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Initial fibroblast attachment to Erbium:YAG laser-irradiated dentine

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Abstract

Bolortuya G, Ebihara A, Ichinose S, Watanabe S, Anjo T, Kokuzawa C, Saegusa H, Kawashima N, Suda H. Initial fibroblast attachment to Erbium:YAG laser-irradiated dentine. *International Endodontic Journal*, **44**, 1134–1144, 2011.

Aims To evaluate the effects of Erbium (Er):YAG laser irradiation on the morphology of resected dentine surfaces, and to investigate fibroblast attachment to laser-irradiated dentine surfaces.

Methodology Dentine blocks obtained from single-rooted human teeth were divided into the following groups after sterilization in an autoclave: (i) Laser group treated with Er:YAG laser irradiation (30 mJ per pulse, 10 pps, 60 s); (ii) L-MTAD group treated with laser irradiation as in (i) plus a mixture of doxycycline, tetracycline isomer and citric acid; (iii) RC-Prep group treated with EDTA gel or cream (RC-Prep) and (iv) Control group left untreated. After each treatment, the dentine blocks were incubated with NIH/3T3 fibroblasts cultured to subconfluency in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and antibiotics. The number of attached cells amongst the groups was analysed statistically at the 5% significance level. The dentine surface morphologies and cell attachments were evaluated by counting assays, histological observations and scanning electron microscopy (SEM).

Results The number of attached cells was significantly higher ($P < 0.05$) in the Laser group than in the RC-Prep and Control groups at 16 h. Dendritic cell extension of the fibroblasts was only observed in the Laser group at 8 h by SEM. In the histological analyses, significantly more attached cells were found on the dentine surfaces treated with laser irradiation.

Conclusions Er:YAG laser irradiation induced morphological alterations in dentine surfaces, which may improve the attachment of fibroblasts to dentine.

Keywords: cell behaviour, endodontic treatment, Er:YAG laser, resected root surface, root-end resection.

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Introduction

Laser irradiation of different wavelengths can be successfully used in endodontics for smear layer removal (Alfredo *et al.* 2009), collagen fibre exposure (Hakki *et al.* 2010) and root canal decontamination (Franzen *et al.* 2009, Akiyama *et al.* 2011). Amongst the dental lasers, the Erbium (Er):YAG laser with a wavelength of 2.94 μm is considered to be a promising tool for root-end resection. The advantages of this type of laser over burs are accurate apical resection (Pozza *et al.* 2009), haemostasis and no vibration and/or discomfort (Stabholz *et al.* 2004, Berrocal *et al.* 2007). In addition, many changes are known to occur in the teeth during the processes of heating and vaporization of water in dentine, cementum and enamel. Laser irradiation has also been shown to produce surfaces with a scale-like texture without producing major

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thermal side effects on the root surface (Nomelini et al. 2009, de Moura *et al.* 2010).

Root-end resection is recommended when failed root canal treatment cannot be retreated (Saunders 2005, Salgado *et al.* 2009). The foremost goal of endodontic surgery is to produce a resected root-end with optimal conditions for subsequent regeneration of the periodontal ligament (PDL) across the resected root-end (Al-Nazhan 2004). For this reason, two critical aspects are the surface topography and chemical treatment of the resected root-end (Bruckmann *et al.* 2005). Various types of burs produce different patterns on the resected root surface (Camargo *et al.* 2010). Different root surface qualities may affect the attachment and orientation of the surrounding tissue and periodontal fibroblasts. Previous studies have shown that cellular behaviour is greatly influenced by the surface properties, including roughness, texture and morphology (Wirth *et al.* 2008, Nakamura *et al.* 2010). Root surface conditioning produces a biocompatible surface that is conducive to periodontal cell colonization (Miyaji *et al.* 2010). After root-end resection, the surrounding cells become activated, undergo proliferation, migrate into the wound site and synthesize new matrix components (Al-Nazhan 2004, Miyaji *et al.* 2010), such that periapical regeneration by bone and cementum deposition and PDL cell attachment to the resected root surface can be achieved (Balto & Al-Nazhan 2003).

At present, chemical and/or mechanical conditioning of the resected surface is utilized to achieve periapical regeneration. Although the results of clinical and morphological studies remain to be fully clarified, the biocompatibilities of resected root surfaces after treatment with acids, low-level Er:YAG laser irradiation or laser irradiation plus antibiotics may be different. Therefore, the purpose of this laboratory study was to determine the influence of laser-treated dentine surface morphologies on the initial fibroblast cell attachment, in comparison with the cell responses to chemical and/or mechanical conditioning of dentine surfaces.

