

Effects of Er:YAG laser irradiation and manipulation treatments on dentin components, part 1: Fourier transform-Raman study

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1 Introduction

Recently, the demand for esthetic restorative dentistry has been increasing. New products and innovative surface treatments such as erbium-doped yttrium-aluminum-garnet (Er:YAG) laser irradiation were developed in an attempt to replace the traditional cavity preparation with drills and the acid etching of enamel and dentin, performed before the advent of adhesive procedures. The Er:YAG laser emits in the mid-infrared region ($\lambda=2.94 \mu\text{m}$), an area of the spectrum where dental tissues have absorption peaks (high absorptivity in water and hydroxyapatite).¹ The dentin is removed by

Abstract. The effects of laser etching, decontamination, and storage treatments on dentin components were studied using Fourier transform (FT)-Raman spectroscopy. Thirty bovine incisors were prepared to expose the dentin surface and then divided in two main groups based upon the decontamination process and storage procedure: autoclaved (group A, $n=15$) or stored in thymol aqueous solution (group B, $n=15$). The surfaces of the dentin slices were schematically divided into four areas, with each one corresponding to a treatment subgroup. The specimens were either etched with phosphoric acid (control subgroup) or irradiated with erbium-doped yttrium-aluminum-garnet (Er:YAG) laser (subgroups: I-80 mJ, II-120 mJ, and III-180 mJ, and total energy of 12 J). Samples were analyzed by FT-Raman spectroscopy; we collected three spectra for each area (before and after treatment). The integrated areas of five Raman peaks were calculated to yield average spectra. The areas of the peaks associated with phosphate content ($P<0.001$), type I collagen, and organic C-H bonds ($P<0.05$) were reduced significantly in group A (control). Analyses of samples irradiated with reduced laser energies did not show significant changes in the dentin components. These results suggest that thymol storage treatment is advised for *in vitro* study; furthermore, 12 J of Er:YAG laser energy does not affect dentin components. © 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3103285]

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thermomechanical ablation where the Er:YAG laser vaporizes its water content, which causes expansion followed by microexplosions.²⁻⁴ Laser-irradiated dentin showed a highly irregular surface, partially opened dentin tubules, and a scaly and flaky surface.⁴

The irradiation of superficial enamel or dentin by Er:YAG laser with lower energy densities prior to bonding procedures has previously been compared with results obtained by acid etching. Scanning electron microscopy images showed that the microstructure produced by laser irradiation is different from that produced by acid etching.^{4,5}

A consensus has not yet been reached regarding guidelines for the correct use of Er:YAG lasers for etching dentin.^{4,6-9} Some studies report that the adhesion strength of laser-irradiated dentin is lower than that of nonirradiated

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dentin.^{8,10,11} These results are due to the lower mechanical properties of dentin after laser irradiation.¹¹ Some authors have also discussed the possible denaturation of collagen fibrils.¹¹⁻¹³ Therefore, there is a need to study the effects of laser irradiation on dentin components by modifying irradiation parameters to determine the ranges which will inflict the least damage.

A significant issue that often does not receive much attention in the design of scientific studies is the type of decontamination method used for teeth studied *in vitro*. The chemical characteristics of teeth after specimen preparation need to be carefully considered. Incorrectly choosing a tooth decontamination method and storage procedure could change the structure of the dental element and the conclusions of the *in vitro* investigation.^{13,14}

Several methods of decontamination are used in scientific investigations. Among these methods, the autoclaving process (sterilization process with humid heat and pressure) is still used in some research groups to prepare the samples.¹⁵ This method is safe and less expensive.¹⁶ As steam autoclaving is available in dental clinics, it is the easiest method of sterilization. Regardless of its damaging effect on collagen,¹⁴ the autoclaving process is still used in some studies to prepare specimens.¹⁵ In some cases the treatment utilized for tooth manipulation is not mentioned, making reproduction of the study impossible.^{7,17,18} Sperandio et al. studied dental manipulation treatments such as gamma irradiation and reported that dentin bond strength was not affected by this treatment.¹⁹ However, Cheung et al. report that collagen molecules are changed by gamma irradiation.²⁰ White et al. used Fourier transform infrared (FTIR) spectroscopy to study the effects of gamma irradiation, ethylene oxide, dry heat, and autoclave sterilization on dentin components.²¹ The authors found a reduction in the phosphate peak after ethylene oxide and autoclave sterilization, indicating a loss of inorganic material on the dentin surface. The intensity of the collagen peak also decreased after autoclave sterilization. A slight increase in peak height for the collagen and inorganic components of the dentin was observed after dry heat sterilization, indicating loss of water on the surface. However, the FTIR spectra indicated no significant change in dentin components after gamma irradiation. Some studies present no firm conclusions and cite the need for more research.¹⁹ Parsell et al. demonstrated that steam autoclaving of sound dentin induced a change in the microhardness of samples, resulting in a softening of the organic matrix.²² This effect was probably due to heat and pressure that caused a disruption of collagen molecules and affected the ionic bonds between collagen and apatite crystals while the molecular structure of the organic matrix remained unaltered.²²

