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Abstract. The effects of laser etching on dentin are studied by microenergy-dispersive x-ray fluorescence spectrometry (μ -EDXRF) and scanning electron microscopy (SEM) to establish the correlation of data obtained. Fifteen human third molars are prepared, baseline μ -EDXRF mappings are performed, and ten specimens are selected. Each specimen received four treatments: acid etching (control-CG) or erbium:yttrium–aluminum–garnet (Er:YAG) laser irradiation (I—100 mJ, II—160 mJ, and III—220 mJ), and maps are done again. The Ca and P content are significantly reduced after acid etching ($p < 0.0001$) and increased after laser irradiation with 220 mJ (Ca: $p < 0.0153$ and P: $p = 0.0005$). The Ca/P ratio increased and decreased after CG ($p = 0.0052$) and GI ($p = 0.0003$) treatments, respectively. CG treatment resulted in lower inorganic content (GI: $p < 0.05$, GII: $p < 0.01$, and GIII: $p < 0.01$) and higher Ca/P ratios than laser etching (GI: $p < 0.001$, GII: $p < 0.01$, and GIII: $p < 0.01$). The SEM photomicrographs revealed open (CG) and partially open dentin tubules (GI, GII, and GIII). μ -EDXRF mappings illustrated that acid etching created homogeneous distribution of inorganic content over dentin. Er:YAG laser etching (220 mJ) produced irregular elemental distribution and changed the stoichiometric proportions of hydroxyapatite, as showed by an increase of mineral content. Decreases and increases of mineral content in the μ -EDXRF images are correlated to holes and mounds, respectively, as found in SEM images. © 2013 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.18.6.068001]

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1 Introduction

Etching of dentin with phosphoric acid is the most traditional mechanism to remove the smear layer (accumulated layer of mechanically polished debris) and to partially demineralize dentin and open the dentinal tubules. This application exposes the collagen layer enhancing the adhesive systems to flow through the demineralized layer, creating the micromechanical retention of composite resin restorations in the microporosities. However, acid etching may make dentin more permeable, and it may be not completely filled with adhesive.¹ Therefore, different strategies, such as erbium:yttrium–aluminum–garnet (Er:YAG) laser irradiation, have been proposed to prepare dentin to enhance the adhesion to tooth tissues.

The Er:YAG laser wavelength of 2.94 μm is highly absorbed by both water and hydroxyapatite, and has great applicability in dental hard tissues.² Absorbed laser pulses give rise to micro-explosions on the dentin surface, so that dental hard tissue

can be effectively removed.³ A number of studies have been performed to verify the use of Er:YAG laser for dentin etching.^{4–11}

The understanding of Er:YAG laser mechanism of interaction with dentin is essential since the adhesion of composite resin could be altered by laser irradiation.^{6,11} Previous investigations on the effects of erbium laser irradiation on dentin and bonding procedures are somewhat controversial. Staninec et al.¹² achieved similar values of shear bond strength with Er:YAG conditioning in comparison with acid etch. Earlier studies on enamel and dentin substrates showed that Er:YAG laser associated with acid etching or self-etching primers produced bond strength values comparable with those achieved with acid etching or self-etching primers.^{13,14} Soares et al.¹¹ found by energy-dispersive x-ray fluorescence spectrometry (EDXRF) mappings that the Er:YAG laser energy of 180 mJ produced a localized increase in Ca and P content on the superficial layer of the dentin (~ 0 to 0.10 mm), which was different from the profile produced by acid etching and laser energies of 80 and 120 mJ, respectively.

Moretto et al.⁶ evaluated the dentin morphology and micro-tensile bond strength of irradiated tissue, and found that the

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erbium laser interacts with the dental hard tissue resulting in a specific morphological pattern of dentin and collagen fibrils that negatively affected the bond strength of composite resin. They found an irregular hybrid layer in the irradiated groups with a greater adhesive deposition in the regions of valleys (bottom of the pulse), while the peaks were covered with a thinner adhesive layer.

Energy dispersive spectroscopy has been used associated with scanning electron microscopy (SEM) analysis to study the effects of dentin irradiation with Er:YAG¹⁵⁻¹⁷ or with Er, Cr:YSGG laser.^{18,19} Also, microenergy-dispersive x-ray fluorescence spectrometry (μ -EDXRF) analysis has been used in previous studies for the same purpose.^{10,11} However, despite chemical and morphological information provided by those previous studies, none of them correlated the chemical data to morphological changes.

