Investigation of Er:YAG laser-activated irrigation for the removal of biofilm and observations of laser-induced cavitation behavior

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Research Article

Keywords: Intraradicular biofilm model, Er:YAG laser, Laser-activated irrigation, Cavitation, Pig model

Posted Date: January 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1068014/v2>

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Abstract

Background

We investigated the biofilm removal effects of LAI using a pig model, focusing on the impact of the fiber tip position, and used a high-speed camera to observe the occurrence and positioning of the cavitation associated with laser irradiation.

Methods

A total of 16 roots of deciduous mandibular second premolars from 4 pigs were used. After a pulpectomy, the canals were left open for two weeks and sealed for 4 weeks to induce intraradicular biofilm. Then, root canal irrigation was performed with Er:YAG laser activation. The fiber tip was inserted at two different positions, i.e., into the root canal in the intracanal LAI group and into the pulp chamber in the coronal LAI group. Intracanal needle irrigation with saline or 5% NaOCl was utilized in the positive control and CNI groups. SEM and qPCR were carried out to evaluate treatment efficacy. For qPCR, ANOVA and a Tukey-Kramer post hoc test were performed with $\alpha = 0.05$. A high-speed camera was used to observe the generation of cavitation bubbles and the movement of the induced bubbles after laser irradiation.

Results

The intracanal and coronal LAI groups showed significantly lower amounts of bacteria than either the positive control or CNI groups. There was no significant difference found between the intracanal and coronal LAI groups. SEM images revealed opened dentinal tubules with the destruction of biofilm in both LAI groups. High-speed camera images demonstrated cavitation bubble production inside the root canal after a single pulse irradiation pulse. The generated bubbles moved throughout the entire internal multi-rooted tooth space.

Conclusions

Coronal LAI can generate cavitation in the root canal with a simply placed fiber inside the pulp chamber, leading to effective biofilm removal. This method could thus contribute to the future development of endodontic treatments for refractory apical periodontitis caused by intraradicular biofilm.

Background

Apical periodontitis refers to purulent inflammation inside the alveolar bone triggered by a bacterial infection of the root canal system, which is the internal structure of the tooth[1]. Treatments require the removal of causative bacteria and their by-products from the root canal system and suppression of the inflammatory processes. Mechanical and/or chemical bacterial removal have been utilized as standard

approaches in these cases under an aseptic environment, and the success rates for these initial root canal treatments have been reported to exceed 90%[2]. Retreatments required in cases of refractory inflammation, but the success rates will typically be lower[3] because of bacterial ingrowth into anatomical complexities such as the lateral canal and isthmus and the subsequent formation of biofilm[4]. The complete elimination of biofilm is almost impossible in this circumstance.

Root canal irrigation is a treatment technique that aims to chemically reduce bacterial loads inside the root canal system, such as in areas that cannot be reached by a mechanical root canal preparation. The standard irrigation method utilizes a syringe and fine needle (i.e., 27-31G) to deliver and reflux the disinfectant solution. Recently, irrigant agitation techniques using ultrasound, sonic vibrations, and lasers have been shown to be more effective in reducing intracanal debris than the syringe technique[5–8]. Notably, removing bacterial biofilm from the root canal surface has remained an issue with these interventions because chemical actions alone are insufficient to remove biofilm and some form of physical manipulation is also needed. Therefore, although the chemical exposure from a root canal irrigation will be effective against planktonic bacteria, irrigation technologies are needed that can eliminate biofilm through the generation of physical force at an adjacent site that is inaccessible to existing mechanical instruments. Among the various types of root canal irrigation techniques that are currently available, photodynamic actions are a possible approach to generating a physical effect during root canal irrigation by means of hydrodynamic force generation.

