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ORIGINAL ARTICLE

Efficacy of ultrasonic and Er:YAG laser activated EDTA irrigation in removing bacteria from ex vivo root canal system

Sharonit Sahar-Helft[1] · Ayşe Sena Kabaş Sarp[2] · Murat Gulsoy[2] · Idan Redenski[1,3] · Adam Stabholtz[1] · David Keinan[4] · Doron Steinberg[3]

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Abstract

Purpose Due to the limited effectiveness of irrigation to reduce the bacterial load inside root canals, the efficacy of activated irrigation techniques was evaluated.

Methods Sixty endodontically prepared single-rooted human teeth were sterilized, infected with *Enterococcus faecalis*, and divided into six groups: (A) endodontic treatment positive control, (B) irrigation with EDTA 17%, (C) Er:YAG laser activated irrigation with tip 1 mm from working length, (D) Er:YAG laser to the coronal third, (E) ultrasonic irrigation applied 1 mm from working length, (F) US irrigation to the coronal third.

Results The positive control group had significantly more bacteria than all other groups. EDTA irrigation had low efficiency. US irrigation 1 mm from the working length was significantly more effective than controls. Laser treatment to the coronal third was the most efficient.

Conclusions Laser activated irrigation caused the greatest bacterial reductions.

Keywords Biofilm · Ultrasonic · Laser · Root canal · Irrigation

Introduction

The major goal of biomechanical cleaning of the root canal system is to eliminate bacteria and irritants which can cause periapical lesions [1]. Biomechanical instrumentation reduces the microbial load; however, complete bacterial eradication is difficult [2–4] because some areas of the canal wall are inaccessible to the instruments, including advanced rotary systems [5]. Ethylenediaminetetraacetic acid (EDTA) is a widely used irrigant in endodontic treatment because it reacts with the calcium ions in dentin and forms soluble calcium chelates**.** The use of antimicrobial canal irrigation agents such as NaOCL with EDTA 17% in combination with mechanical instrumentation further reduces the bacterial content [6]. Nevertheless, some bacteria still persist [7, 8]. Endodontic instrumentation reduces bacteria on the root canal wall; however, a smear layer is created. This layer is amorphous and irregular, containing inorganic debris, pulp tissue, odontoblastic residue, necrotic debris, as well as microorganisms and their metabolic products [9]. Furthermore, the smear layer itself may be infected, preventing efficient cleaning by irrigation [10–12].

Almost 700 bacterial species can be found in the oral cavity [13]. Once the root canal is infected coronally, infection progresses apically until bacterial products or bacteria themselves stimulate the periapical tissues, leading to apical periodontitis. The dominant bacteria remaining following intra-canal disinfection procedures and after root canal treatment is the gram-positive bacteria *E. Faecalis*. This bacterium has been identified in cases of failed endodontic therapy and in canals with persistent infections [14]. *E. Faecalis* has many features which enable survival in the root canal.

✉ Sharonit Sahar-Helft
helft1@bezeqint.net

[1] Department of Endodontics, Faculty of Dental Medicine, School of Dental Medicine, The Hebrew University–Hadassah, P.O.B. 12272, 91120 Jerusalem, Israel

[2] Biophotonics Laboratory, Institute of Biomedical Engineering Çengelköy, Boğaziçi University, Kandilli Kampüs, Istanbul, Turkey

[3] Biofilm Research Laboratory, Institute of Dental Sciences, Faculty of Dental Medicine, School of Dental Medicine, The Hebrew University–Hadassah, Jerusalem, Israel

[4] Department of Endodontics, Sheba Medical Center, Israel Defense Force Medical Corps, Ramat Gan, Israel

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Machine-assisted irrigation techniques include sonic and ultrasonic methods, apical negative pressure irrigation [15], plastic rotary files [16], photo-initiated photoacoustic streaming (PIPS) [17]. The two important factors to consider during irrigation are (1) whether the irrigant can be delivered to the entire root canal system and (2) whether areas inaccessible to mechanical instrumentation, such as lateral canals and isthmuses, can be debrided [16]. Recent studies have shown that ultrasonic activation of irrigants improves debridement compared to conventional syringe irrigation [18]. Increased ultrasonic-device intensity moves the irrigation solution around the file, within the canal more rapidly [19]. De Moor et al. [20] compared the effects of passive ultrasonic irrigation (PUI) and laser-activated irrigation (LAI) on dentin debris concluded that the Er:YAG laser removed debris more efficiently from simulated root canal irregularities.

