Institutional Animal Care and Use Committee at Loma Linda University in California.

Dr. Enrico DiVito formed his dental practice in 1980 in Scottsdale. In 2004, he formed the Arizona Center for Laser Dentistry. He is also the founder and director of the state-accredited Arizona School of Dental Assisting (ASDA). In addition to teaching at ASDA, DiVito is also a clinical instructor at the Arizona School of Dentistry and Oral Health and is responsible for helping to create the Department of Laser Dentistry. He earned his undergraduate degree from Arizona State University in 1980 and is a graduate from the University of the Pacific, Arthur A. Dugoni School of Dentistry with honors receiving several clinical excellence awards. He can be reached at edivito@azcld.com.

Thomas V. McClammy, DMD, MS, graduated from Oregon Health Sciences University, School of Dentistry, in 1982. During the next 15 years, he created a remarkable general practice in Oregon and passionately pursued continuing education courses to fulfill his insatiable desire to learn. In 1996, Dr. McClammy was accepted as a post graduate endodontic resident at Boston University's Goldman Graduate School of Dentistry. He earned a Master's degree in 1999 from his mentor, Dr. Herbert Schilder, who is widely recognized as the "father of modern day endodontics." Currently, Dr. McClammy has a full-time practice dedicated to saving teeth with endodontics and implants in Scottsdale, Arizona, and is well known for his expertise, passion, and extraordinary teaching skills.

References

1. Meire MA, De Prijck K, Coenye T, Nelis HJ, De Moor RJ (2009. Effectiveness of different laser systems to kill Enterococcus faecalis in aqueous suspension and in an infected tooth model. *Int Endod J*. Apr:42(4):351-9.

2. Chávez de Paz LE, Bergenholtz G, Svensäter G (2010). The effects of antimicrobials on endodontic biofilm bacteria. *J Endod*. Jan;36(1):70-7.

3. Noetzel J, Nonhoff J, Bitter K, Wagner J, Neumann K, Kielbassa AM (2009). Efficacy of calcium hydroxide, Er:YAG laser or gaseous ozone against Enterococcus faecalis in root canals. *Am J Dent*. Feb;22(1):14-8.

4. Dewsnup N, Pileggi R, Haddix J, Nair U, Walker C, Varella CH (2010). Comparison of bacterial reduction in straight and curved canals using erbium, chromium:yttrium-scandium-gallium-garnet laser treatment versus a traditional irrigation technique with sodium hypochlorite. *J Endod*. Apr:36(4):725-8.

5. Franzen R, Gutknecht N, Falken S, Heussen N, Meister J (2011). Bactericidal effect of a Nd:YAG laser on Enterococcus faecalis at pulse durations of 15 and 25 ms in dentine depths of 500 and 1,000 µm. *Lasers Med Sci*. Jan:26(1):95-101. Epub 2010 Aug 31.

6. Mohammadi Z (2010). Chemomechanical strategies to manage endodontic infections. *Dent Today*. Feb:29(2):91-2, 94, 96 passim; quiz 99.

7. Upadya MH, Kishen A (2010). Influence of bacterial growth modes on the susceptibility to light-activated disinfection. *Int Endod J*. Nov:43(11):978-87.

8. Kayaoglu G, Ørstavik D (2004).Virulence factors of Enterococcus faecalis: relationship to endodontic disease. *Crit Rev Oral Biol Med*. Sep 1:15(5):308-20.

9. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB (2006). Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. *J Endod*. Feb32(2):93-8.

10. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M (2010). Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. *J Endod*. Sep:36(9):1558-62.

11. De Moor RJ, Meire M, Goharkhay K, Moritz A, Vanobbergen J (2010). Efficacy of ultrasonic versus laser-activated irrigation to remove artificially placed dentin debris plugs. *J Endod*. Sep:36(9):1580-3.

12. Yavari HR, Rahimi S, Shahi S, Lotfi M, Barhaghi MH, Fatemi A, Abdolrahimi M (2010). Effect of Er, Cr: YSGG laser irradiation on Enterococcus faecalis in infected root canals. *Photomed Laser Surg*. Aug:28 Suppl 1:S91-6.

13. Garcez AS, Nuñez SC, Hamblim MR, Suzuki H, Ribeiro MS (2010). Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report. *J Endod*. Sep:36(9):1463-6

14. Benedicenti S, Cassanelli C, Signore A, Ravera G, Angiero F (2008). Decontamination of root canals with the gallium-aluminum-arsenide laser: an in vitro study. *Photomed Laser Surg*. Aug:26(4):367-70.

15. Takeda FH, Harashima T, Kimura Y, Matsumoto K (1998). Efficacy of Er:YAG laser irradiation in removing debris and smear layer on root canal walls. *J Endod*. Aug:24(8):548-51.

16. Divito E, Peters OA, Olivi G (2010). Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation. *Lasers Med Sci*. Dec 1.

17. Yasuda Y, Kawamorita T, Yamaguchi H, Saito T (2010). Bactericidal effect of Nd:YAG and Er:YAG lasers in experimentally infected curved root canals. *Photomed Laser Surg*. Oct:28 Suppl 2:S75-8.

18. Blanken J, De Moor RJ, Meire M, Verdaasdonk R (2009). Laser induced explosive vapor and cavitation resulting in effective irrigation of the root canal. Part 1: a visualization study. *Lasers Surg Med* 41(7):514–519

19. De Moor RJ, Blanken J, Meire M, Verdaasdonk R (2009). Laser induced explosive vapor and cavitation resulting in effective irrigation of the root canal. Part 2: evaluation of the efficacy. *Lasers Surg Med* 41(7):520–523

20. de Groot SD, Verhaagen B, Versluis M, Wu MK, Wesselink PR, van der Sluis LW (2009). Laser activated irrigation within root canals: cleaning efficacy and flow visualization. *Int Endod J* 42 (12):1077–108.

21. Doukas AG, Birngruber R, Deutsch TF (1990). Determination of the shock wave pressures generated by laser-induced breakdown in water. In: Jacques FL (ed) Laser-Tissue Interaction. *SPIE* , Vol. 1202, pp. 61-70.

22. Müller P, Guggenheim B, Attin T, Marlinghaus E, Schmidlin, PR (2011). Potential of shock waves to remove calculus and biofilm. *Clin Oral Invest* 15:959–965.