Photodynamic actions produced by multiple lasers at different wavelengths have been shown to effectively agitate root canal irrigants. An Er:YAG laser emitting at the 2.94 μm wavelength close to the absorption peak of water [9],[10] has been utilized for Laser-activated irrigation (LAI), which effectively removes bacteria from the root canal system[11]. The LAI cleaning mechanism depends on rapid fluid motion[10, 12, 13] in the root canal, which generates subsequent pressure waves through the expansion and collapse of vapor bubbles at the site of the laser irradiation. In addition, the generation of numerous secondary cavitation bubbles can be observed under a high-speed camera after the vapor bubble collapse[14]. Cavitation is a liquid to gas phase transformation phenomenon caused by a decreased pressure due to an increased fluid velocity. A collapse of the cavitation bubbles occurs after this and produces large-amplitude shock waves. Hence, LAI is expected to have properties that not only increase the flow velocity of the irrigant but also generate physical forces upon cavitation collapse on the root canal wall that could be effective for biofilm removal. Since laser agitation of the root canal irrigant can spread throughout the root canal away from the fiber tip, placement of the fiber tip into the pulp chamber without coming close to the apex or into the root canal has been advocated called photon-induced photoacoustic streaming (PIPS) [15, 16].

In the present study, we investigated the biofilm removal effects of LAI using a pig model system, with a particular focus on the impact of the fiber tip position. We also employed a high-speed camera to carefully observe the occurrence and positioning of the cavitation associated with the laser irradiation.

Methods

The current study protocols for animal use were reviewed and approved by the Animal Care and Use Committees of Tohoku University (Permit No. 2018 SHIDO-045). All animal experiments were conducted in accordance with the Regulations for Animal Experiments and Related Activities at Tohoku University.

Sixteen roots from the deciduous mandibular second premolars of four pigs (nine-week-old large white X Landrace breed cross; Japan SLC Inc., Shizuoka, Japan). The light was turned on at 8.00 am and turned off at 6.00 pm each day in our animal facility. The pigs had free access to water at any time and were fed a regular diet (Grandeal B; Zennoh Feed Mills of the Tohoku District, Miyagi, Japan) 3 times per day. All interventions were performed after sedation with medetomidine (0.1mg/kg, IM) and midazolam (0.2mg/kg, IM) followed by inhaled sevoflurane (2-5%), with local injections of 2% lidocaine (1.8 ml, SC) also given to minimize pain. The experimental protocol is presented in Figure 1. All procedures were performed under surgical loupes with LED light (EyeMag PRO; Carl Zeiss, Jena, Germany).

To induce intracanal biofilm is following the previously reported procedure[17]. Briefly, the occlusal surface was flattened using a straight bur and electric engine (Ti-Max X95; NSK, Tochigi, Japan) to prevent tooth fracture and for the ease of working length determination. Following access cavity preparation and obtaining a straight-line access, chemo-mechanical removal of pulp tissue was performed with 5% Sodium Hypochlorite (NaOCl) and K files. The canals were exposed to the oral environment to inoculate the root canal with oral bacteria for two weeks. This was followed by sealing with hydraulic temporary filling material (Lumicon; Heraeus Kulzer, South Bend, IN, USA) and composite resin (MI Flow II; GC, Tokyo, Japan) with the use of adhesive (G-Premio BOND, GC) to create an anaerobic intracanal environment for four weeks to allow the bacterial biofilm to mature. After removal of the temporary filling materials and placement of the dental dam isolation at six weeks after pulp removal, aseptic conditions were established by cleaning the tooth surface with 5% NaOCl and saline. The teeth were then randomly assigned to the different experimental irrigation groups.

The following irrigation protocols were used for the different experimental groups.

CNI group: conventional needle irrigation (CNI) was performed using side port 30G needles with 5% NaOCl. Canals were irrigated for 30 seconds with 5mL of irrigant and left for 30 seconds. This cycle was repeated five times for a total of five minutes of irrigation.