Materials and methods

Specimen preparation

A total of 40 freshly extracted single-rooted human teeth with fully formed apices extracted for periodontal or prosthetic reasons were used in this study. The study protocol was approved by the Ethical Committee of Tokyo Medical and Dental University (#535). After extraction, the PDLs were removed by scrapping the root surface with a scalpel blade, and the teeth were stored at 4 °C in phosphate-buffered saline (PBS). For preparation of dentine discs, the root apex of each tooth was removed and dentine discs with dimensions of approximately $5 \times 5 \times 2$ mm were prepared using a low-speed saw (Isomet; Buehler Ltd., Lake Bluff, IL, USA). A total of 96 dentine discs were prepared, washed three times with phosphate-buffered saline (PBS) and sterilized in a high-pressure steam sterilizer (LSX-300; Tomy Tech USA, Fremont, CA, USA). None of the samples had caries, filling, fracture or endodontic treatment.

Laser device

An Er:YAG laser (Erwin AdvErl; Morita, Kyoto, Japan) was used. The parameters of this device were as follows: wavelength, 2.94 μm; range of output energy settings, 30–350 mJ per pulse; maximum pulse repetition rate, 25 pulses s^{-1} (Hz) and pulse duration, 200 μs. The device employed an optical fibre delivery and a contact probe system. A straight fibre quartz tip with a diameter of 800 μm (P800FL; Morita) was used. The laser apparatus was also equipped with a special water spray system, by which air-mixed water was released to the contact tip to cool the target tissue during the irradiation.

Surface conditioning agents

Two solutions were used for chemical conditioning of the dentine surfaces. The first was an EDTA gel or cream comprising a root canal preparation cream (RC-Prep; Premier Dental Products, Norristown, PA, USA), which contains 10% urea peroxide and 15% EDTA in a special water-soluble base. The second was an MTAD solution (BioPure MTAD; Dentsply Tulsa Dental, Tulsa, OK, USA), which is a root canal irrigant comprising a mixture of a tetracycline isomer, doxycycline and citric acid. The manufacturer claims that the mixed solution cleanses the root canal, removes the smear layer and kills bacteria in instrumented root canals. Sterile saline solution, comprising a sterile solution of 0.9% sodium chloride (pH 6.3; Otsuka, Tokyo, Japan), was employed as a control.

Treatment groups

A total of 96 samples were evaluated. Er:YAG laser irradiation for the Laser and L-MTAD groups was

performed with an energy output of 30 mJ per pulse (energy density: 5.97 J cm^{-2} per pulse) and a pulse frequency of 10 pulses s^{-1}. The Er:YAG laser beam was delivered to the root surface through the fibre quartz tip using a handpiece in a contact mode under water flow rate. The contact tip was moved in a sweeping manner to irradiate the entire dentine surface, and the working time required for this procedure in each sample was 60 s. The samples were randomly divided into four groups. In the Laser group, each sample was treated with Er:YAG laser irradiation for 60 s. In the L-MTAD group, each sample was subjected to Er:YAG laser irradiation for 60 s, and the laser-irradiated surfaces were then treated with the MTAD solution for 60 s. In the RC-Prep group, the EDTA gel or cream was placed on the dentine surface for 60 s. In the Control group, no laser irradiation or treatment was performed. Following each treatment, the samples were thoroughly rinsed with saline solution. After rinsing, a new sterilized cotton pellet was applied to dry each sample.

Scanning electron microscopy (SEM) observation of the dentine surface morphology

After the laser treatment and/or surface conditioning procedures, two samples from each group (n = 8) were randomly chosen for SEM observation to investigate the surface morphology. The dentine discs were fixed for 2 h with 2.5% glutaraldehyde solution and rinsed with 0.1 mol L^{-1} phosphate buffer solution. The discs were then dehydrated in a series of graded ethanol solutions. After washing with 3-methyl butyl acetate, the discs were dried in a critical point drying apparatus (HCP-2; Hitachi, Tokyo, Japan) with liquid CO_2. The samples were sputter-coated with osmium using an NL-OPC80N plasma coater (Filgen, Nagoya, Japan) and observed under an SEM (S-4500; Hitachi) at an accelerated voltage of 15 kV and a magnification of ×1500.

Cell culture

Mouse NIH/3T3 fibroblast cells were seeded on 24-well plates and cultured in Dulbecco's modified Eagle's medium (DMEM; Wako, Osaka, Japan) containing 5% foetal bovine serum (Gibco™; Invitrogen, Grand Island, NY, USA) at 37 °C in a humidified incubator under 95% air and 5% CO_2. After the surface treatments, 88 of the 96 dentine discs were placed in 24-well plates containing 1×10^4 NIH/3T3 cells/well in 500 μL of DMEM.