The alteration of collagen by heating was also discussed by Bachmann et al.²³ They used FTIR spectroscopy to examine the changes in collagen bands of heated and rehydrated dentin. It was observed that dentin collagen partially denatured in temperatures $\leq 175^\circ\text{C}$ and reverted to initial conformation after rehydration. At temperatures between 175 and 225°C partial denaturation occurred; at temperatures higher than 225°C , the collagen is denatured and no reversion is observed after rehydration. The water loss due to heating will reduce structural stability and may cause conformational changes. Therefore, the breakdown of hydrogen bonds is

likely primarily responsible for changing the collagen conformation. With decreased water content, the hydrogen bonds that determine collagen alpha-helix structural stability are lost. However, these bonds can be restored after rehydration.²³

This paper applies Raman spectroscopy to study the components of dentin after specimen decontamination and laser etching. Spectroscopy analysis was chosen because it is a nondestructive analytical method with minimal sample preparation required. Furthermore, Raman spectroscopy provides information about the chemical state of the sample without causing damage.^{4,13,18} This technique permits the structural analysis of samples by identifying specific light-induced molecular vibrations,^{24,25} e.g., the vibrational modes of PO_4^{3-} , OH^- , HPO_4^{2-} , and CO_3^{2-} .²⁶ In addition, the relative intensities of Raman bands allow semiquantitative estimations of these constituents.^{17,27}

Previous reports have evaluated Er:YAG laser irradiation effects by Raman spectroscopy.^{18,28} However, the former studies evaluated only the changes in the intensity of the spectra after laser irradiation used to prepare dental cavities. The influence of tooth sterilization methods has been evaluated by analytical tools such as Fourier transform infrared spectroscopy (FTIR),²¹ a bond strength study,¹⁹ and Raman spectroscopy.¹³ However, there is still a lack of information regarding the effects of these manipulation treatments on dentin components.

This study aims to use FT-Raman spectroscopy to investigate the effects on dentin components of Er:YAG laser etching with modified parameters. In addition, the effects of the decontamination process and storage treatment on dentin components were also studied.

2 Materials and Methods

2.1 Specimen Preparation

This study was approved by the Ethics Committee of the University of Vale do Paraíba (A073/CEP/2007). Thirty bovine incisor teeth were obtained from bovine jaws. All specimens were stored in saline solution (Aster Produtos Médicos LTDA, Sorocaba, SP, Brazil) at 9°C prior to use. After extraction, the remaining soft tissue was removed from the tooth surface with a dental scaler (7/8; Duflex, Rio de Janeiro, RJ, Brazil). The teeth were polished with a paste of pumice (S. S. White, Rio de Janeiro, RJ, Brazil) and water, using a Robinson brush (Viking—KG Sorensen, Barueri, SP, Brazil) in a low-speed hand-piece (KaVo do Brazil SA, Joinville, SC, Brazil).^{4,13}

After the cleaning procedure, teeth were divided into two groups, according to the manipulation treatment (sterilization or storage). Group A consisted of fifteen teeth which were autoclaved at 121°C for 15 min (Biodont—Alpax, Brazil) in a flask filled with sterile saline (Farmavale & Cia—LBS Laborasa Ind. Farm., Ltd., Brazil), which was closed tightly and stored at 9°C .¹³ Group B was comprised of fifteen teeth stored in 0.1% thymol aqueous solution at 9°C for one week. To prepare the dentin specimens, the teeth were washed for 24 h with filtered water to eliminate thymol residues.^{4,13}