Micro x-ray fluorescence is used to obtain the chemical composition of a sample without any specific sample preparation such as the gold coating that is desired for high-resolution images in SEM analysis. Also, the specimens prepared for SEM analysis are coated with carbon to prevent charging. Imaging using μ -EDXRF is nondestructive, and samples can be visualized several times. Moreover, physical or chemical fixations as well as coating of surfaces by sputtering for having a better contrast and conductivity are not necessary. Using the standard procedure for preparation of dentin samples in SEM, a sample is dehydrated in graded acetone series, dried, and coated in sputtering device after its fixation. The occurrence of artifacts caused by shrinking cannot be eliminated in practice, because dentin is strongly sensitive to dehydration.²⁰ Thus the μ -EDXRF analysis became an attractive alternative to evaluate chemical changes on teeth after laser application.

The null hypotheses tested were that there would be differences in composition of inorganic phase (Ca and P) of dentin surfaces prepared using different Er:YAG laser irradiation parameters compared with acid etching. The second aim of the present study was to correlate morphological features obtained by SEM images with the elemental data obtained by μ -EDXRF mappings of dentin inorganic content.

2 Materials and Methods

2.1 Specimen Preparation

This study was approved by the Ethics Committee of the Universidade do Vale do Paraíba (H146/CEP/2009). The specimens were prepared from 15 randomly selected, erupted, non-carious human third molars. The teeth used were recently extracted (less than 3 months) from patients, where extractions of third molars were part of their dental treatment (for periodontal or orthodontic reasons). The teeth were selected according to the following criteria: intact buccal enamel surfaces without developmental defects, cracks, caries, restorations, or fluorosis.

All the specimens were stored in saline solution (Aster® Produtos Médicos LTDA, Sorocaba, SP, Brazil) at 9°C just after extraction to help clean the organic debris.²¹ 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 pumice (S. S. White®, Rio de Janeiro, RJ, Brazil) and water paste using a Robinson brush (Viking-KG Sorensen®, Barueri, SP, Brazil) on a low-speed hand-piece (KaVo® do Brasil SA, Joinville, SC, Brazil), then cleaned and stored in a 0.1% thymol solution

for 1 week. To prepare the dentin specimens, the teeth were washed for 24 h with filtered water to eliminate thymol residues.^{9,10}

After the cleaning procedure, the occlusal one third of the crowns of the samples were sectioned perpendicular to the long axis of the teeth by means of a water-cooled low-speed diamond disc at 250 rpm and with a 100 g load (Isomet 1000—BUEHLER, Lake Bluff, Illinois) as previously described.^{7,8} The surface was ground for 1 min with wet 600-grit silicon carbide paper at 150 rpm to expose the superficial dentin layer and to produce a smooth surface.⁹ Roots were removed with a water-cooled low-speed diamond disc producing one dentin disc-shaped slice with 4 mm thickness for each tooth. All slices were obtained approximately from the same tooth depth.

A reference point was created with a diamond bur (#2—KG Sorensen) in the buccal enamel of the samples [Fig. 1(a)] with a high-speed turbine (KaVo). Ultrasonic cleaning (Maxiclean 1450, Merse, Campinas, SP, Brazil) with distilled water was performed for 5 min, in order to remove excess debris and smear layer. The specimens were then stored in saline solution in a refrigerator at 9°C for 1 week. The sliced surfaces were schematically divided in four areas, corresponding to each treatment group [Fig. 1(a)]. Every specimen received all the studied treatments in order to avoid individual biochemical differences between teeth and to standardize the surface to be treated. Each quadrant of the specimen received a different treatment generating four treatment groups, as described in Table 1.

2.2 Specimen's Selection by μ -EDXRF Mapping Measurements

To eliminate individual variations of each tooth regarding chemical composition, a baseline analysis of Ca and P distribution was performed on 15 selected third molars. The semiquantitative elemental analysis of calcium (Ca) and phosphorus (P) content were done using an μ -EDXRF model μ -EDX 1300 (Shimadzu® Corp., Kyoto, Japan). The equipment calibration and chemical balance were performed as previously reported.^{10,21}

Elemental distribution maps ($n = 60$) were performed on four areas of each human dentin specimen. The maps were scanned covering a selected area of 40×30 points [Fig. 1(b)] with steps of $20 \mu\text{m}$. The energy range of scans was 0.0 to 40.0 eV. The voltage in the tube was set at 15 kV with automatic adjustment of the current and incident beam diameter of $50 \mu\text{m}$.