SEM observation of the cell morphology

The dentine discs were cultured with NIH/3T3 cells for 4, 8 or 16 h. Two samples from each group (n = 24) were randomly chosen to evaluate the influence of the surface treatments in the RC-Prep, Laser and L-MTAD groups on the NIH/3T3 cell morphology by SEM. At the end of the three incubation periods, unattached cells on the dentine surfaces were removed by two rinses with sterile PBS. The samples with attached cells were then fixed, rinsed, post-fixed, dehydrated, dried and coated with gold in a similar manner to the SEM sample preparation. The cells were examined by SEM at an accelerating voltage of 15 kV and a magnification of ×1500.

Numbers of attached cells

Overall, 48 of the 88 dentine discs were used for proliferation assays. The cell numbers were analysed using the WST-8 assay (Cell Counting Kit-8; Dojindo, Kumamoto, Japan), which is a colorimetric assay based on the reduction of tetrazolium salt to water-soluble formazan, and the cells were quantified by spectrophotometry. After 4, 8, or 16 h of culture, the dentine samples were washed with sterile PBS to eliminate unattached cells, and the adhered cells were removed from the dentine discs by incubation with 0.25% trypsin in EDTA. The resulting cell suspensions were placed in 96-well plates in 100 μL of DMEM (Wako, Osaka, Japan) together with 10 μL of a working solution containing WST-8 and incubated for 2 h at 37 °C. The absorbances of the coloured product were measured using a microplate reader (Well Reader SK601; Seikagaku, Tokyo, Japan). Four measurements were taken for each sample.

Histological observations

Four samples from each treatment group were used for ultrastructural analyses. After 16 h of culture, dentine samples with attached cells were rinsed twice with sterile PBS and fixed with 2.5% glutaraldehyde in 0.1 mol L^{-1} phosphate buffer for 2 h at 4 °C. The samples were then rinsed twice with 0.1 mol L^{-1} phosphate buffer for 1 h and post-fixed with 1% osmium tetroxide in 0.2 mol L^{-1} phosphate buffer for 2 h at 4 °C. The samples were dehydrated in a series of graded ethanol solutions for 20 min per solution and covered with 50% Epon in 100% ethanol solution for at

least 4 h. Following polymerization, semi-thin sections for light microscopy that contained NIH/3T3 cells and a cast of the treated surface were cut from the Epon blocks using a glass knife. These sections were stained with toluidine blue and observed under a light microscope (Eclipse™ E800; Nikon, Tokyo, Japan) to determine the original cell orientation to the treated surfaces in each group.

Statistical analysis

The number of attached cells in each group was calculated as the mean ± standard deviation (SD). Differences between the groups were analysed by one-way analysis of variance (ANOVA) together with a *post hoc* Fisher's protected least significant difference. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

SEM observation of dentine surface morphology

Representative SEM images of the four different dentine surfaces are shown in Fig. 1. All dentine surfaces in the Control group basically displayed a smooth appearance and were covered with debris (Fig. 1a). The surfaces in the RC-Prep group also had a smooth appearance, and the smear layer was not completely removed (Fig. 1b). In contrast, the surfaces in the Laser group had irregular and scaly morphologies with intense ablation of the intertubular dentine, removal of the smear layer and open dentinal tubules (Fig. 1c). The surfaces in the L-MTAD group exhibited plugged dentinal tubules without the smear layer, and the MTAD treatment had removed the microstructures produced by the Er:YAG laser irradiation (Fig. 1d).

SEM observation of cell morphology

Representative SEM images of attached cells on the four different dentine surfaces after 4, 8 and 16 h of culture are shown in Figs 2, 3 and 4, respectively. After 4 h of culture, the attached cells in all the groups were round with poorly developed processes (Fig. 2a–d). The cells appeared to adhere via thin filopodia to the four different types of surfaces (Fig. 2a–d). After 8 h of culture, the cells on the surfaces in the Control group were adhered via thin filopodia (Fig. 3a). The cells attached to the surfaces in the RC-Prep group were still round with poorly developed processes (Fig. 3b). In the Laser group, the cells were flattened and attached to the dentine by expanded filopodia (Fig. 3c). Dendritic cell extension of the fibroblasts was only observed in