The buccal enamel surface was removed with a water-cooled low-speed diamond disk at 250 rpm with a 100 g load. The surface was ground for 1 min with wet 600-grit

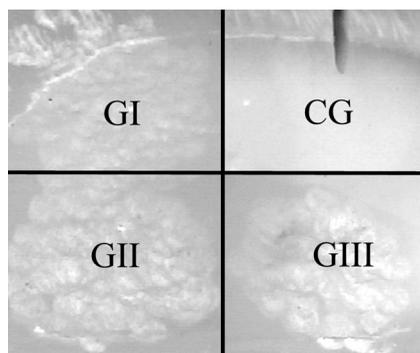


Fig. 1 Digital image of dentin-treated areas.

silicon carbide paper at 150 rpm to expose the dentin layer and to produce a smooth surface.^{4,13} Ultrasonic cleaning (Maxiclean 1450, Merse, Campinas, SP, Brazil) with distilled water was performed for 5 min in order to remove excess debris and the smear layer. The specimens were then stored in saline solution in a refrigerator at 9 °C for one week.

2.2 Surface Treatment

A reference point was created with a diamond disk in the proximal enamel of the samples with a low-speed hand-piece (KaVo do Brazil SA, Joinville, SC, Brazil). The specimens' surfaces were schematically divided into four areas (Fig. 1), and each area of the sample received a different treatment, generating four subgroups as described in Table 1.

Specimens were removed from the saline solution. Laser irradiation was performed in noncontact mode by an Er:YAG laser (KaVo Key Laser II, Germany, $\lambda=2.94 \mu\text{m}$, beam diameter=1 mm) with a no. 2051 hand-piece at a focal distance of 12 mm, with cooling water spray (20 mL/min) and a total energy value of 12 J. Irradiation of the control group quadrant was avoided and a visual distance was maintained between the sides of each quadrant. After the irradiation procedure, acid etching was performed in the control group area

Table 1 Description of group treatments.

Groups	Manipulation process
Group A	Autoclaving 121 °C (15 min, with pressure of 1.1 kgf/cm ²)
Group B	0.1% aqueous thymol solution (one week)
Subgroups	Surface treatments
Control group (CG)	37% phosphoric acid (15 s)
Group I (GI)	Er:YAG laser (80 mJ, 3 Hz, 12 J, 153 pulses)
Group II (GII)	Er:YAG laser (120 mJ, 3 Hz, 12 J, 103 pulses)
Group III (GIII)	Er:YAG laser (180 mJ, 3 Hz, 12 J, 70 pulses)

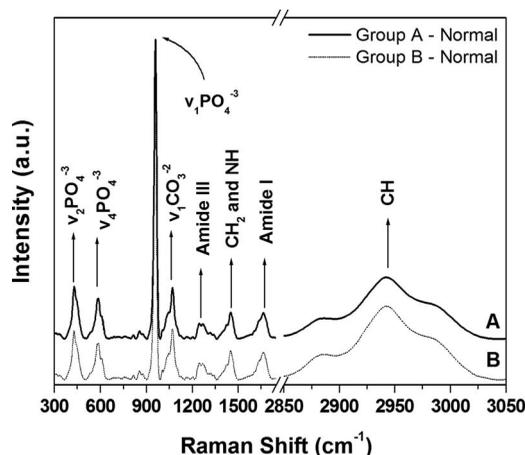


Fig. 2 FT-Raman spectra from experimental groups undergoing decontamination or storage treatments (group A, black line: autoclaved; Group B, dotted line: thymol-treated) in the 300–3050 cm⁻¹ range, after normalization, displaying the main vibrational modes of inorganic and organic dentin components.

using 37% phosphoric acid gel (FGM, Brazil) for 15 s. The etched surface was then rinsed with an air–water spray for 15 s.

2.3 FT-Raman Spectroscopy Analysis

The dentin surfaces were analyzed by FT-Raman spectroscopy before and after treatment. The FT-Raman spectrometer (RFS 100/S®—Bruker, Inc., Karlsruhe, Germany) with a germanium detector cooled by liquid N₂ was used to collect the data. The samples were excited by an air-cooled Nd:YAG laser ($\lambda=1064.1 \text{ nm}$).