Laser technology might improve endodontic treatments [21], especially considering that laser treatment reduces bacterial infection [22]. One of the promising laser techniques for use in endodontics is laser-activated irrigation. Activation of the laser at subablative settings in an aqueous environment can result in the formation of large vapor bubbles which expand up to 1600 times their original volume and then implode [23–25]. Secondary cavitation effects develop after bubble implosion [23]. LAI can remove the smear layer from the root canal wall [26, 27]. The amount of fluid extruded through the apex during LAI is similar to that occurring when standard irrigation needles are used. There are many types of laser available for use in dentistry and endodontics, and the Er:YAG laser is particularly popular.

The aim of this study was to evaluate the efficacy of ultrasonic (US) and Er:YAG activated irrigation in removing bacteria from the main root canal compared to conventional irrigation methods.

Materials and methods

Tooth preparation

Sixty single-rooted human teeth (central incisors) extracted for periodontal reasons were used in this study. The study was approved by the Hadassah Hospital Ethics Committee, approval no. 0118-14-HMO.

After enlarging the canal openings with Gates-Glidden burs, the canals were prepared mechanically using ProTaper files. The root canals were enlarged to the apex with five ProTaper files for 10 cycles. The endodontic procedure was completed with file #40. The canal was irrigated with 1 mL of 2.5% sodium hypochlorite between steps.

After root canal preparation, labial and a lingual grooves were created from the apex to the incisal edge with a #2 round bur. The tooth was split in half using a chisel, and then put in an ultrasonic bath (D-7700, Elma, Germany) for 15 min to remove the smear layer and create a clean surface for bacterial accumulation inside the dentinal tubules. [22]

Bacterial inoculation

Teeth sections were sterilized three times in an autoclave then immersed in brain heart infusion broth (BHIB) and placed in an incubator at 37 °C. Potential bacterial contamination of the broth was checked 72 h later. The sterile teeth were then infected with *E. faecalis* by immersion in BHIB supplemented with *E. faecalis* and incubated at 37 °C with constant shaking. The bacterial suspension was refreshed every 2 days for 21 days. Microscopic examination was performed to ensure the presence of *E. faecalis* without contamination by other bacteria.

Treatment groups

All the infected teeth were mounted in impression material to recreate the natural form of the tooth. The 60 teeth were randomly divided into six groups:

1. Group A (mechanical preparation—positive control, PC). Mechanical preparation up to file #40.
2. Group B (17% EDTA). Root canals were irrigated with 10 mL of 17% EDTA solution for 60 s using a syringe with a 27 gauge needle.
3. Group C (LAI with fiber tip 1 mm from working length (WL). Root canals were radiated with an Er:YAG laser (Light instrument, Yokneam, Israel) at a wavelength of 2940 nm. Using sapphire tip 17-mm 400-μm plane-ended. Pulse length 158 μs, pulse energy 50 mJ, frequncy 10 Hz for 60 s. The water spray was turned off. An irrigation solution of 17% EDTA was injected during radiation.
4. Group D (LAI with fiber tip in the coronal third of the canal). Root canals were irrigated and radiated with the Er:YAG laser as above for Group C, but the fiber tip was only inserted in the coronal third of the canal. Continuous irrigation with 10 mL of 17% EDTA was applied manually for 60 s.
5. Group E (US irrigation applied 1 mm short of WL). US irrigation was applied with a stainless steel #25/.00 file (Irri-Safe; Acteon, Merignac, France) placed 1 mm from the working length, driven by an US device (Suprasson PMax; Satelec, Acteon) at a power setting of 5 for 60 s. Continuous irrigation with 10 mL of 17% EDTA was applied manually for 60 s.
6. Group F (US irrigation applied to the coronal third of the root canal). US irrigation was applied as for group D, but placed in the coronal third of the canal. Continuous irrigation with 10 mL of 17% EDTA was applied manually for 60 s.

Plating and counting

After each treatment, the canals were swabbed using three #35 paper points (Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA) which were then placed in 800-μl sterile PBS with three glass beads.

Each sample was mixed and then sonicated in a water bath for 10 min. Subsequently, 20 μl of the supernatant fluid from the paper-point liquid was transferred to an ELISA plate well with 180 μl BHI and diluted six times. Next, 50 μl of liquid from each well was spread on BHI agar plates [28].

After 3 days of incubation, colonies were counted with a manual counting viewer. The colony-forming unit (CFU) values for each sample were calculated together with the mean standard deviations for each group [29].