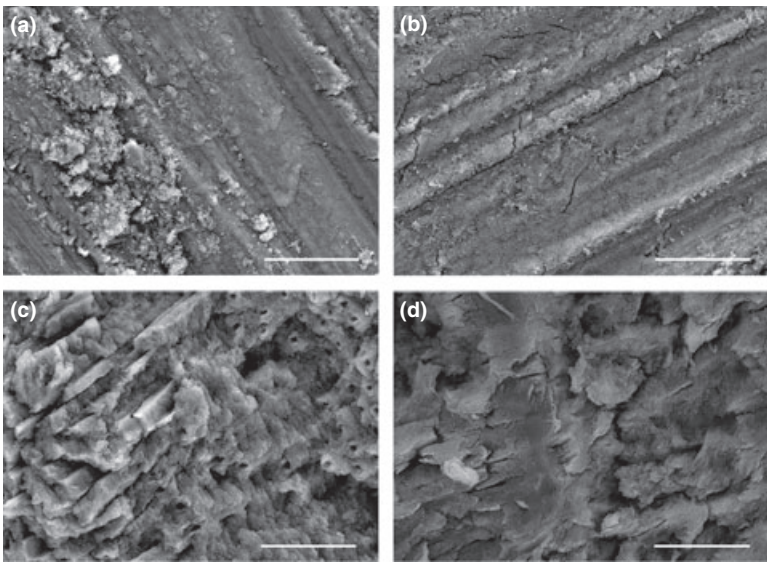

Figure 1 Representative SEM images of the dentine disc surfaces after the treatments. (a) The surface in the Control group shows a smooth appearance with a smear layer. (b) The surface in the RC-Prep group displays a smooth appearance, but the smear layer is not completely removed. (c) An irregular microstructure with open dentinal tubules is revealed on the laser-treated surface in the Laser group. No smear layer is seen. (d) The surface in the L-MTAD group shows plugged dentinal tubules with removal of the irregularities observed in the Laser group. Original magnification: ×1500. Scale bars: 20 μm.

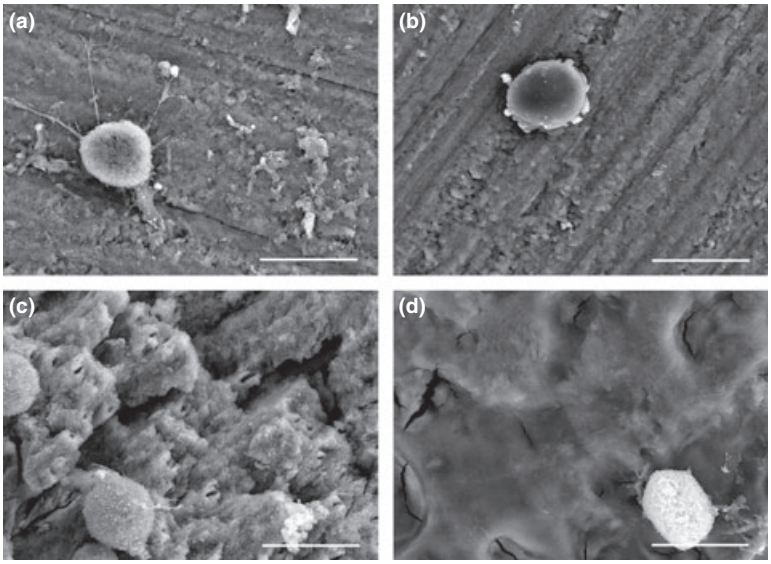

Figure 2 Representative SEM images of fibroblasts after 4 h of culture. (a) Control group. (b) RC-Prep group. (c) Laser group. (d) L-MTAD group. The cells have started to adhere to the dentine surfaces at this time-point. Original magnification: ×1500. Scale bars: 20 μm.

