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Er:YAG Laser Modification of Root Canal Dentine: Influence of Pulse Duration, Repetitive Irradiation and Water Spray

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Abstract. The aim of this study was to determine the effects of varying parameters of Er:YAG laser irradiation with and without water spray cooling on root canal dentine in vitro. After horizontally removing tooth crowns from extracted human teeth, roots were axially sectioned into thin slices, exposing the root canal surface. An Er:YAG laser delivered 10–30 J/cm^2 into a 0.4-mm diameter laser spot on the root canal surface. Single pulses of different lengths (80–280 μs) were applied with and without water spray cooling/irrigation, and sequences of three pulses at a repetition rate of 30 Hz were applied at selected pulse parameters. The irradiated samples were investigated using both confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). At most irradiation conditions, the root canal dentine surface was ablated. Three-dimensional images from CLSM revealed that the cavity walls were not smooth. Depths of the cavities revealed significant differences between the cavities. No debris was observed at the surface of cavities at any irradiation condition. Strong melting and recrystallisation, or unusually flat surfaces with open dentinal tubules were obtained with sequences of three pulses without water cooling. CLSM is an effective tool for investigation of laser effects on root canal dentine. By varying the irradiation conditions, the Er:YAG laser can induce different modifications of root canal surface, which may be very interesting for root canal preparation.

Keywords: Ablation; Confocal laser scanning microscopy; Dentistry; Endodontics; Hard tissue; Morphological change; Scanning electron microscopy

INTRODUCTION

Root canal preparation is usually performed using hand instruments and/or rotary instruments [1]. However, preparing root canals using those instruments is not easy, and often leaves a smear layer and debris on the surface of the root canal. The smear layer is composed of debris, composed of fractured bits of dentine and soft tissue from the canal. In clinical practice, chemical irrigation is used to remove the smear layer, but it is very difficult to achieve a complete removal. Moreover, ordinary root canal preparation methods are not able to disinfect the root canal surface.

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As a possible solution, novel modalities for root canal therapy utilising laser irradiation have recently been investigated. Two studies of root canal disinfection using lasers have been performed [2,3]. Le Goff et al. reported the effect of CO_2 laser on animal teeth contaminated with an endodontic bacterial strain [2]. Berkiten et al. investigated the disinfection ability of Nd:YAG laser on the root canal walls [3]. Harashima et al. [4] evaluated the efficacy of Nd:YAG laser for removing smear layer and suggested that it was useful and that it caused melting of internal structures on the instrumented root canal walls. Blum et al. [5] used the Nd:YAP laser and reported that laser has a potential in ensuring optimal canal cleanliness. Machida et al. [6] applied the KTP:YAG laser to remove the smear layer and debris from the root canal surface, and indicated its effectiveness and thermal safety. One possibility is ablation of the root canal dentine with the Er:YAG laser (wavelength 2.94 µm), which is used clinically for cavity preparation. However, only a few studies thus far have investigated the feasibility of root canal treatment using this laser [7–12].

The main purpose of the present study was to determine the surface and subsurface modifications of root canal dentine after Er:YAG laser irradiation under variable conditions. The exposed surface of human root canal dentine was irradiated in vitro by an Er:YAG laser with various pulse durations and fluences, with and without water spray cooling/irrigation. Water cooling is clinically advisable to reduce the risk of thermal damage to the adjacent root canal and periodontal structures [11]. The irradiated surface was investigated using both confocal laser scanning microscopy (CLSM) [13,14] and scanning electron microscopy (SEM). In contrast with the latter, more customary method, CLSM does not require the samples to be dehydrated, and therefore does not produce artefactual cracks. Furthermore, CLSM has the potential to analyse the subsurface layer of irradiated dentine, and produce three-dimensional images of the ablation craters.

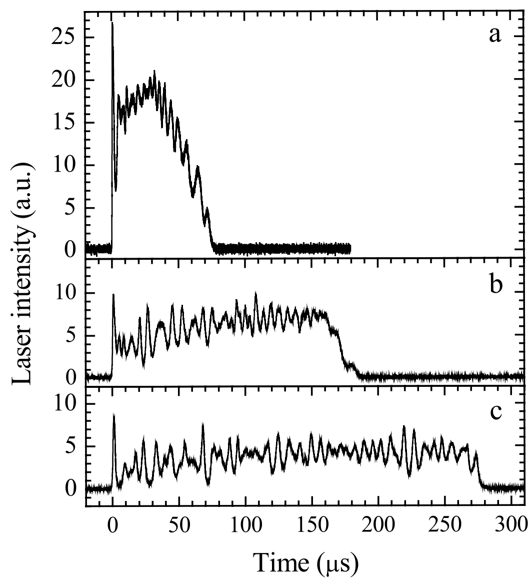

Fig. 1. Temporal dependence of Er:YAG laser intensity in the three pulsed modes used in the study: (a) VSP, (b) LP and (c) VLP.

MATERIALS AND METHODS

Sample Preparation

Extracted human teeth were stored in demineralised water with 0.01% (w/v) thymol. After removing the tooth crown horizontally, the roots were sectioned axially using a low-speed saw with water coolant (Isomet, Buchler, IL, USA). In this manner, approximately 600 µm thick slices were prepared, exposing the surface of the root canal for laser irradiation.

