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ORIGINAL ARTICLE

Comparative analysis of root surface smear layer removal by different etching modalities or erbium: yttrium–aluminum–garnet laser irradiation. A scanning electron microscopy study

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Abstract The purpose of this study was to evaluate the effect of erbium:yttrium–aluminum–garnet (Er:YAG) laser (2.94 μm) irradiation on the removal of root surface smear layer of extracted human teeth and to compare its efficacy with that of citric acid, ethylenediamine tetra-acetic acid (EDTA), or a gel containing a mixture of tetracycline hydrochloride (HCl) and citric acid, using scanning electron microscopy (SEM). Thirty human dentin specimens were randomly divided into six groups: G1 (control group), irrigated with 10 ml of physiologic saline solution; G2, conditioned with 24% citric acid gel; G3, conditioned with 24% EDTA gel; G4, conditioned with a 50% citric acid and tetracycline gel; G5, irradiated with Er:YAG laser (47 mJ/10 Hz/5.8 J/cm^2/pulse); G6, irradiated with Er:YAG laser (83 mJ/10 Hz/10.3 J/cm^2/pulse). Electron micrographs were obtained and analyzed according to a rating system. Statistical analysis was conducted with Kruskal–Wallis and Mann–Whitney tests ($P<0.05$). G1 was statistically different from all the other groups; no statistically significant differences were observed between the Er:YAG laser groups and those undergoing the other treatment modalities. When the two Er:YAG laser groups were compared, the fluency of G6 was statistically more effective in smear layer removal than the one used in G5 (Mann–Whitney test, $P<0.01$). Root surfaces irradiated by Er:YAG laser had more irregular contours than those treated by chemical agents. It can be concluded that all treatment modalities were effective in smear layer removal. The results of our study suggest that the Er:YAG laser can be safely used to condition diseased root surfaces effectively. Furthermore, the effect of Er:YAG laser irradiation on root surfaces should be evaluated in vivo so that its potential to enhance the healing of periodontal tissues can be assessed.

Keywords Comparison studies · Laser/therapeutic use · Periodontal therapy · Scaling · Smear layer

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Introduction

Removal of diseased cementum by scaling and root planing has been advocated as part of periodontal therapy [1, 2]. However, studies have indicated that root debridement may not completely remove contaminated cementum [3, 4]. Therefore, several approaches for additional root surface treatment have been proposed. Conditioning of the root surface after scaling and root planing has been suggested as

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a promising procedure for removing endotoxins and smear layer, as well as to compensate for the limitations inherent in mechanical root surface therapy. In vitro [5–10] and in vivo [11, 12] studies have tested a number of chemical agents for adjunctive detoxification and smear layer removal of diseased root surfaces, including citric acid, ethylenediamine tetra-acetic acid (EDTA) and tetracycline hydrochloride (HCl). Even though in vitro [5–10] and in vivo [11–12] studies have shown the effectiveness of chemical root conditioning agents, clinical studies in humans have not [13–15].

Two of the goals of periodontal therapy are to convert the diseased root surface into a substrate, which is biologically hospitable to epithelial and connective tissue cell adherence and attachment, and to regenerate the lost periodontal tissues [16]. Laser therapy has been studied in different applications in dentistry, especially in periodontics. Several studies have demonstrated the effectiveness of the erbium:yttrium–aluminum–garnet (Er:YAG) laser in removing the smear layer left on the dentin surface after mechanical root canal preparation [17], as well as calculus removal, with no signs of thermal damage [18–20]. On the other hand, some studies have been designed to evaluate the effect of laser irradiation on the removal of the root surface smear layer and the exposure of dentinal tubules after root planing [20–22].

Although conventional periodontal treatment improves clinical parameters, it is not effective in eliminating all periodontal pathogens. Er:YAG laser scaling has been suggested as an alternative to conventional scaling and has been reported to exhibit high bactericidal properties [23]. The purpose of this study was to evaluate the effect of Er:YAG laser irradiation on the removal of the smear layer left after root planing of the cementum of extracted human teeth and to compare its efficacy with citric acid, EDTA, or a gel containing a mixture of tetracycline and citric acid, using scanning electron microscopy (SEM).

Material and methods

Collection and preparation of specimens

This study was approved by the Ethics Committee of the Instituto de Pesquisas Energéticas e Nucleares (CNEN/SP), Brazil. Fifteen healthy premolars and impacted third molars were extracted. The teeth were washed with sterile saline solution immediately after extraction for the removal of loosely adherent material. They were stored in sterile saline solution at 37°C for a maximum of 6 months to avoid dehydration.

