Braz Dent J (2006) 17(1): 15-19 ISSN 0103-6440

Molecular Analysis of Er:YAG Laser Irradiation on Dentin

Luís Eduardo Silva SOARES[1,2]
Aldo BRUGNERA JUNIOR[3]
Fátima ZANIN[3]
Marcos Tadeu Tavares PACHECO[4]
Airton A. MARTIN[1]

[1]*Laboratory of Biomedical Vibrational Spectroscopy, Research and Development Institute (IP&D), UNIVAP, São José dos Campos, SP, Brazil*
[2]*Discipline of Restorative Dentistry, Faculty of Dentistry, UNIVAP, São José dos Campos, SP, Brazil*
[3]*Dental Laser Center, Research and Development Institute (IP&D), UNIVAP, São José dos Campos, SP, Brazil*
[4]*Research and Development Institute (IP&D), UNIVAP, São José dos Campos, SP, Brazil*

The aim of this study was to evaluate by dispersive Raman spectroscopy the mineral and organic components of human dentin before and after laser irradiation and acid etching. The occlusal enamel of six non-carious human third molars was removed providing 6 dentin discs, which were divided in four quadrants each of them receiving a different surface treatment: etching with a 37% phosphoric acid gel (control); irradiation by Er:YAG laser (KaVo Key Laser II) with 80 mJ, 3 Hz, 30 s (group I); 120 mJ, 3 Hz, 30 s (group II); and 180 mJ, 3 Hz, 30 s (group III). The Raman spectra of normal (untreated) and treated dentin were analyzed and the mineral and the organic component were evaluated. Results were submitted to statistical analysis by ANOVA and Tukey's test at 5% significance level. The minerals and organic content were less affected in the control group and group I ($p>0.05$). Group II presented more reduction in mineral content ($p<0.01$) whereas in group III the inorganic ($p<0.05$) and organic ($p<0.01$) content were more affected. Dispersive Raman spectroscopy provided valid information of dentin chemical constituents with non-chemical sampling preparation.

Key Words: dentin, collagen, Er:YAG laser, dispersive Raman spectroscopy.

INTRODUCTION

Acid etching refers to application of a 37% phosphoric acid gel to tooth structure for 15 s followed by vigorous rinsing for the same time (1). Alternative types of dental surface treatment for prior to the adhesive protocol have been currently proposed among which Er:YAG laser irradiation, which can also be used for cavity preparation (1). The 2.94-micron wavelength of Er:YAG laser falls in an area of the electromagnetic spectrum where water and OH^- have their absorption peaks (2-4). According to Hibst and Keller (5), depending on energy output, Er:YAG laser irradiation of dental tissues can promote partial removal of dental substance upon water vaporization, microexplosions and ejection of both organic and inorganic tissue particles, thus creating a microretentive pattern on the lased surface. Enamel exhibits a crater-like appearance and dentin exhibits open dentinal tubules (1).

Several morphological and chemical studies have been performed in an effort to understand and obtain a clearer picture of the dentin-resin interface and demineralization mechanism. Due to the very small thickness of the hybrid layer, methods to analyze this effect must have a very high resolution and sensitivity. Raman micro-spectroscopy is a technique that fulfils both requirements and has been used to study the composition and structure of sample bonding (6-8). The acquired spectra are attributed to molecules rather than to single elements. Dehydration of the samples is not required and the measurements can be performed under room conditions (8).

Correspondence: Dr. Luís Eduardo Silva Soares, Laboratório de Espectroscopia Vibracional Biomédica, Instituto de Pesquisa e Desenvolvimento (IP&D), Universidade do Vale do Paraíba (UNIVAP), Av. Shishima Hifumi, 2911, 12244-000 São José dos Campos, SP, Brasil. Tel: +55-12-3947-1165. e-mail: lesoares@univap.br

The purpose of this *in vitro* study was to evaluate by dispersive near-infrared Raman spectroscopy, the chemical changes in the mineral and organic dentin contents after etching with conventional phosphoric acid or Er:YAG laser irradiation.