Statistical analysis

The two US and laser irrigation groups were subjected to pairwise comparisons according to treatment type or application length. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA) after testing for normality using Shapiro–Wilk and Kolmogorov–Smirnov tests. A Kruskal–Wallis test was performed followed by a Mann–Whitney *U* test for precise comparison. ANOVA and Student's *t* test were performed for parametric data groups. All statistical tests were performed at the 95% confidence level ($P = 0.05$).

Results

In this study, three cleaning techniques were compared. The mean CFU value of the PC group was significantly higher than all other groups ($P < 0.05$) (Fig. 1). The EDTA solution was significantly less efficient at removing bacteria than the activated groups ($P < 0.05$) (Fig. 2).

From Figs. 2 and 3, it is clear that US irrigation applied 1 mm from WL (group E) was more effective than its application to the coronal third of the canal. These figures also compare LAI with fiber tip 1 mm from WL and LAI with fiber tip in the coronal third, with significant differences ($P < 0.05$) between the two laser groups (C,D). While both US (groups E, F) and Er:YAG laser (groups C, D) reduce bacteria from the main root canal, LAI of the coronal third of the canal was the most efficient (group D).

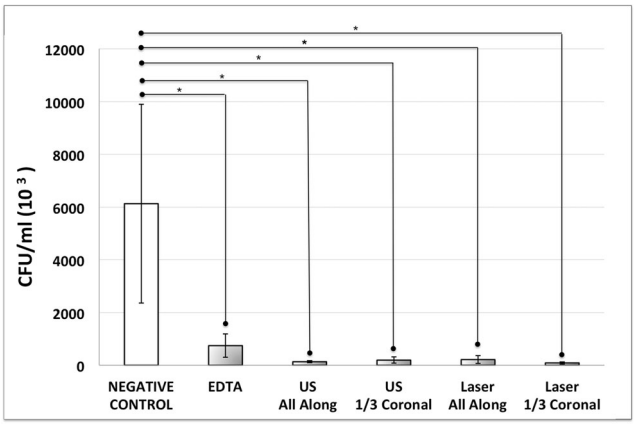

Fig. 1 CFU of *E. faecalis* in all groups were compared with Kruskal–Wallis statistical test and Mann–Whitney *U* test. The CFU value of the PC group was significantly higher than all other groups ($P < 0.05$). * indicates significant differences

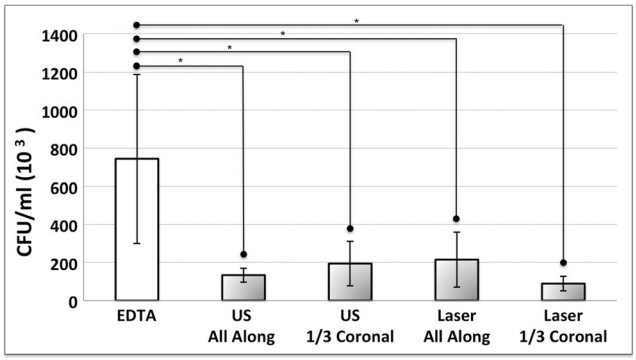

Fig. 2 CFU of *E. faecalis* in all groups compared with EDTA, group B. EDTA had the lowest removal efficiency ($P < 0.05$). Groups C–F, Kruskall–Wallis test and Mann–Whitney *U* test. * indicates significant differences

Discussion

The antibacterial effectiveness of several root canal cleaning techniques at different working lengths was compared. Significant differences in removal of bacteria from the canal were found between treatments.

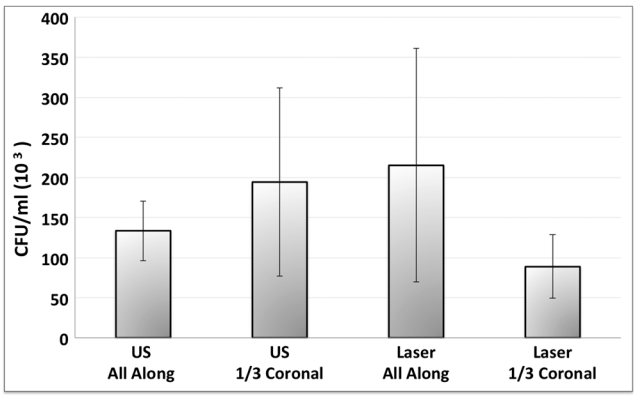

Fig. 3 CFU values of *E. faecalis* in all groups. Laser radiation applied to the coronal third of the canal with EDTA showed the best reduction of bacteria ($P < 0.05$, Kruskall–Wallis test and Mann–Whitney *U* test) group D