Intracanal LAI (I-LAI) group: irrigants were activated using an Er:YAG laser (Erwin AdvErl EVO; Morita, Kyoto, Japan) using a cone-shaped 300 µm diameter tip (R300T). Canals were irrigated for 30 seconds with 5 ml of the solution using a side port 30G needle and the irrigants were activated for LAI with the laser settings at 30mJ and 20pps without air and water supply. This cycle was repeated five times for a total of five minutes of irrigation and subsequent agitation. During laser irradiation, the tip was inserted 2 mm short of the working length and was moved slowly up and down a 3 mm length to the coronal side as with the conventional LAI irrigation.

Coronal LAI (C-LAI) group: this irrigation protocol had the same conditions as the I-LAI group except for the laser fiber tip position, which was inside the pulp chamber and kept stationary. During laser

irradiation, the solution in the pulp chamber keeping the volume, so that a light-cured resin built up around the crown (Dentto-Dam, MEDICLUS, Korea), and NaOCl was then added with a syringe.

Positive control group: the teeth irrigated using saline were used as the positive control group. After root canal irrigation, each canal was rinsed with saline for 30 seconds. The teeth were then extracted, and the remaining bacteria in each root were evaluated using real-time PCR (n=2 each) and SEM (n=2 each).

Quantifications of the bacteria present in the root canals were performed based on previously described methods. Briefly, the sample roots were immersed in liquid nitrogen immediately after tooth extraction and the tooth crown was resected. For the bacterial quantification, the intact deciduous mandibular second premolar was obtained from a pig used for other purposes as the sound tooth. Two roots from a sound tooth were preserved intact without receiving any root canal procedure. The roots were then crushed with SK mill (Tokken, Chiba, Japan) to acquire powdered samples. Total DNA was extracted from each powdered root sample using a Cica Geneus DNA extraction Kit (KANTO chemical co.; Tokyo, Japan) in accordance with the manufacturer's instructions. The presence of bacteria was verified in the experimental samples by qPCR using the bacterial primers 357F and 908R22. These assays were performed using a real-time PCR apparatus (CFX Connect; Bio-Rad Laboratories, Hercules, CA, USA). Amplifications were conducted for 40 cycles at 95°C for 15 seconds followed by 65°C for 1 minute, with the fluorescence signals measured at the end of each cycle. A standard curve was generated by subjecting 10-fold dilutions of a known concentration of *E. faecalis* DNA to the same qPCR protocol. The bacterial counts in all experimental groups were calculated using threshold cycle (Ct) values plotted against the standard curve. Statistical analysis was performed using ANOVA followed by a Tukey Kramer post-hoc test with an α value of 0.05 (BellCurve for Excel; Social Survey Research Information Co., Ltd. Tokyo, Japan) to detect significant differences in the bacterial populations.

SEM sample preparation was conducted according to previously described methods[17, 18]. Briefly, after resecting the tooth crown, the mesial and distal roots were separated with a diamond disc. The distal root was then split into halves and immersed in 2.5% glutaraldehyde to fix the root canal biofilm, and then rinsed with PBS and treated with 1-ethyl-3-methyl-imidazoliumtetrafluoroborate. The samples were dried in a vacuum desiccator for one day and sputter-coated with platinum. The surfaces of the root canal wall and intraradicular biofilm were observed using SEM (VE-8800; Keyence Inc., Osaka, Japan) at a 10kV acceleration.

To observe the generation and movement of bubbles inside the root canal through the LAI, images were taken using a high-speed digital camera (FASTCAM Mini AX200; Photron, Tokyo, Japan) attached to a macro lens (SP 90mm F/2.8 Di; Tamron, Saitama, Japan). The LED light source was placed diagonally in front of the root canal model. The Er: YAG laser was equipped with R300T fiber tip with an output setting of 30 mJ at 20PPS and no water or air supply.