MATERIAL AND METHODS

Six human non-carious third molars were used in this study. The teeth were obtained from patients needing extractions as a part of their dental treatment plan, as approved by the Ethics in Research Committee of University of Vale do Paraíba (UNIVAP) (Protocol No. 01/14384-8). Soft tissue remnants were removed from tooth surface with periodontal curettes (7/8; Duflex, Rio de Janeiro, RJ, Brazil). The teeth were polished with water/pumice slurry (SS White, Rio de Janeiro, Brazil) in Robinson bristle brushes (Viking KG Sorensen, Barueri, SP, Brazil) in a low handpiece (KaVo do Brasil SA, Joinville, SC, Brazil.). The teeth were washed and stored in aqueous 0.1% thymol solution at 9°C for 1 week. Thereafter, the teeth were washed for 24 h with filtered water to eliminate thymol residues. To obtain dentin discs, the occlusal third of the crowns was sectioned perpendicular to the long axis of the teeth using a water-cooled Isomet slow-speed diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA) at 250 rpm and with 200 g load. Dentin surface was polished on wet #600-grit silicon carbide paper (3M, St. Paul, MN, USA) at 150 rpm in a polishing machine (Knuth-Rotor, Struers, Copenhagen, Denmark) under constant water cooling for 1 min to produce a standard smear layer (9,10). The specimens were ultrasonicated in an ultrasonic cleaner (Cole-Parmer 8891; Cole-Parmer Instrument Co., Vernon Hills, IL, USA) with distilled water for 5 min to remove excess debris, thorough washed and stored in saline at 9°C. The roots were removed with the water-cooled Isomet slow-speed diamond saw (Isomet 1000; Buehler) thus producing a 4-mm thick dentin disc *per* tooth. The discs were divided into for quadrants, each submitted to one of the dentin treatments shown in Table 1.

For Er:YAG laser irradiation, a reference point was made on the outer enamel buccal surface of the dentin discs with a # 2 diamond bur (KG Sorensen, Barueri, São Paulo, SP) at high-speed handpiece (KaVo do Brasil). KaVo Key Laser II Er:YAG laser device (KaVo, Biberach, Germany; $\lambda = 2.94$ μm; beam diameter = 1 mm) was used. The specimens were removed from saline storage and irradiated on non-contact mode with a #2051 handpiece at 12 mm focal distance, 2 mL/min waterflow and 15 J energy density. Care was taken for the laser beam not to reach the acid-etched control quadrant and enough space was left between each treated area. Er:YAG laser parameter settings are shown in Table 1. The control area was acid-etched and rinsed with air/water spray for 15 s (Table 1).

Dentin surfaces (quadrants) were analyzed by dispersive near-infrared Raman spectroscopy before and after acid or laser ethcing. Four spectra *per* each group area were collected before surface treatment. The procedure was repeated after dentin treatment and four spectra *per* each group area were accumulated as well. The samples were excited in the near infrared region by a Ti:Saphire laser (Model 3900S, Spectra-Physics, Mountain View, CA, USA; λ = 785 nm), pumped by an Argon laser (Stabilite 2017; Spectra-Physics, λ = 514 nm). Ti:Saphire laser output was limited to 80 mW in the sample holder. The spectral slit was set to 200 μm. A liquid nitrogen-cooled charge coupled device (CCD) detector collected the 96 Raman spectra of the dentin discs. For each measurement, 10 readings with 1-s integration time were recorded.

Averages of the 96 spectra were obtained from each group. The fluorescence for dispersive Raman data was removed by a polynomial fitting from the 24 average spectra and the relative peak areas were calculated by using the Microcal Origin software (Microcal Software, Inc., Northampton, MA, USA). The semi-quantitative evaluation of the changes in mineral and organic contents was done by calculating the relative intensity ratio of the peaks at 961 and 1670 cm^{-1} regarding to the 1046 cm^{-1} peak (7). This procedure is based on the assumption that the ratio of the integrated intensities of antisymmetric and symmetric stretching mode of phosphate ion should not change (9).

Raman spectroscopy results were analyzed

Table 1. Surface treatment groups.

Group	Surface treatment
Control	37% phosphoric acid - 15 s
I (Er:YAG Laser)	80 mJ, 3 Hz, 15 J, 190 pulses
II (Er:YAG Laser)	120 mJ, 3 Hz, 15 J, 128 pulses
III (Er:YAG Laser)	180 mJ, 3 Hz, 15 J, 86 pulses

statistically by one-way ANOVA at a 95% confidence level and Tukey-Kramer multiple comparison post-hoc test using GraphPad Instat software (GraphPad Software Inc., San Diego, CA, USA) to identify significant differences between normal and treated dentin data.

RESULTS

The dispersive Raman spectra provided information on chemical identity of both mineral and organic dentin components. Figure 1 shows the typical Raman spectrum of deep dentin surface. The stronger peak at 960 cm^{-1} is associated to the crystalline hydroxyapatite $Ca_{10}(PO_4)_6(OH_2)$ component. The peak at 1670 cm^{-1} (Fig. 1) is associated to the C=O vibrations of type I collagen (2).