The power of the Nd:YAG laser incident on the sample was 100 mW. The spectral resolution was set to 4 cm⁻¹, and for each area (before and after treatment) three spectra were collected with 100 scans, totaling 720 spectra.⁴

Before treatment, the averages of three spectra per treatment area were calculated, resulting in 240 spectra. For the qualitative and semiquantitative spectral analysis, the average spectra were baseline corrected and then normalized using the 960-cm⁻¹ peak.^{26,29,30} The changes of organic and inorganic dentin components were analyzed by comparing the integrated areas of the Raman peaks centered at 434 cm⁻¹ (p1), 1103 cm⁻¹ (p2), 1667 cm⁻¹ (p3), and 2940 cm⁻¹ (p4) to the peak at 1071 cm⁻¹ (p5). The integrated areas of the peaks were calculated by Microcal Origin5.0® software (Microcal Software, Inc., Northampton, MA, USA).

2.4 Statistical Analysis

The measurements obtained from the integrated area under the Raman peaks (p1–p5) were statistically analyzed using InStat software (GraphPad Software, Inc., San Diego, CA, USA). The one-way ANOVA test at a 95% confidence level and the Tukey–Kramer multiple comparisons test were applied to test the significance of the relative area evaluation between the normal and treated dentin data. Statistical analyses were initially performed using the difference between nor-

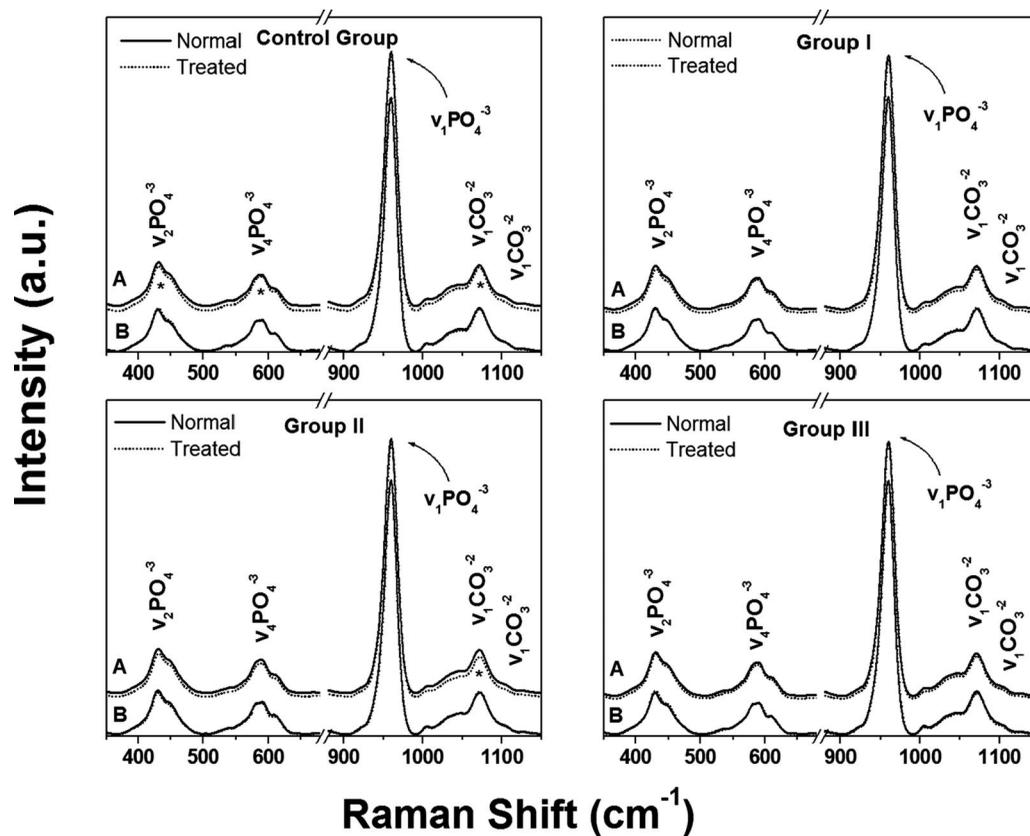


Fig. 3 Dentin Raman spectra in the 300–1200 cm^{-1} range, with inorganic peaks (p1: 430 cm^{-1} , p2: 1104 cm^{-1} , and p5: 1071 cm^{-1}) of autoclaved (A) and thymol-treated (B) specimens. The black lines correspond to the Raman spectrum before treatments and dotted lines correspond to the spectra collected after the following treatments: acid etching (control group—CG) and Er:YAG laser irradiation with 80 mJ (GI), 120 mJ (GII), and 180 mJ (GIII) of laser energy. We used an average of three acquisitions per area for each sample in group A (15 samples) and group B (15 samples). An asterisk denotes a clear reduction in spectral intensity after treatment.

mal and treated values of the relative area. Comparisons between the groups were also performed considering only the treated values.