Two types of root canal models were used i.e., a single or two canal model. A single root canal model made of epoxy resin with apical size #80, 02taper, and length 15 mm was used to observe the characteristics of the bubbles generated with a laser pulse. The fiber tip was placed 12mm from the apex,

and the root canal was filled with water. The frame rate, image size, and recording duration were set at 40,000 frames per second (fps), 384×256 pixels, and 217ms, respectively. For the two root canal model, an artificial maxillary premolar model (TrueTooth # 5-002, Dental Engineering Laboratories, CA, USA) was used to observe the induction of bubbles and changes in their behavior over time. The tooth was prepared with an access opening, straight-line access, and root canal preparation with a size #50 Reciproc Blue (VDW, Munich, Germany). The fiber tip was placed at the center of the pulp chamber as assuming C-LAI. During laser irradiation, the irrigant was continuously supplied with a syringe. The frame rate, image sizes, and recording duration were set at 750 fps, 1024×1024 pixels, and 29s, respectively.

Results

Figure 2 shows the qPCR results representing the remaining bacterial amount in the root canal after experimental root canal irrigation. The I-LAI group (6.11×10^3) and the C-LAI group (5.02×10^3) showed a significantly lower level of bacteria than the positive control group (8.08×10^3) or the CNI group (7.62×10^3). There was no significant difference between the I- and C-LAI groups in this regard. Compared to the sound teeth (4.95×10^3), the bacterial counts in the positive control group and the CNI group were significantly higher, while those in the both LAI groups did not differ significantly.

SEM images of the root canal wall taken after root canal irrigation are shown in Figure 3. A multi-layered biofilm that covered the entire root canal was observed in the positive control group. Some dentinal tubule structures could be seen in the CNI and I-LAI groups due to biofilm destruction although residual biofilm was observed. Only a small amount of biofilm remained in the C-LAI group, and dentin tubule structures could be seen on the entire root canal wall of these teeth.

Bubble generation and collapse after a single pulse of irradiation inside a single root canal model are shown in Figure 4. An additional movie file shows in more detail (see Additional file 1). At the tip, the bubbles generated at $t=0$ ms most notably developed in the crown direction, shrank, and then finally disappeared at $t=0.525$ ms. Additionally, several cavitation bubbles were observed from $t=0.625$ ms to $t=0.825$ ms at the central part of the canal appearing at around 3 to 8 mm from the fiber tip. Subsequently, cavitation bubbles were repeatedly observed in the same range indicated above from 0.875 ms to 1.15 ms. The bubble at the fiber tip was found to have shrunk the most at $t = 0.5$ ms, with a bubble appearing at a distance of about 5 mm approximately 0.05 ms later. We surmised from this that bubbles are generated in the tubules by the pressure wave generated during their shrinking and re-expansion at the tip end, in addition to the associated depressurization. In this case, the velocity of the pressure wave can be calculated to be about 100 m/s.

The movement behavior of the bubbles over 30 sec is depicted in Figure 5 and additional movie file (see Additional file 2). After laser irradiation, the bubbles generated in the pulp chamber reached the root canal orifice in 1.03s and the root apex in 1.75 sec in the left root canal. In the right root canal, the bubbles reached the root canal orifice in 1.15 sec and the root apex in 7.64 sec. The number and size of the bubbles in the root canal increased with time. The generated bubbles and their movement could be

observed across entire internal tooth space. A slight irrigant extrusion from the root apex was observed at t=24 sec.

Discussion

Removing biofilm from inside a root canal, which is the cause of refractory apical periodontitis, is still challenging when conducting a root canal retreatment[4, 19]. The most reliable biofilm removal method is mechanical disruption, although no instrumentation technique to achieve this can reach all of the root canal surfaces[20]. In addition, biofilms are observed in anatomical complexities such as the isthmus and lateral canal[21]. Hence, non-contact biofilm removal techniques continue to need a more reliable retreatment protocol. We have previously reported an *in vivo* intraradicular biofilm model in the pig [17], which can closely replicate human biofilms in terms of morphology and microbiota, to evaluate the effectiveness of biofilm removal protocols and found that LAI is an effective intervention in this regard¹⁷. In the present study, we further found that the cavitation bubbles produced by C-LAI were effective in biofilm removal using *in vivo* intraradicular biofilm model, which indicated that LAI has the potential to apply the non-contact removal technique.