Laser Irradiation

This study was performed using the 'Fidelis' Er:YAG laser (Fotona, Ljubljana, Slovenia), which permits the user to choose between four laser 'modes', featuring various pulse durations at nearly square temporal profiles, and repetition rates from 2 to 50 Hz. In addition, a manufacturer-provided customisation of the system enabled us to externally control the laser to generate repeatable pulse sequences at high repetition rates. Beam delivery via the instrument's articulated arm and a non-contact focusing handpiece (RO-8, Fotona, Ljubljana, Slovenia) was used to irradiate perpendicularly nominally 0.4-mm-diameter spots on the root canal surface.

Laser pulses generated in three different laser 'modes' were used in the study: VSP ('very short pulse', pulse duration t_p=80 µs), LP ('long pulse', t_p=180 µs), and VLP ('very long pulse', t_p=280 µs). In each laser mode, the output energy was set at around 35 mJ to produce an estimated energy density of 28–29 J/cm^2 on the dentine surface. The temporal dependence of laser intensity in these three laser 'modes', as detected with an InSb photodetector, is presented in Fig. 1. One or two external attenuators were used to reduce the sample irradiation fluence by 31% (one attenuator) or 52%, to 13–14 J/cm^2 (with two attenuators), while preserving the spatial and temporal structure of the laser pulse. In addition, using the custom external control, we programmed the laser to emit sequences of three pulses with the temporal profile resembling the LP laser mode, at a repetition rate of 30 Hz (see Table 1 for an overview of the irradiation conditions). All fluence values used were lower than the manufacturer-recommended settings for efficient ablation of caries and dentine in cavity preparation application (45–100 J/cm^2, repetition rates up to 12 Hz).

Table 1. Overview of the laser irradiation parameters

Laser mode	t_p (μs)	E_{laser} (mJ)	F_0 (J/cm^2)	F_1 (J/cm^2)	F_2 (J/cm^2)
VSP (very short pulse)	80	35	28	20	13
LP (long pulse)	180	34	28	19	13
VLP (very long pulse)	280	37	29	20	14
Sequence (3 × LP, 30 Hz)	180	30	—	18	12

At each of the above laser settings, two sites on the exposed root canal surface were irradiated without water cooling. Then, the complete experimental protocol was repeated using a dental water spray cooling/irrigation system at a water flux of 4 ml/min, for a total of 44 irradiated sites on 15 tooth sections.

Confocal Laser Scanning Microscopy (CLSM)

Laser-irradiated samples were stained using Rhodamine 123 (Sigma, MO, USA) at a concentration of 10^{-5} M in phosphate buffered saline (PBS, Sigma, MO, USA) for 24 h. After staining, they were rinsed two to three times with PBS, and fixed on slide glasses with cyanoacrylate glue.

Stained samples were examined with a Zeiss LSM 410 inverted laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany). The objective lense used was the Plan-Neofluar 20 × bright field (Carl Zeiss, Oberkochen, Germany). The 488-nm line of a continuous argon laser was used for sample excitation, and the fluorescence emission was isolated with a long-pass filter at 590 nm. A number of optical sections parallel to the sample surface (i.e. horizontal) were obtained for each sample. The distance between individual optical sections was 2–10 μm, depending on the depth of the cavity. The manufacturer-supplied software enabled generation of vertical cross sections, as well as three-dimensional (3-D) images through the cavity, from stacks of acquired horizontal sections.

Scanning Electron Microscopy (SEM)

SEM was performed to identify the microstructural effects of laser irradiation on the root canal dentine surface. After being imaged by CLSM, the samples were dehydrated in a graded series of aqueous ethanol (50, 70, 90 and 100% ethanol) for 10 min at each concentration. Then, they were mounted on stubs using colloidal silver liquid (Ted Pella, CA, USA), and gold coated on a PAC-1 Pelco advanced coater 9500 (Ted Pella). Micrographs of the root canal dentine surface were taken with a Philips 515 (Mohawk, NJ, USA) SEM.

RESULTS

Confocal Laser Scanning Microscopy

At all irradiation conditions tested in this study, root canal dentine was ablated and small cavities were prepared by laser irradiation when no water spray cooling/irrigation was used. Fluorescence from the surface and subsurface of the resulting cavities was detected by the CLSM in well-defined planes, perpendicular to the axis of the microscope objective. Figure 2(a–d) presents a set of such optical sections, acquired from ablation cavity caused by a single LP pulse (t_p = 180 μs) at the energy density of F = 28 J/cm^2, and no water spray. The images were acquired with the 20 × bright-field objective from horizontal planes separated by 4 μm, and roughly correspond to cross sections of the ablation cavity. The size bars in the images mark the lateral dimension of 100 μm. Figure 2(f) shows a 3-D representation of the same cavity, viewed along the cavity axis, composed from the acquired CLSM images.