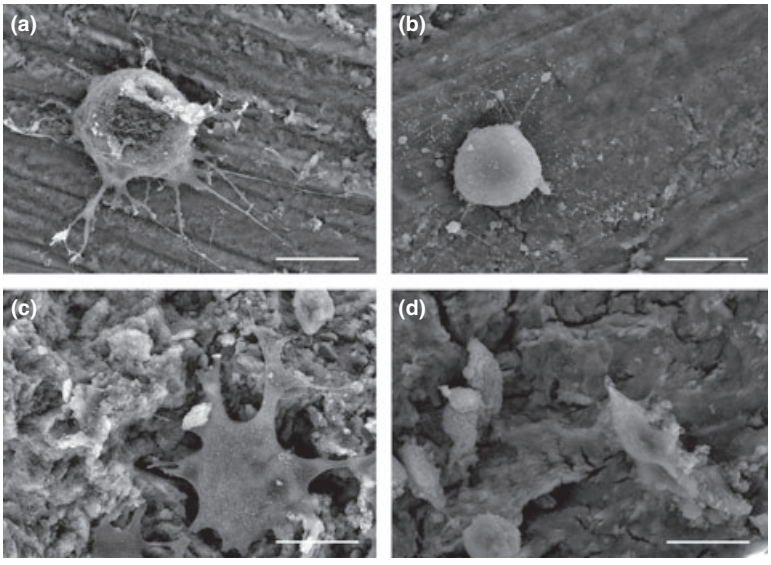

Figure 3 Representative SEM images of fibroblasts after 8 h of culture. (a) In the Control group, the cell adheres to the dentine surface via thin filopodia. (b) In the RC-Prep group, the attached cell is still round with poorly developed processes. (c) In the Laser group, the attached cell is flattened with dendritic cell extensions. (d) In the L-MTAD group, the cell adheres to the dentine surface via poorly developed processes. Original magnification: ×1500. Scale bars: 20 μm.

the Laser group (Fig. 3c). The cells on the surfaces in the L-MTAD group had poorly developed processes (Fig. 3d). After 16 h of culture, the cells continued to proliferate and adhered to all the dentine surfaces (Fig. 4a–d). No obvious differences in the cell morphologies were observed between the Control group and the three treatment groups after 16 h of culture (Fig. 4a–d).

Attached cell numbers

The numbers of cells attached to the four different surfaces after 4, 8 and 16 h of culture are shown in Fig. 5. The numbers of attached cells after 4 h of culture did not differ significantly amongst the groups. Cell attachment started to increase by 8 h of culture in the Laser group. However, the numbers of attached cells did not differ significantly amongst the groups after 8 h of culture. The adhesion rate was highest on the surfaces in the Laser group after 16 h of culture ($P < 0.05$). The Laser group exhibited the highest number of attached cells (1.135 ± 0.229 cells per sample, $n = 4$), followed by the L-MTAD group (0.805 ± 0.149 cells per sample). These groups differed significantly ($P < 0.05$) from the RC-Prep group, which exhibited the lowest number of attached cells (0.445 ± 0.076 cells per sample).

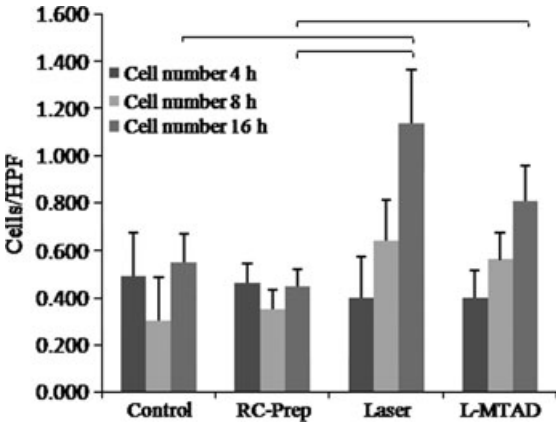

Figure 5 Numbers of fibroblasts attached to the dentine surfaces in the control and experimental groups. The ordinate represents the number of attached cells/high-power field (HPF) after 4, 8 and 16 h of culture. All data are means ± SD ($n = 4$). Statistically significant differences between the groups are indicated by horizontal lines ($P < 0.05$).

Histological observations

Representative histological images of the four different surfaces after 16 h of culture are shown in Fig. 6. In the Control group, the untreated dentine surfaces exhibited a smooth appearance with a thin layer stained by toluidine blue and few attached cells (Fig. 6a). The dentine surfaces in the RC-Prep group appeared to be smooth and were similarly stained to the surfaces in the Control group with few attached cells (Fig. 6b). In the Laser group, a thick and darkly stained layer with a micro-irregular border was observed on the whole surface, and cells with well-extended processes were observed on the scaly surface (Fig. 6c). In the L-MTAD group, the micro-irregular border was not