The Er:YAG laser is immediately absorbed by water upon irradiation and generates bubbles that create pressure waves which will agitate the cleaning solution via the high-velocity irrigant flow [8, 13, 14, 22]. LAI was significantly more effective in cleaning root canals, especially in removing root canal debris and intracanal bacteria, than CNI or ultrasonic agitation[11]. Moreover, the vapor bubble- and cavitation bubble-induced turbulence associated with LAI can cause shearing stress on the root canal wall surface[11, 23]. In our current study, LAI showed the capacity to remove biofilm along with excellent cleaning effects, indicating that it can generate physical forces inside root canal systems.

The fiber tip used in our current investigations has a conical tip and is designed to distribute 80% of the irradiated laser energy laterally and 20% axially. When such a conical-shaped tip is inserted into the root canal system, regarded as a I-LAI approach, the space between the fiber tip and the root canal wall is insufficient to induce irrigant flow. Most of the irradiated energy in this case goes directly to the root canal wall[14, 24]. On the other hand, the C-LAI or PIPS methods reserve a sufficient amount of space because the fiber tip is placed into the pulp chamber so that the energy is absorbed by the irrigant and can therefore produce a high irrigant velocity[25]. In our present study, we found that a C-LAI can simultaneously observe the generation of bubbles and disperse them throughout the whole root canal system. Also, bacterial reduction of C-LAI is comparative to the I-LAI, meaning that C-LAI was possible to achieve a sufficient flow velocity to agitate the solution into the entire root canal system. The results suggested that C-LAI is both a safer and simpler method of root canal cleaning that can treat multiple root canals simultaneously with less impact of the laser irradiation on the root apex due to the insertion of the fiber into the root canal.

The observation of cavitation bubbles after a single pulse of irradiation revealed bubble generation at a distance from the fiber tip. This phenomenon is not simply due to the movement of bubbles but is

considered a cavitation occurrence caused by a decrease in the pressure inside the root canal. Although the velocity of the pressure wave, calculated at about 100 m/s, was smaller than the velocity in the cavitation flow of several hundred m/s, it still seemed capable of generating cavitation because of the pressure wave limits arising from the friction forces in a narrow spatial structure and gas-liquid mixed phase.[26]. This phenomenon of cavitation bubbles and physical reaction occurrences away from the fiber tip accords with the previously reported behavior of laser-induced cavitation in the liquid phase[27]. Hence, it can be considered that during biofilm removal, C-LAI not only agitates the irrigants but also mechanically detaches the biofilm from the root canal wall by generating shock waves due to cavitation bubbles.

The pulse width of the C-LAI used in our present study was about 300 μ sec, which is larger than the 50 μ sec pulse width used in PIPS. The Er:YAG laser is capable of tooth structure ablation with irradiation energy increases, thus PIPS is designed to have a narrow pulse width to optimize the agitation of the irrigant without tooth ablation[15]. However, it was found previously that PIPS cannot easily generate a shock wave inside the root canal system[28]. Hence, with the long pulse width used in this study, we assumed that the increased irradiation energy contributed to the generation of a sufficient pressure wave for cavitation bubbles to occur. Thus, cavitation by laser irradiation can generate a physical force on the root canal wall and is expected to become a novel treatment technique for non-contact biofilm removal in the retreatment of refractory periapical periodontitis cases. In addition, C-LAI can be clinically applied as an effective, safe, and relatively simple root canal treatment technique because it can shorten the treatment time by cleaning multiple root canals simultaneously by positioning the fiber tip in the pulp chamber.

Establishing more effective root canal cleaning methods will improve the success rates of root canal retreatment. Although additional investigations are needed to further optimize laser irradiation methodologies, and verify the safety of these approaches, our current evidence indicates that C-LAI will more safely improve irrigant activation for better biofilm removal without the need for other complicated techniques.