The manufacturer-supplied software enables also reconstruction of cross sections through the cavity, perpendicular to the root canal surface. Figure 3(a–g) presents such vertical sections (of the same ablation cavity as shown in Fig. 2), positioned in equidistant distances of 20 μm from its centre towards the periphery. In addition, a 3-D lateral view of one half of the cavity is presented in Fig. 3(h). The images reveal the uneven depth and rather rough

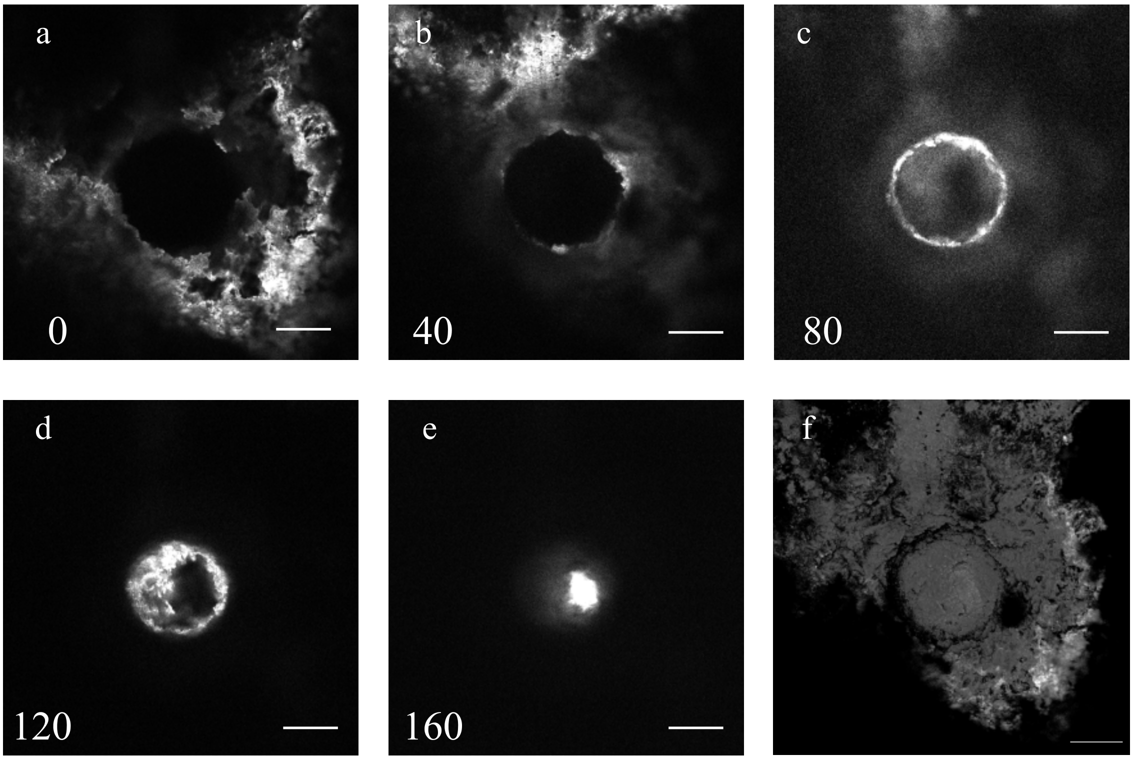

Fig. 2. CLSM images of an ablation cavity (LP, 28 J/cm^2, no water spray), as acquired at depths of (a) 0 μm, (b) 40 μm, (c) 80 μm, (d) 120 μm and (e) 160 μm. (f) shows a 3-D presentation of the same cavity, composed from 48 optical sections, separated by 4 μm. The size bars in the images are 100 μm long (objective magnification: 20×).