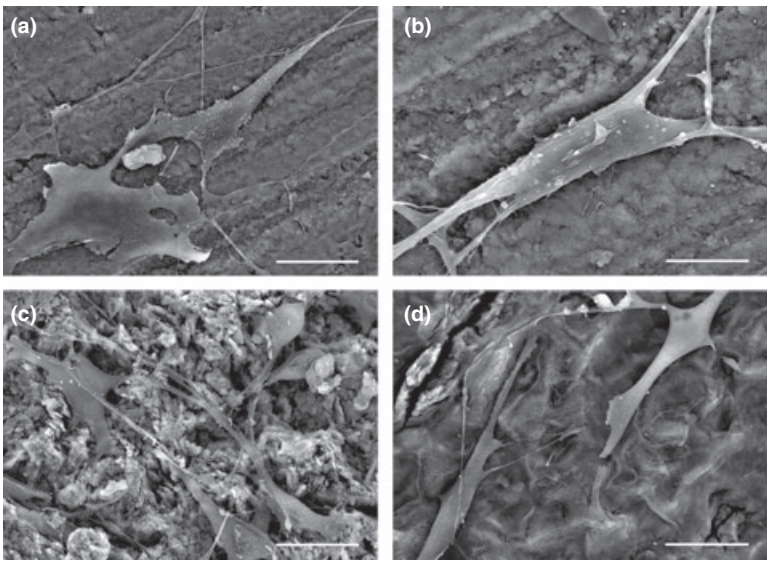

Figure 4 Representative SEM images of fibroblasts after 16 h of culture. (a) Control group. (b) RC-Prep group. (c) Laser group. (d) L-MTAD group. The cells continue to proliferate and adhere to the sample surfaces. No obvious differences in the cell morphologies are observed amongst the groups. Original magnification: ×1500. Scale bars: 20 μm.

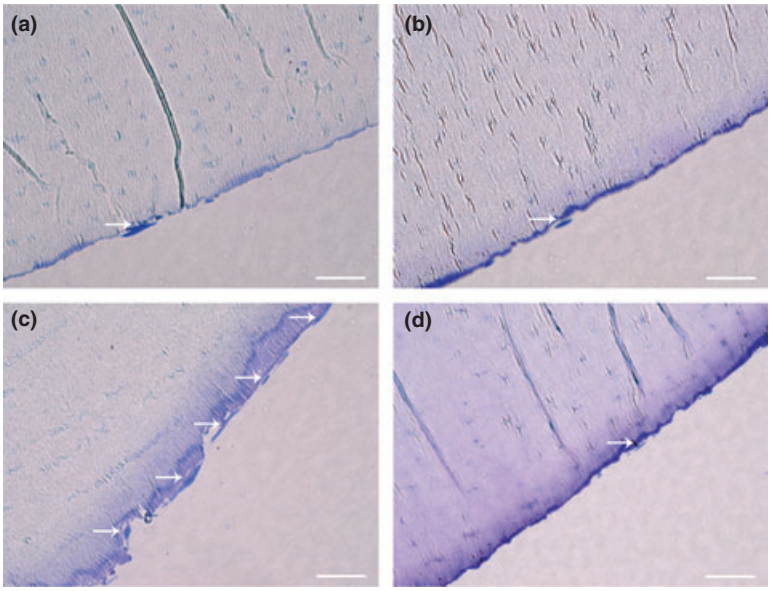

Figure 6 Representative photomicrographs of histological sections of dentine surfaces with attached cells following Er:YAG laser irradiation and/or chemical conditioning. (a) Control group. (b) RC-Prep group. (c) Laser group. (d) L-MTAD group. The untreated surface in the Control group shows a thin layer stained by toluidine blue with few attached cells, whilst the laser-treated surface in the Laser group exhibits a thick and darkly stained layer and contains cells with well-developed processes. The arrows indicate attached cells on the dentine surfaces. Original magnification: ×40. Scale bars: 20 μm.

present, but a thicker and darkly stained layer was observed (Fig. 6d). Few attached cells were seen on the L-MTAD surface (Fig. 6d).

Discussion

After root-end resection, the ultimate goal was to achieve periapical repair including PDL cell attachment onto the resected root surface. Therefore, it is desirable to obtain a resected root-end with favourable conditions for the subsequent regeneration (Al-Nazhan 2004). Laser irradiation has been used in root-end resection because of its benefits (Berrocal *et al.* 2007, Pozza *et al.* 2009). Most previous studies employing laser techniques for root-end resection mainly concentrated on the sealing ability to reduce the penetration of dye or bacteria (Stabholz *et al.* 2004, Alfredo *et al.* 2009), and little is known about the cellular responses to the laser-altered surfaces. In this study, the modifications induced by Er:YAG laser irradiation as well as the effects of the altered surfaces on cell behaviour were evaluated.