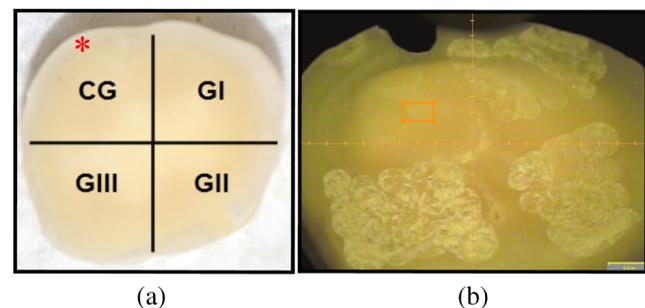


Fig. 1 (a) Exposed superficial dentin layer of third molar with a reference point (red asterisk). The dentin disc-shaped slices obtained had the surfaces schematically divided into four areas (CG, GI, GII, and GIII). (b) Characteristic of dentin surface after acid etching and laser irradiation showing the limits of area mapping (bordered square).

Table 1 Description of the experimental treatments and parameters.

Groups	Surface treatments
Control group	37% phosphoric acid (15 s)
Group I	Er:YAG laser (100 mJ, 10 Hz, 3 s)
Group II	Er:YAG laser (160 mJ, 10 Hz, 3 s)
Group III	Er:YAG laser (220 mJ, 10 Hz, 3 s)

After mappings, the data of each specimen were processed by the equipment software (Shimadzu® microEDX MP ver. 1.03, Shimadzu Corp.). The software provided the average values for each component. After that, five specimens, which showed irregular chemical distribution of Ca and P throughout dentin surface, were excluded. Next, the 10 selected specimens were submitted to four experimental treatments (Table 1).

2.3 Surface Treatment

Specimens were removed from the saline solution and laser irradiation was performed in a noncontact mode by an Er:YAG laser (Twin Light, Fontana Medical Lasers, Slovenia, $\lambda = 2.94 \mu\text{m}$) at a focal distance of 15 mm with a cooling water spray (20 mL/min). This laser system operates from 200 to 450 μs of pulse duration. Irradiation of the control group quadrant was avoided, and a visual distance was maintained between the sides of each group. The pulse repetition rate of 10 pulses per second (10 Hz) was used, and three different radiant pulse energy parameters were selected (Group I: 120 mJ; Group II: 160 mJ; and Group III: 220 mJ). Laser irradiation was performed in a sweeping motion, and by this way, 3 s was the time to irradiate the whole target area of the specimen. After the irradiation procedure, acid etching was performed on the control group area using 37% phosphoric acid gel (FGM, Joinville, SC, Brazil) for 15 s. The etched surface was then rinsed with an air-water spray for 15 s. To prevent the flow of phosphoric acid onto the areas allocated for groups I, II, and III, acid etching was performed with gel, and the rinsing procedure was done with the specimen tilted in the opposite

direction to the irradiated areas, thus avoiding contamination with acid during the rinsing procedure.^{7,8} μ -EDXRF area mappings ($n = 40$) were performed again to determine the mineral content after dentin treatments [Fig. 1(b)] with the same conditions used in first measurements.

2.4 SEM Analysis

After treatments, one specimen was randomly selected for SEM observation. The specimen was mounted on aluminum stubs, and the carbon was thermally evaporated on to the specimens, and then examined using a Supra 50 VP Scanning Electron Microscope (EVO-MA10, Carl Zeiss® STM, Oberkochen, Germany) with an acceleration voltage of 20 kV. The SEM images were captured at the magnification of 5000x.