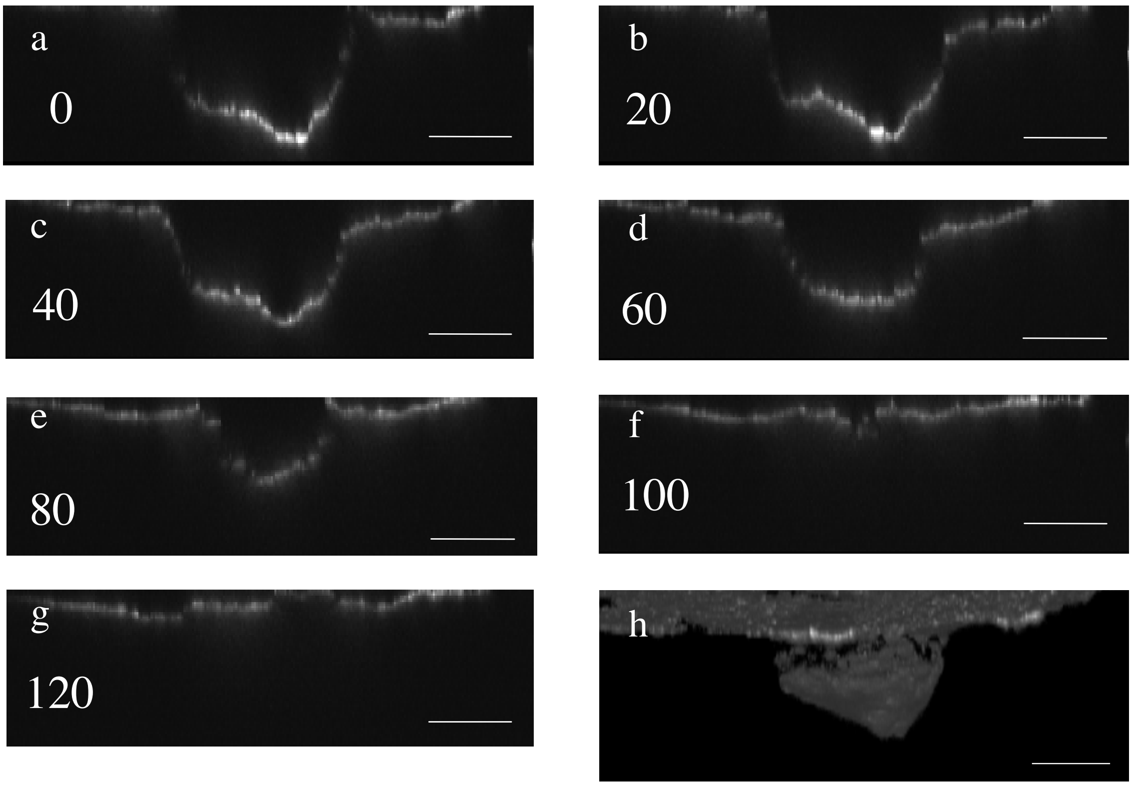

Fig. 3. Vertical cross sections of the laser ablation cavity from Fig. 2, from the centre (a), towards its periphery (g), as reconstructed from 48 horizontal optical sections. The presented sections are equidistant at 20 μm, the size bars are 100 μm long. (h) The composed 3-D lateral view of one-half of the same ablation cavity (LP, 28 J/cm^2, no water spray).

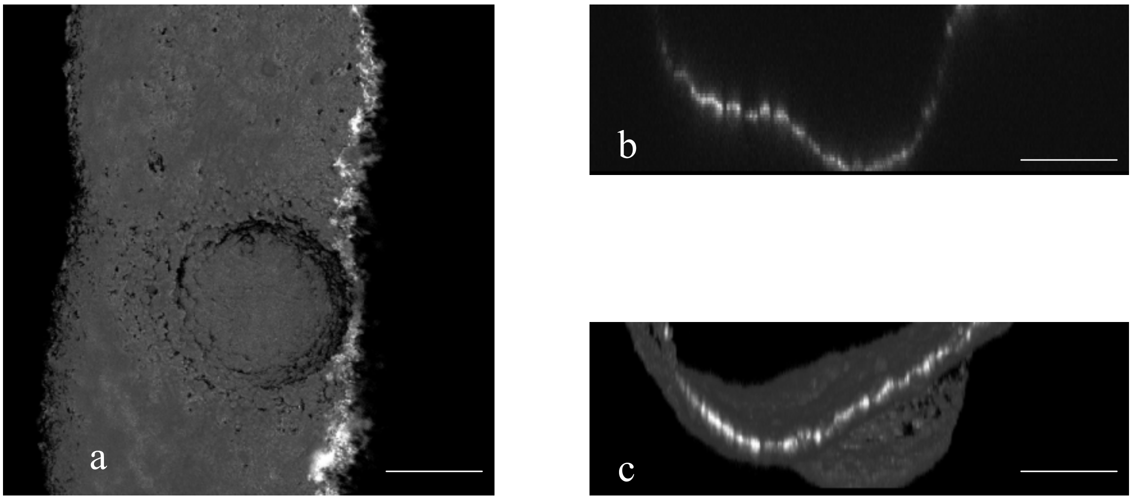

Fig. 4. (a) 3-D presentation of a laser ablation cavity (VSP, 28 J/cm^2, with water spray), as composed from 50 horizontal CLSM images, separated by 4 μm. (b) Vertical cross section through the centre, and (c) 3-D lateral view of one half of the same ablation cavity. The size bars are 100 μm long.

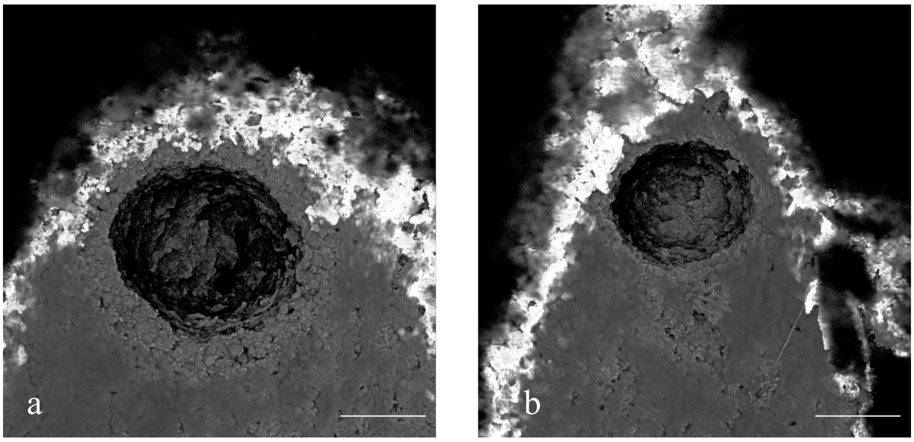

Fig. 5. 3-D presentation of laser ablation cavities produced with sequences of three laser pulses (LP, 12 J/cm^2, 30 Hz), without (a) and with (b) application of dental water spray. These images were composed from 46 and 20 CLSM scans (for a and b, respectively), obtained with the _0× objective from horizontal planes separated by 6 μm. The size bars are 100 μm long.

surface of this particular cavity (LP, 28 J/cm^2, no water spray).