For the 960 cm^{-1} intensity, non-statistical significance ($p>0.05$) were found between normal dentin (N) and treated dentin (T) in the control specimens (Fig. 1 A). The same result was found in group I ($p>0.05$) (Fig. 1 B). However, significant differences were found between normal dentin (N) and treated dentin (T) in group II ($p<0.01$) (Fig. 1 C). For group III, specimens treated with the laser energy of 180 mJ showed statistically significant reduction in the 960 cm^{-1} peak area ($p<0.05$) (Fig. 1 D).

Comparisons for the peak area at 1670 cm^{-1} (Fig. 1) showed no statistically significant difference ($p>0.05$) between normal dentin (N) and treated dentin (T) in the control group (Fig. 1 A). The specimens treated with the laser energies of 80 and 120 mJ also presented non-significant statistical differences ($p>0.05$) between normal and treated dentin (Figs. 1 B e 1C). The specimens irradiated by Er:YAG laser at 180 mJ presented relative peak area reduction for type I collagen component with statistically significant difference ($p<0.01$) (Fig. 1 D).

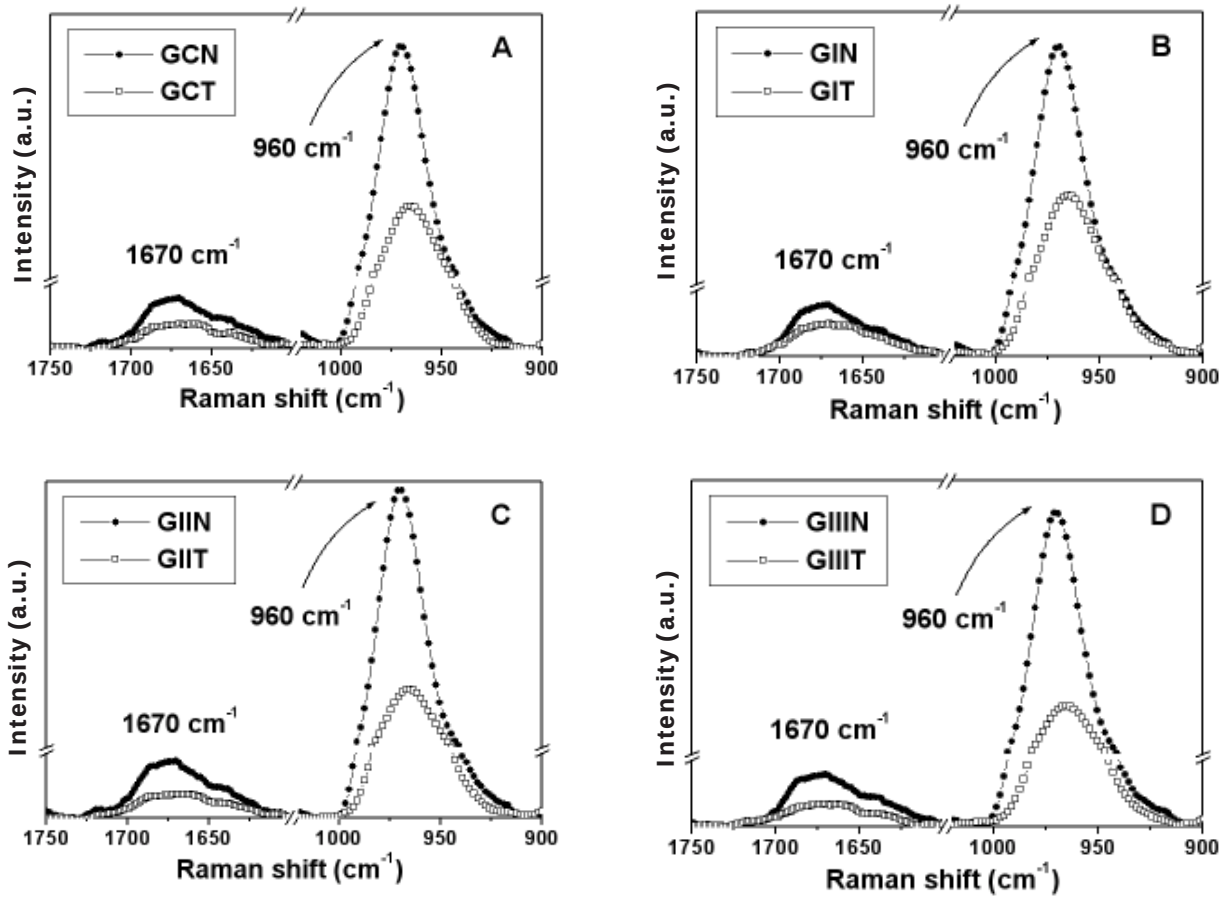

Figure1. Dispersive Raman spectra of the dentin specimens before (N =normal) and after (T= treated) surface treatment. A: control group (GC); B: group I (GI – 80 mJ, 3 Hz, 30 s); C: group II (GII – 120 mJ, 3 Hz, 30s); and D: group III (GIII – 180 mJ, 3 Hz, 30s) before (N – normal) and after (T – treated) surface treatment. The fluorescence signal was subtracted by polynomial fitting.