Traditional endodontic techniques involve mechanical instrumentation in combination with irrigation solutions to clean and decontaminate the root canal system [30]. These instruments contact about 40% of the root canal walls when a rotary technique is used [31]. Irrigation and instrumentation facilitate the removal of pulp tissue and microorganisms. EDTA is a widely used irrigant in endodontic treatment because it reacts with the calcium ions in dentin and forms soluble calcium chelates, and therefore was employed in the current study. Longer exposures than 1 min can cause excessive removal of both peritubular and intratubular dentin [32]. Sodium hypochlorite (NaOCl) is the most popular as a root canal irrigant due to its antimicrobial and tissue dissolution properties.

However, NaOCl has various limitations, including tissue toxicity, inability to remove the smear layer, inability to fully eradicate microbes from the infected canals, allergic potential, and risk of emphysema on overfilling [33]. Numerous protocols have been suggested to improve outcomes, e.g., improved bactericidity, penetration, and tissue dissolving ability were reported from ultrasonic activation of NaOCl [34]. Sanchez et al. (1997) showed that sodium hypochlorite 5.5% has a high absorption of laser below 350 nm [35], and LAI is effective when the laser beam is highly absorbed into the irrigating liquid. However, the creation of an intense bubble stream close to the apex of the root canal may result in extrusion of the irrigant [27]. For these reasons, NaOCl was not used in our study.

The advantages of US irrigation are undeniable. Kuah et al. [36] demonstrated the efficiency of a 1-min application of EDTA combined with US at removing the smear layer and debris in the apical region of the root canal. Sahar-Helft et al. [37] showed that combining US irrigation with EDTA improves smear-layer removal in all parts of the canal. The ability of EDTA to remove the biofilm is limited when applied as an irrigation solution with a syringe, but when activated by US bacteria were effectively removed from the root canal. This effect depended on the depth of insertion of the US irrigation file (Figs. 2 and 3).

Furthermore, various laser systems used in dentistry such as Nd:YAG, Er:YSGG, diode, CO_2, and Er:YAG can potentially clean and disinfect the root canal system after biomechanical instrumentation. In most cases, the effect is directly related to the energy level of the radiation and depth within the canal [38].

Due to the limitations of irrigation alone, the penetration of irrigant in the apical third and beyond the main canal is limited [39], and activation is suggested to improve their distribution in the canal system and increase irrigation effectiveness.

we compared the removal of bacteria from the root canal system using different irrigant activation techniques. The CFU value of the PC group was significantly higher than all other groups ($P < 0.05$). We found that EDTA removed bacteria from the root canal less effectively than when activated by US or laser (Figs. 1 and 2).

In the present study, in agreement with others, LAI was more effective than US irrigation and conventional needle irrigation in the removal of bacteria [24–26]. Interestingly, the most effective means of reducing bacteria were with EDTA and laser applied to the coronal third of the canal (Fig. 3) (group D). This is probably because the laser beam was absorbed by a thick layer of irrigation solution that was instantly super-heated at high pressure, causing it to vaporize. This high-pressure vapor expands rapidly, providing an opening in front of the fiber for the laser beam. As the laser continues to emit energy, the light passes through the bubble and evaporates the water surface at the front of the bubble. In this way, it "drills" a channel through the liquid until the pulse ends, after about 140 μs. This well-known mechanism has been referred to as the "Moses effect in the microsecond region" [40].

CFU and SEM were performed in this study in order to evaluate any changes in the structure of the biofilm formed on the main root canal walls (SEM) and the changes in live bacteria (CFU). In next studies, PCR and confocal laser scanning microscopy will allow us to evaluate the EDTA, Laser, PUI effects on biofilm formation in the main root canal.

Here, we used the Er:YAG laser on endodontically prepared teeth. EDTA 17% irrigation was applied to the coronal part of the root canal with a syringe during the laser treatment. A coronal reservoir of irrigant formed in the pulp chamber as well as along 5 mm of the coronally prepared root canal.

However, it is important to note that the bacteria were not eliminated from the entire canal surface. This incomplete bacterial elimination could be attributed to the limited removal of bacteria from canal irregularities [41].

Conclusions

All activation regimes resulted in higher bacterial reduction from the main root canal than syringe irrigation alone. The greatest reduction was obtained with LAI in coronal third of the main root canal. The results highlight the importance of application length in activation procedures.

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Compliance with ethical standards

The study was approved by the Hadassah Hospital Ethics Committee, approval no. 0118-14-HMO.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study. [Optional].

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