3 Results and Discussion

Figure 1 shows a macro morphological aspect of dentin-treated areas obtained by a digital image. Visually the group III area is more irregular than the other areas. Figure 2 shows that the main features of the Raman scattering of dentin were consistent with previous results.^{4,13,17,26–28} In the raw Raman spectra of untreated specimens in both decontamination groups, the strongest peak at 960 cm^{-1} arose from $\nu_1\text{PO}_4^{3-}$. The peak at 580 cm^{-1} and its shoulder at 610 cm^{-1} were assigned to $\nu_4\text{PO}_4^{3-}$ vibrations. The peaks at 1245 and 1450 cm^{-1} are related to the amide III and C–H bending vibrations.^{4,13,17,26–28}

The selected range of Raman spectra from the inorganic and organic components of dentin are shown in Figs. 3 and 4, respectively. The Raman spectrum has been vertically shifted for clarity. In Fig. 3, the peak at 1071 cm^{-1} is attributed to type B carbonate ($\nu_1\text{CO}_3^{2-}$) vibrations.^{4,13,17,26} The peaks at 430 and 1104 cm^{-1} are related to $\nu_2\text{PO}_4^{3-}$ and $\nu_1\text{CO}_3^{2-}$ type A modes of phosphate and carbonate, respectively.^{4,13,17,26} In

Fig. 4, the bands at 1665 and 2940 cm^{-1} are attributed to the organic components of dentin, i.e., amide III and CH_2 vibrations, respectively.^{4,13,17,26}

Except for the absolute change in the intensity of the Raman peaks, no other effect was observed in the spectrum. The Raman spectra of inorganic components showed a reduction in the intensity after treatment for group A_{CG} (Fig. 3). With regard to the organic component in the A_{III} group, the relative intensity of CH bonds decreased more than in the other groups (Fig. 4).

Analyses of the integrated areas of the Raman peaks related to inorganic and organic components are shown in Tables 2 and 3, respectively. Significant changes in inorganic material were found in group A specimens. The integrated area of the peak centered at 430 cm^{-1} (p1) was significantly reduced for the specimens of group A_{CG} ($P < 0.001$). However, for the same peak in all group B specimens, the integrated area showed no significant change after surface treatment. For the peak centered at 1104 cm^{-1} (p2), the integrated areas remained unchanged after treatment for both group A and group B (Table 2).

Raman data analysis of the dentin organic components (1665 and 2940 cm^{-1}) showed the most significant changes in group A specimens. The integrated area of the peak centered