In the conventional method for surface preparation of the resected root-end, various types of burs have been used. However, little information exists about the effects of the surface topography after using Er:YAG laser irradiation for surface preparation. In the present study, an energy output of 30 mJ per pulse was selected as the minimum setting for the device (minimum on the control panel: 30 mJ per pulse), and a pulse repetition rate of 10 pps was selected based on the most effective and safe setting for clinical use of Er:YAG laser therapy (Schwarz *et al.* 2003). As citric acid, tetracycline and EDTA have been shown to remove the debris and smear layer (Ruggeri *et al.* 2007, Ring *et al.* 2008, Yasuda *et al.* 2010), MTAD and RC-Prep were used for surface conditioning in the present study. During surgical endodontic treatment, modification of the conventional treatment method may be necessary for rapid wound healing.

Root surface conditioning removes the smear layer and provides a surface conducive to mechanical adhesion and cellular mechanisms for growth and attachment. The root surfaces were treated with saline, citric acid, tetracycline hydrochloride, minocycline and EDTA gel to produce different surface textures (Babay 2001, Ring *et al.* 2008). The SEM observations revealed that application of RC-Prep to the dentine surface resulted in a smooth surface appearance with incomplete removal of the smear layer. Although the

effect of EDTA is well documented for its effective removal of the smear layer (Ring *et al.* 2008), this treatment cannot be applied to endodontic surgery because the manufacturer has advised against the use of EDTA when mineral trioxide aggregate is used as the root-end filling material because EDTA can chelate various metallic ions. Ballal *et al.* (2009) examined the cytotoxic effects of EDTA and demonstrated that there was a significant decrease in cell viability. Some studies have shown that RC-Prep produces less smear layer in the coronal and apical third (Prati *et al.* 2004, Tunga *et al.* 2011). However, those studies compared RC-Prep with NaOCl, H_2O_2, MTAD solution, not 17% EDTA conditioning. In the present study, RC-Prep application did not completely remove the smear layer, and this was in agreement with the literature which shows that RC-Prep is not as efficient as 17% EDTA solution. Furthermore, RC-Prep treatment of the dentine surface resulted in the lowest number of attached cells amongst the experimental groups. As all the specimens were prepared using the same procedure to maintain the same conditions amongst the experimental groups, the slight roughness produced by the low-speed sectioning disc was not responsible for the results obtained in the RC-Prep group. Therefore, the reasons for these results may be the experimental methods. Given the observations, further *in vitro* and *in vivo* studies are required to investigate clinically the severity of cytotoxicity of 17% EDTA to cell biology, and the necessity of complete removal of the smear layer, and collagen fibre exposure for optimum attachment.

In the present study, MTAD solution, which is a root canal irrigant comprising a mixture of a tetracycline isomer, doxycycline and citric acid, was applied as an additional treatment to increase the biocompatibility of the lased surfaces. Clinical studies have already demonstrated favourable wound healing of periodontal pockets following Er:YAG laser therapy and revealed that the lased surfaces displayed characteristic microstructures (Schwarz *et al.* 2003, 2008). The affected layer on the root surface produced by Er:YAG laser irradiation was generally biocompatible (Mizutani *et al.* 2006). However, Maruyama *et al.* (2008) found that application of tetracycline solution to laser-treated cementum surfaces increased its biocompatibility by completely removing the microstructure. The results of the present study for the surfaces in the L-MTAD group revealed that the irregular morphology of the lased surfaces was removed by MTAD treatment, resulting in a basically smooth appearance. Furthermore, the present study showed that application of MTAD to laser-treated dentine surfaces resulted in the second highest number of attached cells at 16 h by removing the microstructures from the lased surfaces.

Previous studies have revealed that Er:YAG laser irradiation with different parameters produced glazed microstructures that provided roughness to the root surface without adverse thermal damage (Hakki *et al.* 2010, Camargo *et al.* 2010). In the present study, the Laser group exhibited typical micro-irregularities. These findings are consistent with the previous investigations (Stabholz *et al.* 2004, Galli *et al.* 2009, Nomelini *et al.* 2009) and can be attributed to the laser photothermal mechanism, which can cause the dissolution of mineral components and fusion of amorphous particles, resulting in microscopic irregularities. Some studies have reported faster adhesion and growth of fibroblasts on laser-treated surfaces compared with mechanically debrided surfaces and that Er:YAG laser irradiation induced a homogeneous roughness on the root surface (Feist *et al.* 2003, Pourzarandian *et al.* 2004, Schwarz *et al.* 2008), as confirmed in the present study. In addition, Er:YAG laser irradiation removes the smear layer and leaves the dentinal tubules open. This removal may be responsible for the highest number of attached cells after 16 h of culture. Moreover, the pilot study demonstrated that Er:YAG laser irradiation exposed collagen fibres on the dentine surface. These findings are supported by the previous investigations showing that cell attachment is affected by the smear layer produced during mechanical root-end resection (Maruyama *et al.* 2008, Pozza *et al.* 2009).