2.5 Statistical Analysis

Statistical analysis of the μ -EDXRF results was performed using InStat® software (GraphPad Software, San Diego, California). The Kolmogorov and Smirnov test verified the normal distribution of the sample data. The standard deviations were assessed by Bartlett’s test. Statistical comparisons of Ca and P weight percentages and the Ca/P ratio between nontreated and treated values (in the same group of treatment) were performed using the unpaired *t*-test with Welch correction. The Kruskal–Wallis Test (nonparametric ANOVA) was used to evaluate, after treatments, the Ca and P weight percentages and the Ca/P ratio between control and laser groups and among laser groups.

3 Results

μ -EDXRF spectra reveal the mineral dentin content (Ca and P) and Ca/P ratio of dentin before and after treatments (Table 2). The unpaired *t*-test with Welch correction showed significant reduction in weight percentages of Ca and P ($p < 0.0001$) and a significant increase in the Ca/P ratio ($p = 0.0052$) after acid etching (CG). No significant changes were found in inorganic dentin content after laser irradiation at 100 mJ (GI) ($p > 0.05$). However, the Ca/P ratio significantly decreased in GI specimens ($p = 0.0003$). The laser irradiation with 160 mJ (GII) did not result in significant changes in inorganic dentin content as well as in the Ca/P ratio ($p > 0.05$). The laser

Table 2 Mean and standard deviations (SD) ($n = 10$) of the average content of calcium (Ca) and phosphorus (P) percentages in the dentin and the Ca/P weight ratios obtained by x-ray fluorescence before (nontreated—N) and after (treated—T) treatments. Statistical comparisons were performed: (a) between N and T data (significant statistical comparisons are highlighted in gray); (b) after treatments between control and laser groups (different capital letters shows statistical differences); and (c) among laser groups (the same small letters indicate absence of statistical difference).

Groups	Ca (wt%)		P (wt%)		Ca/P	
	Mean (S.D.)	p-Value	Mean (S.D.)	p-Value	Mean (S.D.)	p-Value
CGN	29.36 (1.31)	$p < 0.0001$	15.91 (0.51)	$p < 0.0001$	1.85 (0.03)	$p = 0.0052$
CGT	23.37 (1.04)A		12.15 (0.82)A		1.93 (0.07)A	
G1N	30.03 (3.08)	$p = 0.8128$	16.15 (1.28)	$p = 0.2011$	1.86 (0.05)	$p = 0.0003$
G1T	29.74 (2.34)Ba		16.82 (0.93)Ba		1.77 (0.04)Ba	
G2N	29.96 (1.84)	$p = 0.1244$	16.55 (1.43)	$p = 0.0709$	1.82 (0.07)	$p = 0.4243$
G2T	31.59 (2.60)Ba		17.62 (0.98)Ba		1.79 (0.05)Ba	
G3N	28.93 (0.99)	$p < 0.0153$	15.72 (0.37)	$p = 0.0005$	1.84 (0.03)	$p = 0.0645$
G3T	32.22 (3.42)Ba		17.90 (1.30)Ba		1.80 (0.06)Ba	

irradiation with 220 mJ (GIII) resulted in significant increase in the Ca ($p < 0.0153$) and P ($p = 0.0005$) content after irradiation, however, without any changes in Ca/P ratio ($p > 0.05$).

The Kruskal–Wallis test showed significantly lower quantities of Ca and P after dentin acid etching (CG) than after laser etching (GI: $p < 0.05$, GII: $p < 0.01$, and GIII: $p < 0.01$). A significant increase in the Ca/P ratio was found in CG than in the laser-treated specimens (GI: $p < 0.001$, GII: $p < 0.01$, and GIII: $p < 0.01$). No significant differences were found among laser-treated groups in the Ca and P weight percentages and in the Ca/P ratio for the comparisons ($p > 0.05$).

The graphic images produced by surface area mappings showed that phosphoric acid etching produced a homogeneous demineralization pattern for the average content of Ca and P in the dentin surface [Fig. 2(a)–2(d)]. Elements were evenly distributed throughout the dentin. In contrast, Er:YAG laser etching produced an irregular demineralization pattern with some patches of concentrated elements (Figs. 3–5).

The laser irradiation at 100 mJ resulted in small changes in elements distribution throughout the surface [Fig. 3(a)–3(d)]. The Er:YAG laser irradiation at 160 and 220 mJ pulse energies (groups II and III) produced similar μ -EDXRF images. The laser irradiation at those parameters created an irregular distribution of Ca and P components on the dentin surface with concentrated demineralization areas (blue and cyan areas) (Figs. 4 and 5). The images also evidenced mineralized areas with the highest maximum Ca and P values for the group III (Fig. 5) (64.22% and 32.52%, respectively).