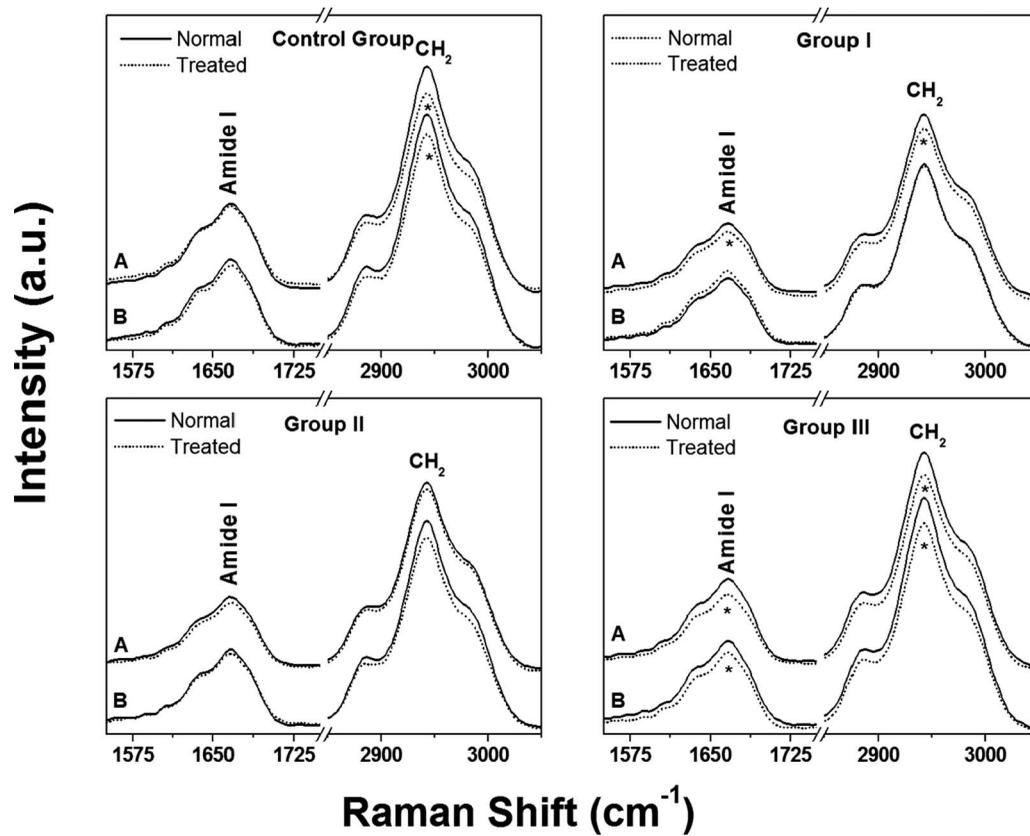


Fig. 4 Dentin Raman spectra in the 1500–3100 cm^{-1} range, with organic peaks at (p3: 1665 cm^{-1} and p4: 2940 cm^{-1}) of autoclaved (A) and thymol-treated (B) specimens. The black lines correspond to the Raman spectrum before treatments and dotted lines correspond to the spectra collected after the following treatments: acid etching (control group—CG) and Er:YAG laser irradiation with 80 mJ (GI), 120 mJ (GII), and 180 mJ (GIII) of laser energy. We used an average of three acquisitions per area for each sample in group A (15 samples) and group B (15 samples). An asterisk denotes a clear reduction in spectra intensity after treatment.

at 1665 cm^{-1} (p3) was reduced in all treatment groups. Comparing the normal and treated areas after treatment revealed a significant reduction in this peak for groups A_{CG} ($P < 0.05$) and A_{GIII} ($P < 0.001$) (Table 2). For the peak centered at 2940 cm^{-1} (p4), a significant statistical reduction in the integrated area was observed after treatment in group A_{CG} ($P < 0.05$) (Table 3).

Our study used FT-Raman spectroscopy to evaluate two dental manipulation/storage methods and the effects of Er:YAG laser etching on dentin components. The results suggest that the autoclaving process significantly changed the inorganic and organic components of dentin as compared to the uses of thymol after dentin etching. Dentin components such as phosphate ($\nu_2\text{PO}_4^{3-}$ vibrations), amide I, and CH bonds were probably affected by the heat and pressure of the autoclaving treatment (121 °C and 1.1 kgf/cm^2 , respectively), as evidenced by significant relative area reduction. These results should be considered when the autoclaving treatment is chosen for specimen sterilization; the method can be inadequate for tooth preparation, modifying dentin structures required for the adhesion process.

The second evaluation focused on Er:YAG laser-etching of dentin. Previous studies with Raman spectroscopy of dentin laser etching showed that the inorganic and organic content of dentin were more drastically affected by higher energy den-

sity values (15.3 and 22.9 J/cm^2).^{4,13} Considering these results, the pulse laser energies in the present study were modified for a new evaluation of laser irradiation effects on dentin.

Table 2 Mean and standard deviation of integrated areas of dentin inorganic Raman peaks before (normal) and after (treated) treatment.