The surface topography of the resected root-end may affect the behaviour of the surrounding cells (Bruckmann *et al.* 2005, Miyaji *et al.* 2010, Nakamura *et al.* 2010) and provide a surface that is conducive to mechanical adhesion and cellular mechanisms for growth and attachment. PDL fibroblasts changed from the state of being round with microvilli growth to flattening of the cell mass with filopodia (Balto & Al-Nazhan 2003). These different states are not discretely separable but are different phases of a continuous process. In the present study, SEM observations after 8 h showed the presence of attached cells with different phases in the different groups. Dendritic cell extension of the fibroblasts was only observed in the Laser group. The laser-treated surfaces may be favourable for the duration of these phases and the degree of overlapping of these events. Furthermore, the morphological roughness of the lased surfaces enhanced the adhesion of fibroblasts, and a significantly higher number of

attached fibroblasts were observed at 16 h. In addition, regarding the effects of the morphological micro-irregularities, there has been speculation that the scale-like structure of lased surfaces would be advantageous for initial cell and tissue attachment, as fibrin and blood clots are formed more easily on lased surfaces (Sasaki *et al.* 2002, Theodoro *et al.* 2006) and this would provide a scaffold for the ensuing cell attachment. Moreover, the surface transformation caused by Er:YAG laser irradiation may expose chemical substances in the dentine that are highly selective for chemotaxis of fibroblasts (Fernyhough & Page 1983). These modifications induced by Er:YAG laser irradiation could be a direct consequence of root conditioning by exposure of certain extracellular constituents acting on the attachment mechanism of fibroblasts (Crespi *et al.* 2006) or an indirect effect by the increased fixation of cells on the mineralized surface (Scheven *et al.* 2002).

Similar results were obtained by Feist *et al.* (2003), who studied the adhesion and growth of cultured human gingival fibroblasts on root surfaces treated by both Er:YAG laser irradiation and curettage. Fibroblasts adhered to and grew on all the treated surfaces, but the surfaces lased at 60 mJ per pulse and 10 Hz exhibited a significantly higher number of attached cells, and the authors reported that a high energy level of Er:YAG laser irradiation delayed early fibroblast adherence. These observations further demonstrate the importance of appropriate laser exposure settings for initial cell attachment and are in agreement with the previous clinical studies showing that excessive exposure to laser light can have potentially damaging effects that may negate any initial benefits of laser exposure (Pourzarandian *et al.* 2004, Theodoro *et al.* 2006, Galli *et al.* 2009).

Gouw-Soares *et al.* (2004) conducted a study on root-end resection performed with burs, Er:YAG laser irradiation or CO_2 laser irradiation. The use of lasers resulted in smoother surfaces and more homogeneous dentine fusion, which occluded tubules and decreased permeability. However, Camargo *et al.* (2010) demonstrated that the use of a bur was a significantly faster method than laser irradiation or ultrasound but provided a regular surface that interfered with the adaptation to the remaining filling material. In addition, Nd:YAG laser irradiation altered the biocompatibility of the cementum surface, making it unfavourable for fibroblast attachment (Trylovich *et al.* 1992), whilst Fayad *et al.* (2004) found morphological changes on the dentine surface after CO_2 laser irradiation, including charring and melting of the dentine mineral phase, and concluded that these changes may have played a role in the absence of PDL cell attachment to the root surfaces. These authors used Nd:YAG and CO_2 laser irradiation at higher energy power than that used in the present study and obtained different results. This may be explained by the specific properties of the Er:YAG laser, which produces no major thermal side effects such as the carbonization and/or cracking of the root surface that are usually observed after CO_2 or Nd:YAG laser irradiation. In addition, low-energy laser irradiation was selected.

As cell attachment is affected by the smear layer produced during mechanical root-end resection (Scheven *et al.* 2002, Maruyama *et al.* 2008, Shapira & Halabi 2009), the findings of the present study suggest that Erbium:YAG laser irradiation may induce modification of the resected dentine surface morphology with complete elimination of the smear layer. However, more extensive *in vivo* studies are required to confirm the results.

Conclusions

Erbium (Er):YAG laser irradiation induced morphological alterations of dentine surfaces, thereby improving fibroblast attachment after low-energy laser irradiation. Fibroblast attachment is mainly induced by the removal of the smear layer and exposure of the collagenous matrix of dentine. Further laboratory and *in vivo* studies are required to better understand the effects of the surface modifications after Er:YAG laser irradiation on cell mechanisms.

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