One transverse notch was made at the cemento-enamel junction on the mesial and distal root surfaces of the premolars and on the buccal and lingual root surfaces of the molars. A second transverse notch was made 5 mm apical to the first. The cementum located between the two notches was removed with a high-speed handpiece and copious water irrigation. The freshly exposed dentin was then planed manually with an assortment of standard periodontal curettes (Hu-Friedy Co., Chicago, IL, USA). The root planing was performed by the same experienced operator. Two dentin specimens measuring 3 mm square and 1 mm thick were prepared from each tooth with a diamond saw (Isomet, Buehler®, Chicago, IL, USA) under continuous distilled water irrigation. A total of 30 dentin specimens were obtained and stored in distilled water at 4°C until required for treatment.

Experimental procedures

A total of 30 dentin specimens were randomly divided into six groups. The five specimens in each group were then treated as follows:

Group 1 (G1). Control group. Irrigation with 10 ml of physiologic saline solution.

Group 2 (G2). Topical application of 24% citric acid gel, pH 1, for 2 min, followed by irrigation with 10 ml of physiologic saline solution.

Group 3 (G3). Topical application of 24% EDTA gel at pH 7 for 2 minutes, followed by irrigation with 10 ml of physiologic saline solution.

Group 4 (G4). Topical application of a gel containing a mixture of 50% tetracycline HCl and 50% citric acid at pH 1 (Biotechnol, Bauru, SP, Brazil) for 2 min, followed by irrigation with 10 ml of physiologic saline solution.

Group 5 (G5). Irradiation with pulsed Er:YAG laser with wavelength of 2.94 μm (KaVo Key Laser, KaVo, Biberach, Germany) according to the following parameters: energy at 80 mJ as indicated on the display, resulting in transmitted energy of 47 mJ at the tip of the handpiece (#2056, transmission factor of 57%), repetition rate of 10 Hz for 15 s (total of 150 pulses), and fluency of each pulse 5.8 J/cm^2. The specimens were then immediately irrigated with 10 ml of physiologic saline solution.

Group 6 (G6). Irradiation with pulsed Er:YAG laser with wavelength of 2.94 μm (KaVo Key Laser, KaVo) according to the following parameters: energy at 140 mJ as indicated on the display, resulting in transmitted energy of 83 mJ at the tip of the handpiece (#2056, transmission factor of 57%), repetition rate of 10 Hz for 15 s (total of 150 pulses), and fluency of each pulse 10.3 J/cm^2. The

specimens were then immediately irrigated with 10 ml of physiologic saline solution.

In groups 2, 3 and 4 (G2, G3, G4), cotton pellets moistened with the chemical agent were placed over each dentin specimen without any rubbing motion for a total of 2 min.

Laser irradiation conditions

In G5 and G6 the dentin specimens were irradiated with a handpiece with a special application tip (1.65 mm× 0.5 mm). The laser was focused with the tip positioned perpendicular to, and in contact with, the surface of the dentin, in simulation of its use during actual periodontal surgery. The specimens were irradiated under continuous water irrigation by movement of the tip in a sweeping motion. The specimens were fixed in equipment specifically designed to receive the treatment. The irradiation was done manually in simulation of clinical conditions.

Preparation and analysis of specimens for SEM

After treatment, the specimens from all groups were fixed in 2.5% glutaraldehyde in a phosphate buffer (pH 7.3) for 24 h and then washed three times for 10 min each in the phosphate buffer. The specimens were then dehydrated in a graded series of aqueous ethanol solutions (50%, 70%, 85%, 95%, and 100% ethanol) for 10 min each. The samples were dried overnight at room temperature. They were mounted on SEM stubs and sputter-coated with a gold–palladium alloy under a vacuum (Balt-Tec SCD-050, Chicago, IL, USA) for 120 s. A central root surface section representative of each specimen was examined and photographed at ×3,000 magnification with a scanning electron microscope (XL 30, Phillips, Eindhoven, Netherlands).

Statistical analysis

A single blind evaluation of the SEM micrographs was conducted by three qualifieded examiners according to the rating system presented in Table 1. Smear layer removal scores were independently analyzed, with G1, G2, G3, G4, G5 and G6 considered as independent variables. We used the non-parametric Kruskal–Wallis test to compare the rank of the evaluated groups. This was followed by a non-parametric Mann–Whitney test when the Kruskal–Wallis test suggested a significant difference between groups ($P<0.05$). The statistical analysis was conducted with computer software (Bioestat, Manaus, Amazonas, Brazil).