DISCUSSION

Transmission electron microscopy and tensile bond strength analyses have provided morphological and mechanical characterization of the lased dentin (1,4,10,11). However, there is a lack of chemical studies regarding the effects of Er:YAG laser irradiation on dentin structure at molecular level. In the present study, Er:YAG lased dentin was studied by the dispersive Raman spectroscopy, which is a non-destructive spectroscopic technique that provides accurate information on the chemistry of analyzed surfaces.

Transmission electron microscopic studies of dentin irradiated by Er:YAG laser with 180 mJ energy pulse and 2 Hz frequency (4) showed that the superficial portion of the laser-modified layer was composed of a scaly surface layer in which collagen fibrils were completely melted and vaporized. Along the basal portion of the laser-modified layer, the remaining denatured collagen fibrils were fused and poorly attached to the underlying dentin substrate. The presence of this fused layer resulted in lower shear bond strength values (4).

A previous study has shown that Er:YAG laser irradiation of dentin with 140 mJ energy pulse and 4 Hz frequency did not improve the tensile bond strength of Prodigy and Z100 restorative systems (1). In view of this, lower laser energies were selected for this study to assess their effects on dentin components. The frequency of 3 Hz was chosen because it was an intermediate frequency compared to those used in previous other studies (1,4).

It was observed by the Raman spectra, how different laser energies affect the tooth components that are involved in the adhesion process. Reductions in the inorganic and organic components were observed by the changes in the relative area of the peaks at 961 and 1670 cm^{-1}. Mineral content changes associated to the hydroxyapatite and changes in the collagen content, showed that the specimens irradiated with 180 mJ were more affected. The control areas (15-s phosphoric acid etching) and the areas irradiated by Er:YAG laser with 80 mJ (group I) had a more conservative etching, with less mineral and organic dentin content removal. Laser energy of 120 mJ (group II) showed intermediate results, affecting the mineral content in a greater extension than group I. Dentin treatment by Er:YAG laser with 180 mJ (group III) modified the hydroxyapatite and collagen content, with statistically significant difference from the other groups ($p<0.05$).

Dispersive Raman spectroscopy showed the changes that occurred in dentin upon Er:YAG laser irradiation. Specimen preparation is fairly simple as no specific dimensions or translucency requirements are necessary. Raman measurements can be carried out in normal atmospheric conditions with no need of high vacuum. Furthermore, because it is a non-destructive technique, specimens can be used for multiple analyses.

In this study, dispersive Raman spectroscopy showed that the mineral and organic dentin contents were less affected by acid etching and Er:YAG laser irradiation with 80 mJ. Pulse energy of 180 mJ produced greater reduction of organic and inorganic contents associated with more reduction in the peak areas at 961 and 1670 cm^{-1}.

RESUMO

O objetivo deste estudo foi avaliar por espectroscopia Raman, os componentes mineral e orgânico da dentina humana antes e após o condicionamento ácido e a irradiação com laser de Er:YAG. Seis discos de dentina foram obtidos de 6 terceiros molares humanos hígidos após remoção da superfície oclusal. Cada disco foi dividido em quatro regiões (quadrantes) de tratamento: condicionamento com ácido fosfórico a 37% (controle), irradiação com laser de Er:YAG (KaVo Key Laser II) com 80 mJ, 3 Hz, 30 s (grupo I); 120 mJ, 3 Hz, 30 s (grupo II) e 180 mJ, 3 Hz, 30 s (grupo III). Os espectros Raman da dentina normal e tratada foram analisados e os componentes mineral e orgânico foram avaliados. Os resultados foram submetidos a análise estatística pela ANOVA e teste de Tukey com intervalo de confiança de 95%. O conteúdo mineral e orgânico foi menos afetado nos grupos controle e I ($p>0,05$). O grupo II apresentou maior redução no conteúdo mineral ($p<0,01$) enquanto que a irradiação com laser Er:YAG 180 mJ (grupo III) reduziu mais o conteúdo inorgânico ($p<0,05$) e orgânico ($p<0,01$). A espectroscopia Raman forneceu informações dos conteúdos químicos da dentina sem preparação química dos espécimes.

ACKNOWLEDGEMENTS

This work was supported by IP&D (UNIVAP), FAPESP (Grant number 2001/14384-8) and CNPq (Grant number 302393/2003-0).

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Accepted November 10, 2005