Figure 4 shows an overview of CLSM images of another ablation cavity (VSP, F=28 J/cm^2), where water spray cooling was applied during the laser irradiation. 3-D views of the cavity, reconstructed from 50 horizontal CLSM scans, (a) separated by 4 μm, (b) in vertical cross section through the centre and (c) in a 3-D lateral view of one half of the same ablation cavity are shown. Although the general shape of the cavity is similar to that seen in Figs 2 and 3 (reflecting the intensity profile of the laser), it features significantly rounder, smoother cavity walls.

In Fig. 5, no significant difference can be observed between the cavities produced with sequential Er:YAG laser irradiation (3 × LP, 12 J/cm^2, 30 Hz), without (a) and with (b) application of water spray cooling.

The depths of all ablation cavities, as assessed using CLSM, are collected in Fig. 6. The symbols mark the average values determined from two samples, and error bars mark the standard error of the mean. The end points of each bar, therefore, correspond to individual values for the two craters, and only one crater was available where no error bar is plotted. For the sequential irradiation, one-third of the cavity depth is plotted, to yield the ablation depth per pulse. The plotted curves are not based on theory and serve only as a visual guide.

The cavities resulting from single laser pulses of three different durations (VSP, LP, or VLP) and sequences of three LP pulses without

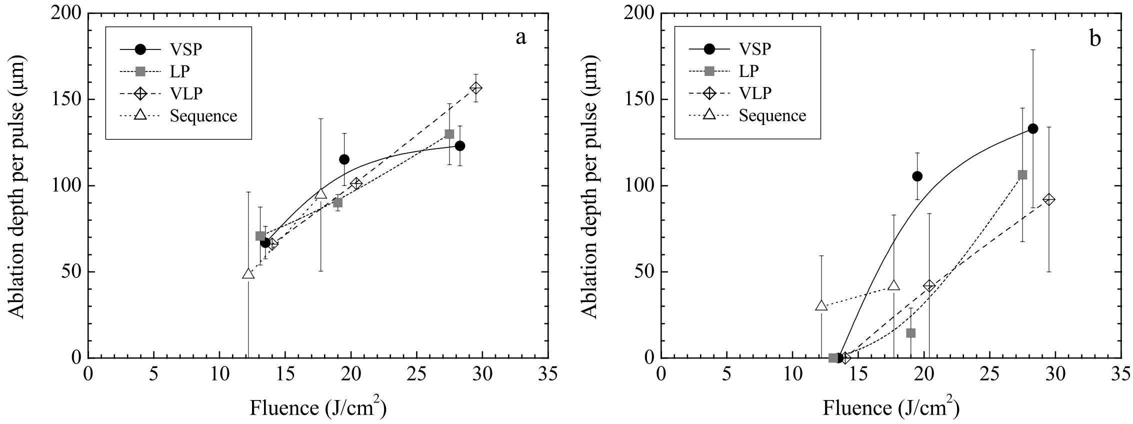

Fig. 6. Ablation depth per pulse as a function of single-pulse fluence for irradiation with individual pulses in three laser 'modes' (VSP, LP, VLP), and sequences of three LP pulses at 30 Hz repetition rate, without (a) and with (b) application of the water spray.