Table 1 The rating system used to analyze the micrographs

Score	Content
1	No smear layer and open dentinal tubules
2	No smear layer and partially open dentinal tubules
3	No smear layer and obliterated dentinal tubules
4	Moderate smear layer and open dentinal tubules
5	Moderate smear layer and partially open dentinal tubules
6	Heavy smear layer and open dentinal tubules
7	Heavy smear layer and partially open dentinal tubules

Results

Group 1 (untreated control)

One specimen had a moderate smear layer and partially open dentinal tubules (score 5). The other four specimens had heavy smear layers with partially open dentinal tubules (score 7). Scratches caused by manual instrumentation were observed.

Group 2 (24% citric acid)

All specimens displayed open dentinal tubules. Three had no smear layer (score 1) (Fig. 1); the other two had moderate smear layers (score 4).

Group 3 (24% EDTA)

No smear layer and partially open dentinal tubules (score 2) were noted in three specimens, with a more regular surface than that of the controls. On the two remaining specimens, no smear layer and open dentinal tubules were found (score 1).

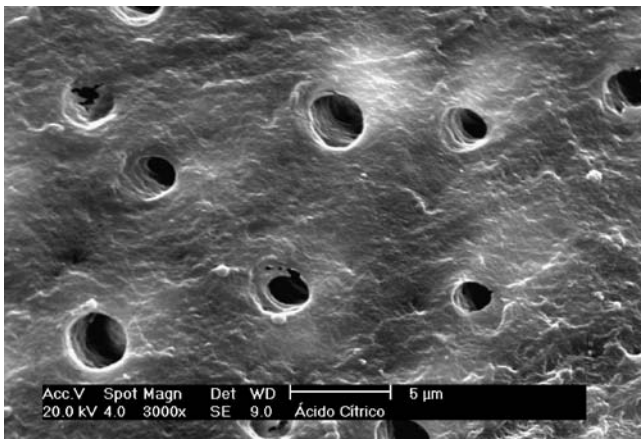

Fig. 1 Group 2 (citric acid gel): irregular surface, no smear layer and open dentinal tubules. ×3,000

Group 4 (mixture of 50% tetracycline HCl and 50% citric acid)

Four specimens had no smear layer and partially open dentinal tubules (score 2). The last specimen showed no smear layer and open dentinal tubules (score 1). All specimens had smooth, regular surfaces.

Group 5 (Er:YAG at 5.8 J/cm^2)

Four specimens displayed no smear layer and obliterated dentinal tubules (score 3) (Fig. 2). Only one had partially open dentinal tubules and no smear layer (score 2). Irregular surfaces were observed on all specimens, without cracking, fissuring, or carbonization.

Group 6 (Er:YAG at 10.3 J/cm^2)

All specimens presented with no smear layer and open dentinal tubules (score 1) (Fig. 3). Irregular surfaces were observed in all specimens, without cracking, fissuring, or carbonization. The specimens in this group were similar in appearance to those in group 5. However, only the specimens in this group had open dentinal tubules uniformly distributed throughout the irradiated area.

Data analysis

There were limitations in the statistical analysis, because this was a descriptive study comparing various treatments. We used analysis of variance (ANOVA) for our data, considering a 5% error to confirm the power of the study. Therefore, the results demonstrated that with a sample size of 3.96 ($P<0.05$) the power of the study would be 95%. Considering each group as an independent variable, the non-parametric Kruskal–Wallis test showed a significant difference among the evaluated groups regarding smear layer scores ($P<0.01$). The results of the statistical tests are depicted in Table 2. The non-parametric Mann–Whitney test demonstrated that groups 2, 3, 4, 5 and 6 exhibited significantly lower smear layer removal scores than did G1 (control group); G2, G3, G4 were not statistically different from G5 or G6; and G6 exhibited statistically significant lower smear layer removal scores than G5 did ($P<0.01$).

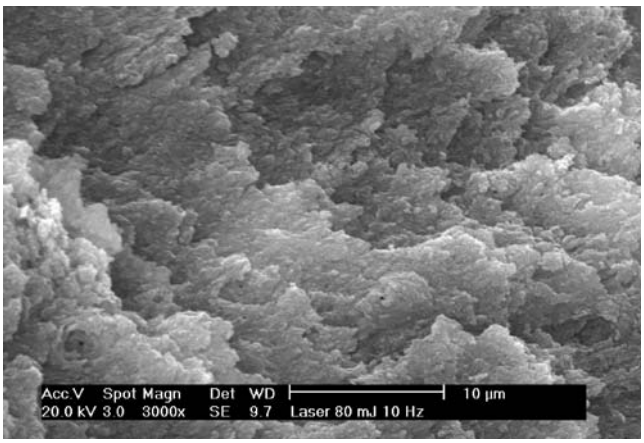

Fig. 2 Group 5 (Er:YAG laser, 5.8 J/cm^2 per pulse): irregular surface, no smear layer and obliterated dentinal tubules. ×3,000