Conclusions

A non-contact debridement modality is required to establish a reliable biofilm removal technique for orthograde root canal retreatments. Our current report is the first to indicate that C-LAI can generate secondary cavitation in the root canal, which can lead to the removal of biofilm through the simple placement of an optic fiber inside the pulp chamber. C-LAI method is thus likely to contribute substantially to the future development of endodontic treatments for patients who suffer from refractory apical periodontitis caused by an intraradicular biofilm.

Abbreviations

LAI

laser-activated irrigation
PIPS
photon-induced photoacoustic streaming
NaOCl
sodium hypochlorite
CNI
conventional needle irrigation
PPS
pulse per second
FPS
frames per second

Declarations

Ethical approval and consent to participate

This study was reviewed and approved by the Animal Care and Use Committees of Tohoku University Graduate school of Dentistry (Permit No. 2018 SHIDO-045). Animal studies were followed the ARRIVE 2.0 guidelines. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Consent to participate was not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests in relation to this study.

Funding

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Japan Society for Promotion of Science (Grant Number 20K09970). The funding source had no role in any part of the research process.

Author contributions

TN and YY conceived and designed the study. TN, YY, KH, TT and VVS acquired the experimental data. MN, SS, YK, MK and VVS contributed to the analysis and interpretation of the data. TN and YY drafted the

manuscript. TN, YY and MS revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are extremely grateful to Ms. Teruko Sueta for her assistance with the animal experiments.

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Figures

Figure 1

Experimental outline for the current study in the pig model.

After the removal of the pulp tissue, the canal was exposed to the oral environment for 2 weeks and to an anaerobic intracanal environment for four weeks to allow for bacterial biofilm maturation. Six weeks after pulp removal, the pig teeth were irrigated by one of four different test protocols. Qualitative evaluations by SEM and quantitative assessment by qPCR were performed (a).

CNI, conventional needle irrigation; LAI, laser activated irrigation.

Figure 2

Quantitative evaluation of the number of bacteria in the root canal by qPCR

The I-LAI and C-LAI groups had a significantly lower bacterial concentration than either the positive control or CNI groups. There was no significant difference found between the I-LAI and C-LAI groups. The number of bacteria in the positive control and CNI groups was significantly higher than that in the sound teeth. However, the bacterial levels in the both LAI groups were not significantly different from that in the sound teeth.

Figure 3

Typical images (a-1, b-1, c-1, and d-1) and SEM images (30x: a, b, c, and d-2; 1000x: a, b, c, and d-3; 3000x: a, b, c, and d-4) from each study group.

In the high magnification image, a residual layer of biofilm was observed to cover the root canal wall in the positive control group, and the structure of the root canal wall could not be confirmed (a-4). In the CNI group and I-LAI group, some openings of the dentinal tubules were observed compared with the positive control group, but some biofilm remained (b-4, c-4). In the C-LAI group however, almost no biofilm

remained, and opening of both the dentinal tubules and the reticulated intertubular dentin structure could be observed (d-4).

Figure 4

(a) Setting used for the observation of bubble behavior in a single pulse with a high-speed camera. (b) Single canal model. (c) Captured images showing the generation and disappearance of vapor bubbles at the tip, and the generation of cavitation bubbles. Recording conditions were set at 40,000 fps, 384×256 pixels, and 217ms. At the tip, the bubbles generated at $t=0\text{ms}$ notably developed in the crown direction, shrank, and finally disappeared at $t=0.525\text{ms}$ (i). Additionally, several cavitation bubbles could be observed from $t=0.625\text{ms}$ to $t=0.825$ at the central part of the canal (ii). The cavitation bubble was observed to generate and disappear again within the same time range from 0.875 ms to 1.15 ms (iii).

Figure 5

Observation of bubble behavior over time in a two root canal model.

The number and size of the bubbles in the root canal increased with time from 0 sec at the time of laser irradiation, and bubbles were observed over a wide range from the coronal area to the apex of both root canals. A slight overflow of the solution was observed from the apex at 24 sec (red arrow). Recording conditions were set at 750 fps, 1024×1024 pixels, and 29 sec.

Supplementary Files

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