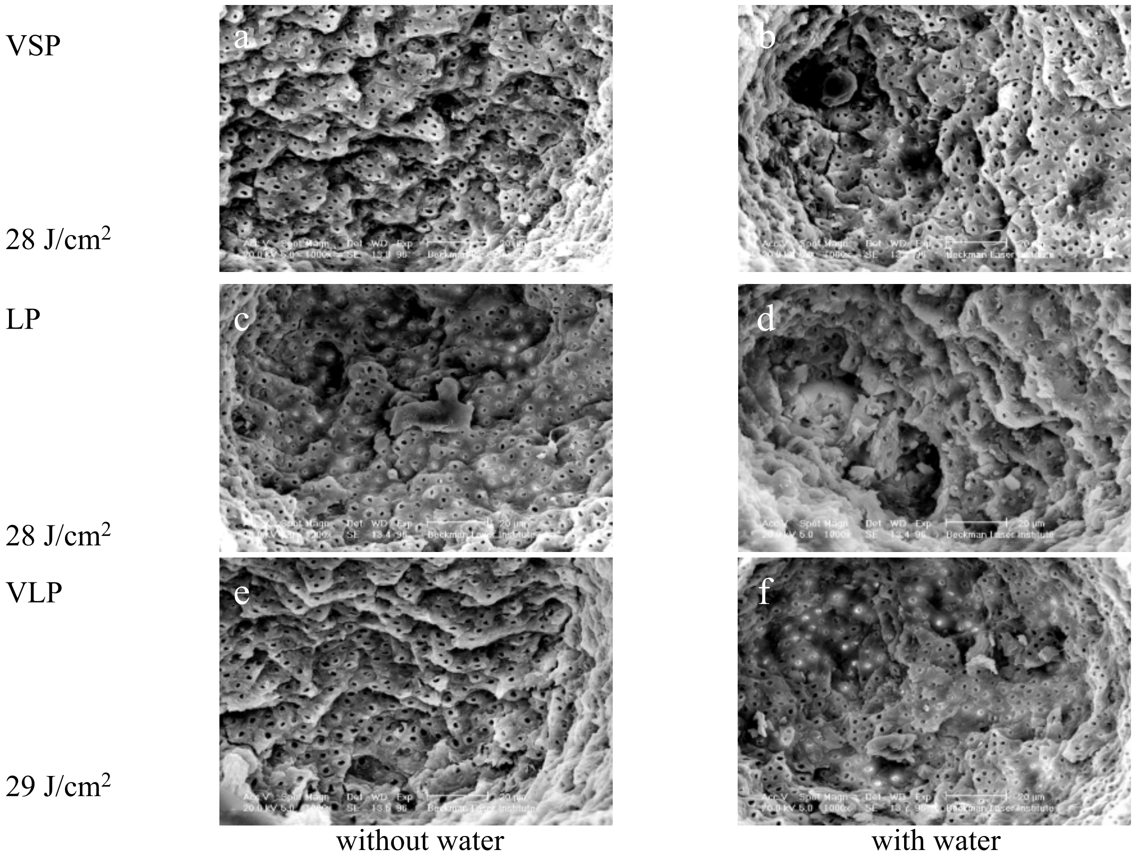

Fig. 7. SEM images of the bottom of ablation cavities, produced by irradiation with individual Er:YAG laser pulses in three 'modes': (a), (b) VSP, 28 J/cm^2; (c), (d) LP, 28 J/cm^2; (e), (f) VLP, 29 J/cm^2. (a, c, e) no water cooling; (b, d, f) with application of water spray cooling. (Original magnification: 1000×)

water spray cooling (Fig. 6a) display similar fluence dependences. However, the VSP data (pulsewidth $t_p = 80$ ms) indicate a greater ablation effect at moderate pulse fluences ($F \sim 20$ J/cm^2), but also the strongest saturation (i.e. diminution of ablation efficiency, causing a deviation from linear dependence) with increasing fluence values, as compared to the LP and VLP pulses. Within the experimental margin of error, there is no difference between the two latter data sets. The greatest variability of the results is observed with the sequential irradiation.

Very similar observations can be made from results obtained when water spray cooling was applied during the irradiation (Fig. 6b). The