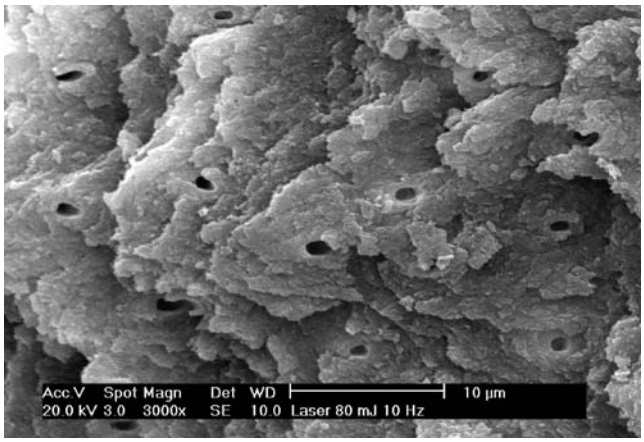

Fig. 3 Group 6 (Er:YAG laser, 10.3 J/cm^2 per pulse): irregular surface, no smear layer and open dentinal tubules. ×3,000

Discussion

The specimens in group 1, which had been treated by manual root planing alone, a showed heavy smear layer, in accordance with the findings of several previous studies [4, 24, 25]. All the groups that had undergone root surface modification treatment (G2, G3, G4, G5, and G6) in our study were effective in removing the smear layer after root planing when they were compared with the control group.

Table 2 Rank (Kruskal–Wallis test) and median values of smear layer removal score with comparison among the groups G1, G2, G3, G4, G5 and G6

Group	Median	Rank (20.31; $P<0.01$)[c]
G1 ($n=6$)	7	28.0
G2 ($n=6$)	1	13.4[a]
G3 ($n=6$)	1	11.7[a]
G4 ($n=6$)	2	13.6[a]
G5 ($n=6$)	3	20.3[a,b]
G6 ($n=6$)	1	6.0[a,b]

[a] Statistically significant differences between treatment and control groups ($P<0.01$; Mann–Whitney test)

[b] Statistically significant differences between groups ($P<0.01$; Mann Whitney test)

[c] Kruskal–Wallis test

The dentin specimens of G2, treated with 24% citric acid, showed complete smear layer removal and total exposure of the dentinal tubules (Fig. 1). These findings are corroborated by several in vitro studies [6, 8, 9, 26, 27], which have demonstrated the effectiveness of citric acid when used at the same concentration for root surface conditioning. Some in vivo studies [11, 12, 28] have demonstrated improved healing of the connective tissue attachment with the use of citric acid root surface conditioning. However, others [13] have not found any statistically significant difference when citric acid was used in conjunction with guided tissue regeneration surgery.

All dentin specimens in G3, treated with 24% EDTA, showed complete smear layer removal, which was in accordance with the findings of several other studies [7, 29, 30]. However, there was minimal change in the diameter of the dentinal tubules. That is, the diameters of the dentinal tubules of the specimens in G3 were smaller than those of the specimens in G2. However, Blomlöf et al. [7] have demonstrated that 24% EDTA gel is able to penetrate into the dentinal tubules and is more effective in exposing collagen fibers than is citric acid. The findings from G3 were similar to the ones from G4 specimens, which had been treated with a mixture of tetracycline and citric acid. High intensity lasers have been shown to be very effective at reducing the number of periodontal pathogens on the root surface [31, 32], and the Er:YAG laser has demonstrated a high bactericidal potential at low energy level [33].

Since removal of bacterial plaque is an important procedure in the treatment of periodontitis, treatment with the Er:YAG laser should be considered as a therapeutic option for the treatment of the root surface after scaling and root planing and to compensate for the limitations inherent in mechanical root surface therapy. Both of the laser irradiation energies we used were effective in smear layer removal. However, the fluency used on the specimens of G6 removed significantly more smear layer than that used on G5. The fluency used on G5 exposed dentin tubules in only one specimen, while all the others showed dentin tubules that had been totally obliterated (Fig. 2). On the other hand, all G6 specimens showed exposure of the dentinal tubules with no smear layer inside them (Fig. 3). It is possible to speculate that the energy applied to the specimens in G6 might also have exposed dentin collagen fibrils as a consequence of the thorough smear layer removal observed in this study. According to some authors [16, 24, 25], the exposure of the dentin collagen fibrils could favor the establishment of new connective tissue attachment.