Group	430 cm^{-1} / 1071 cm^{-1}		1104 cm^{-1} / 1071 cm^{-1}	
	Normal	Treated	Normal	Treated
A _{CG}	2.42 (0.3) ^a	1.92 (0.2) ^a	0.38 (0.1)	0.38 (0.1)
A _{GI}	1.89 (0.2)	1.84 (0.1)	0.36 (0.1)	0.34 (0.1)
A _{GII}	1.90 (0.2)	1.83 (0.1)	0.31 (0.1)	0.30 (0.1)
A _{GIII}	1.92 (0.2)	1.96 (0.2)	0.34 (0.1)	0.35 (0.1)
B _{CG}	1.84 (0.3)	1.92 (0.2)	0.92 (0.2)	0.92 (0.2)
B _{GI}	1.91 (0.3)	2.00 (0.2)	0.97 (0.3)	0.92 (0.2)
B _{GII}	1.74 (0.2)	1.86 (0.1)	0.93 (0.2)	0.96 (0.3)
B _{GIII}	1.86 (0.2)	1.90 (0.2)	0.98 (0.2)	0.98 (0.3)

^aStatistically significant difference between normal and treated data (by row).

Table 3 Mean and standard deviation of integrated areas of dentin organic Raman peaks before (normal) and after (treated) treatment.

Group	1665 cm ⁻¹ /1071 cm ⁻¹		2940 cm ⁻¹ /1071 cm ⁻¹	
	Normal	Treated	Normal	Treated
A_CG	1.18 (0.3) ^a	0.94 (0.2) ^a	2.68 (0.4) ^a	2.30 (0.5) ^a
A_GI	1.04 (0.1)	0.86 (0.1)	2.16 (0.3)	1.97 (0.4)
A_GII	0.97 (0.2)	0.79 (0.1)	2.16 (0.3)	2.14 (0.3)
A_GIII	1.22 (0.2) ^a	0.88 (0.3) ^a	2.58 (0.4)	2.31 (0.3)
B_CG	1.10 (0.2)	1.12 (0.2)	2.97 (0.6)	2.60 (0.4)
B_GI	0.95 (0.2)	0.92 (0.2)	2.44 (0.5)	2.25 (0.2)
B_GII	0.83 (0.2)	0.85 (0.2)	2.35 (0.6)	2.17 (0.4)
B_GIII	1.10 (0.2)	1.05 (0.2)	2.72 (0.7)	2.44 (0.4)

^aStatistically significant difference between normal and treated data (by row). The different letters indicate statistically significant differences between treatment groups (by column).

Total energy was set to 12 instead 15 J. We substituted human teeth with bovine specimens due to the difficulty of obtaining human teeth for research and the similarity of bovine specimens.

The evaluation of the effects of acid or Er:YAG laser-etching demonstrated that those treatments produced significant changes only in specimens that were autoclaved prior to treatment. Studying the relative areas of two inorganic and two organic peaks allowed qualitative and semiquantitative analyses of the dentin response to tooth decontamination and to Er:YAG laser-etching treatment.

The significant changes in dentin collagen caused by acid-etching in autoclaved specimens could result from a softening of the dentin matrix mediated by heat and pressure (121 °C and 1.1 kgf/cm²). For group A_GIII, these changes were probably caused by laser thermal effects in addition to the heating effect of autoclave treatment. For group A_CG, the results are in agreement with previous studies obtained by dispersive Raman spectroscopy.¹³

Raman spectroscopy showed that the surface treatments did not significantly alter the dentin components in the specimens previously treated with thymol solution. Fewer studies have used Raman spectroscopy to evaluate Er:YAG laser irradiation effects on enamel and dentin.^{4,13,18,28} Carmelino et al.²⁸ reported changes limited to the spectra intensity caused by high Er:YAG laser energies and frequencies used in cavity preparation, as compared to traditional preparation using diamond drills. Yamada et al.¹⁸ also showed changes limited to peak intensity and luminescence after Er:YAG laser irradiation. Our study, however, showed the comprehensive results of an integrated area evaluation of inorganic and organic dentin peaks before and after Er:YAG laser treatment.

The heating due to autoclave treatment and laser irradiation may have broken the collagen bonds of dentin irradiated with Er:YAG laser pulses, as suggested by the largest reduction in peak area to be observed in the present study.

4 Conclusions

The results of this study suggest that thymol storage treatment is advised for the treatment of specimens to be used for *in vitro* study. The use of 12 J of Er:YAG laser energy does not affect the dentin components in specimens treated with thymol. After acid etching, the collagen component showed significant changes in response to autoclave treatment, as evidenced by the decrease in the integrated areas of the peaks ascribed to the organic matrix (1670 and 2940 cm⁻¹). Raman measurements of samples irradiated with reduced laser energies showed that laser etching did not produce significant changes in phosphate, carbonate, or collagen dentin components in specimens treated with thymol.

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