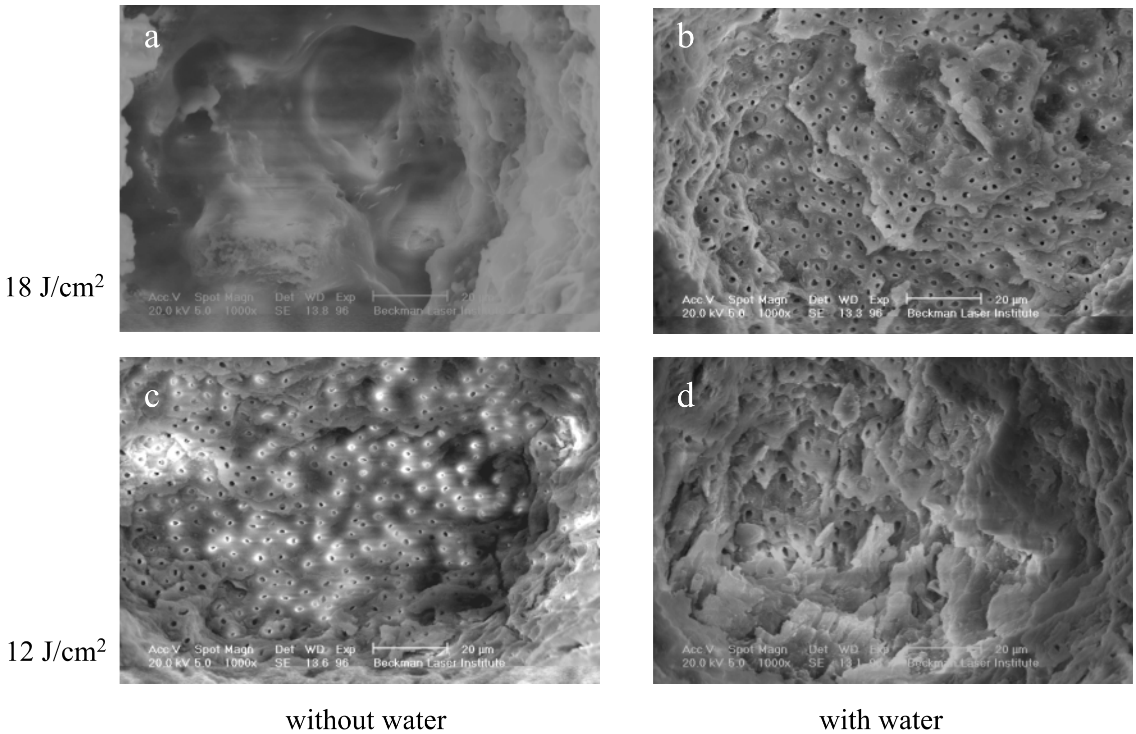

Fig. 8. SEM images of the root canal dentine at the bottom of ablation cavities, resulting from irradiation with 30-Hz sequences of three LP pulses with single-pulse fluence of (a), (b) 18 J/cm^2; (c), (d) 12 J/cm^2. (a, c) no water cooling, (b, d) with application of water spray cooling. (Original magnification: 1000×)

ablation effect at moderate pulse fluences is again greatest in the VSP mode, which displays a significant saturation at higher fluence values, whereas the LP and VLP results are comparable within the experimental error. By comparing the results in Fig. 6(a) and (b), we see that the application of water spray cooling/irrigation increased the ablation threshold for all laser operation modes. Nevertheless, the differential ablation efficiency (i.e. increase in ablation depth per unit increase of pulse fluence) seems somewhat higher when the water spray is used, in particular for the VSP laser mode.

A comparison of the cavity diameters has revealed that, in addition to increasing the ablation threshold, water spray cooling/irrigation also reduces the cavity diameter, regardless of the laser mode [14].

Scanning Electron Microscopy

No debris was observed in any of the ablation cavities obtained with single-pulse irradiation at 28–29 J/cm^2, regardless of the pulse duration or application of the water spray (Fig. 7). In all presented examples, the root canal dentine is cleanly ablated, with dentinal tubules open, and no evidence of melting or recrystallisation.

In contrast, a large variety of surface morphologies resulted when sequences of lower-fluence pulses were applied with or without water spray cooling (Fig. 8). Very strong melting of dentine and recrystallisation is evident at single-pulse fluence of 18 J/cm^2 and no water spray (Fig. 8a). In contrast, just moderately annealed, but very flat surface with open dentinal tubules is observed at 12 J/cm^2 (Fig. 8c). With aplication of water spray cooling, the thermal effect is strongly reduced at both fluence values. At 18 J/cm^2, a nice flat surface with just slightly annealed ‘edges’, but open dentinal tubules results (Fig. 8b), whereas at 12 J/cm^2, the micromorphology of the surface is essentially identical to that observed after single-pulse irradiation (Fig. 8d).

DISCUSSION

During routine root canal treatment using hand and rotary instruments and chemical irrigation, debris may remain on the root canal walls [15]. In this study, no smear layer was observed on the surface of the root canal dentine after Er:YAG laser irradiation, just as reported by Takeda et al. [8]. On the other hand, it has been reported that irrigation with water followed by Er:YAG laser irradiation increases dentine permeability, which leads to greater cleanliness and more open tubules [10]. This finding may be an important factor in achieving disinfection of the root canals and a higher mechanical bonding between the root canal sealer and dentine [10]. Since the Er:YAG laser was reported to ablate carious dentine more readily than sound dentine [16], it might be particularly suitable for removal of caries from root canal dentine.

In addition, the Er:YAG laser has a high bactericidal potential at a low energy level [17,18]. Mehl et al. [18] reported that Er:YAG laser radiation (pulse energy 50 mJ; repetition rate 15/s) for 15 or 60 s could effectively reduce *Staphylococcus aureus* and *Escherichia coli*, and concluded that Er:YAG laser radiation exerted very effective antimicrobial properties in dental root canals, depending on the duration of irradiation. The antibacterial effectiveness of the Nd:YAG, Ho:YAG and Er:YAG laser in infected root canals were compared, and the most favourable results were obtained by the Er:YAG laser – achieving a mean bacterial elimination of 99.64% [19]. Thus, the Er:YAG laser has the potential to achieve multiple effects of shaping, cleaning and disinfecting the root canal.

Matsuoka et al. [9] evaluated the efficacy of a fibrescope for the assessment of remnant debris on the root canal walls, and suggested that it was effective for evaluation of root canals of intact teeth. In this study CLSM was used to evaluate the surface of the irradiated root canal. Since this method does not require the samples to be dehydrated, it avoids producing artefactual cracks. Depth of cavities could be determined from CLSM 3-D reconstructions of the cavity shape and cross sections. Moreover, CLSM can visualise subsurface dentine. These properties of CLSM render this modality ideal for the investigation of dental structures, particularly for the non-invasive visualisation of surface and subsurface microstrucures in laser-irradiated hard tissues.