The analysis of the micrographs in this study demonstrated that laser irradiation was effective in removing the smear layer, although it did cause irregularities on the root surface. Despite the presence of the irregularities, carbonization, craters, cracks and fractures were not observed, in accordance with findings in other studies [18, 19, 34].

The use of lasers in scaling and root planing has been controversial. Some studies using the neodymium:yttrium–aluminum–garnet (Nd:YAG) laser have found that it caused the formation of craters, cracks, and fissures, and brought about fusion and melting [35, 36], especially when used at a high energy level [37].

The possibility of fibroblast attachment on root surfaces treated by Nd:YAG laser depends on the amount of laser energy delivered or the laser–target distance [38].Therefore, the higher the delivered energy, the less appropriate the root surface will be for the attachment of collagen fibers.

Some papers [18, 19] have demonstrated that the Er:YAG laser has many advantages over the Nd:YAG laser, especially when used on mineralized tissues. Among the lasers that work by explosive ablation, the wavelength of Er:YAG is the most strongly absorbed by mineralized tissues. Among all lasers emitting in the near- and mid-infrared spectral range, the absorption of the Er:YAG laser in water is the greatest, because its 2.94 μm wavelength coincides with a large absorption band for water [19]. Therefore, the Er:YAG laser would ablate hard tissues containing some water more effectively and would cause less thermal damage to the adjacent tissues than either the Nd:YAG or the carbon dioxide (CO_2) laser [19]. The interaction mechanisms with the mineralized tissues have been described as photothermic, in the case of Nd:YAG and CO_2, and photomechanical in Er:YAG. The absorbed energy depends on the absorption coefficient of each molecule in the tissue. The Nd:YAG laser is not well absorbed by water or hydroxyapatite; thus, energy is transformed into heat, which increases the root surface temperature. One of the advantages of the use of the Er:YAG laser is that it is possible to remove calculus and a superficial layer of infected cementum without a significant increase in pulpal temperature, especially if irrigation is used [39]. Another advantage of this laser is that it is capable of removing smear layer without causing carbonization or formation of craters, as demonstrated by the results from G5 and G6 in our study. Smear layer removal may favor clot stabilization in the earliest stages of periodontal healing by increasing adhesion of blood cells and fibrin to the root surface [5] or even improving the retention and contact of substances, such as enamel matrix protein on the root surface. The advantage of the use of the Er:YAG to remove the smear layer is that it has a high bactericidal potential. Irradiation with Er:YAG laser produced a rather rough surface, but the relationship between root smoothness and wound healing remains ambiguous [40]. An increase in surface roughness could result in faster colonization and maturation of the dental plaque, supragingivally,

while the effect is less dramatic subgingivally, probably because this environment already offers more niches for bacterial adhesion and survival [40]. On the other hand, some studies [41–43] have shown an increase in fibroblast adhesion to root surfaces irradiated by Er:YAG laser that could be related to the smear layer removal or to the exposure of collagen fibers. Theodoro et al. [44] found adhesion of elements of blood on irradiated root surfaces with Er:YAG laser in vitro, but with no difference when compared with a control group [(conventional scaling and root planing (SRP)], showing that irradiation of root surfaces did not interfere with adhesion of blood clots. During Er:YAG laser irradiation, the power setting, pulse repetition, energy, exposure time, water cooling and fluency must be regulated appropriately so that detrimental effects to the root surface are avoided. Within the limits of this study, it can be concluded that all the treatment modalities were effective in smear layer removal. When either of the Er:YAG laser groups was compared with the groups that were treated with 24% acid citric gel, 24% EDTA gel, or a mixture of tetracycline HCl with 50% citric acid gel, no statistically significant differences in smear layer removal were observed when assessed by the Mann–Whitney test. However, when comparing the two Er:YAG laser groups, we observed that the fluency used for group 6 (10.3 J/cm^2) was statistically more effective in smear layer removal than the one used for group 5 (5.8 J/cm^2). Also, the root surfaces irradiated by Er:YAG laser demonstrated more irregular contours than the ones treated by chemical agents. The results of our study suggest that the Er:YAG laser can be safely used to condition diseased root surfaces effectively. Furthermore, the effect of Er:YAG laser irradiation on root surfaces should be evaluated in vivo so that its potential to enhance the healing of periodontal tissues can be assessed.

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