The depths of cavities resulting from three single-pulse laser modes (VSP, LP, VLP) are quite similar between themselves, both with and without the application of water spray cooling/irrigation. However, in both cases, VSP laser pulses ($t_p \sim 80$ ms) display the highest ablation efficiency at moderate pulse fluences (~ 20 J/cm^2), but also the strongest saturation at higher fluence values. Such behaviour is in perfect agreement with earlier systematic measurements on human dentine, as well as theoretical considerations of the involved mechanisms [20,21]. On one hand, it results from diffusion of heat from the laser–tissue interaction layer during the laser pulse. This effect, which is more pronounced with longer laser pulses, reduces the amount of energy available for explosive dentine ablation, and therefore diminishes more the ablation efficiency of the longer laser pulses (LP, VLP). On the other hand, scattering and absorption of laser radiation on the ejected debris is strongest with the shortest laser pulses; however, it diminishes the ablation efficiency primarily at larger pulse fluences, where the deeper ablation cavities trap the ejected debris more effectively [20,21].

Our results demonstrate that the application of water spray cooling/irrigation increases the single-pulse ablation threshold for all pulsewidths under test (from 5–10 J/cm^2 to >13 J/cm^2, see Fig. 6a, b). This matches nicely to earlier observations in ablation of tooth dentine with sequences of ten 200–300 μs long Er:YAG laser pulses [22], and can be attributed to the energy necessary to ablate the strongly absorbing water film on the sample surface [23,24]. On the other hand, the water spray can substantially increase differential ablation efficiency (i.e. increase of ablation effect per increase of pulse fluence). This effect has been observed in previous studies on laser ablation of enamel, and was tentatively attributed to reduced enamel desiccation [20,22]. However, although this explanation is viable for experiments involving repetitive laser irradiation, we would expect the influence of this factor to be minimal in single-pulse ablation experiments, such as presented in this study. Therefore, one or several of the earlier suggested non-thermal ablation mechanisms, such as intense acoustic transients, or fast water jets associated with the collapse of laser-induced water bubbles, might indeed be at work here [22,24–26]. In an extensive study, Ashouri et al. [27] have recently investigated the influence of the water layer on ablation with free-running and Q-switched Er:YAG and Er:YSGG lasers, as well as Ho:YAG and 9.6-μm TEA CO_2 laser. The authors have concluded that 'water augments the ablation of dental enamel by aiding in the removal of loosely attached deposits of non-apatite mineral phases from the crater surface, thus producing a more desirable surface morphology'.

With sequences of three laser pulses, the ablation depth per pulse is observed to be essentially the same as with single pulses, when no water spray was used (Fig. 6a). This might be incidental, however, since one would expect the incomplete thermal relaxation of the sample between successive pulses to reduce the ablation threshold for subsequent laser pulses, whereas the possible desiccation of the root canal dentine would certainly tend to increase it. Since such additional effects are introduced when the water spray is used, the relation between the single-pulse and repetitive ablation effect can depend on numerous parameters, such as the number of pulses in the sequence, repetition rate, water spray characteristics, etc. For example, the reduced ablation threshold for the repetitive, as compared to single-pulse, Er:YAG irradiation (Fig. 6b), might indicate that the water film on the dentine surface, which is removed by the first laser pulse, does not reconstitute before the next pulse arrives, thus diminishing the effect of ablation threshold increase with the water spray.

High-resolution SEM images of the dentine surface structure reveal a clean ablation and no debris on the cavity walls after single-pulse irradiation, regardless of the pulse duration and water spray application (at 28–29 J/cm^2). In contrast, a very smooth, possibly slightly annealed, surface was observed at some sequence-irradiated sites, with and without application of the water spray. It might be very interesting to further explore this regime for root canal preparation. Pecora et al. [28] reported that dentine treated with Er:YAG laser displayed greater adhesion of sealers than dentine treated with EDTAC. The latter, in turn, was greater than dentine which received no treatment. Therefore, the sealing Er:YAG laser modification of the dentine surface ability of root canal fillings might be considerably affected by Er:YAG laser modification of the dentine surface [28].

A non-contact hand piece was used in this study, and only a few investigations have been reported thus far using fibre tips in contact mode [7,29]. As reported earlier, it is very difficult to treat the canal walls with standard fibre tips, which emit primarily in the forward direction [7,30]. Therefore, it seems imperative that a suitable side-firing fibre tip should be developed before Er:YAG or similar lasers can be applied clinically for treatment of root canals.

CONCLUSION

In conclusion, confocal laser scanning microscopy is an effective tool for investigation of laser effects on root canal dentine. The cavities resulting from Er:YAG laser irradiation at different pulse durations (80–280 μs) without water spray cooling display similar fluence dependences. However, the shortest pulses feature a larger ablation effect at moderate pulse fluences ($\sim$20 J/cm^2), but also the most pronounced saturation. This is even more pronounced when water spray is applied during the irradiation, which markedly increases the ablation threshold. Scanning electron microscopy indicates clean ablation and no debris on the cavity walls after single-pulse irradiation at $\sim$30 J/cm^2, regardless of the pulse duration or water spray application. However, a very smooth surface with no debris and open dentinal tubules is observed at some repetitively irradiated sites (at 30 Hz), with and without application of the water spray. It might be very interesting to further explore such regimes for root canal preparation.

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