Group III images show a more irregular distribution of components throughout the surface than the other treatments with concentrated demineralization areas (blue and cyan areas) and

areas with concentrated mineralization (yellow and red areas) (Fig. 5).

SEM image of acid-etched dentin [Fig. 6(a)] revealed a flat and smooth surface with tubule orifices free of smear plugs and with widened tubule orifice due to removal of peritubular dentin inorganic phase at the opening of the tubules. The inter-tubular dentin was undisturbed and the surface was homogeneously demineralized. In contrast, SEM micrographies of the laser-irradiated specimens revealed high irregular surfaces, partially opened dentin tubules, and a scaly and flaky surface [Fig. 6(b)–6(d)]. Groups I and II dentin surfaces showed partially opened dentin tubules and the presence of holes and mounds [Fig. 6(b) and 6(c)]. Er:YAG laser irradiation at 220 mJ (GIII) produces a higher number of partially opened dentin tubules than in other laser groups [Fig. 6(d)].

4 Discussion

In the present study, a different pattern of dentin etching was found between phosphoric acid and Er:YAG laser treatments. The obtained μ -EDXRF results provided by mappings showed that acid etching significantly reduced the calcium (Ca) and phosphorus (P) dentin content, and showed a significant increase in the Ca/P ratio (Table 2, Fig. 2). The acid etching also removed more mineral than the Er:YAG laser irradiation. This result is significant because the Ca and P present in hydroxyapatite crystals are the major inorganic components of dental hard tissue.²² Alterations in the Ca/P ratio may change the original ratio between organic and inorganic components that, in turn, change the permeability and solubility characteristics of dentin.²³ The increase in the Ca/P ratio found in the present study after acid etching indicates that the mineral content of

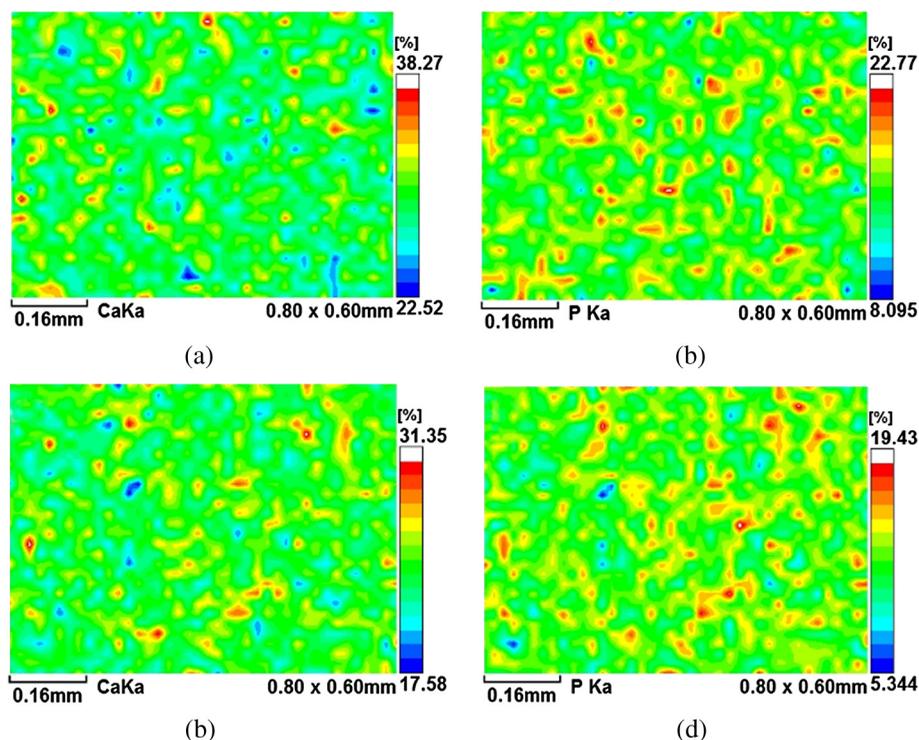


Fig. 2 Microenergy-dispersive x-ray fluorescence spectrometry (μ -EDXRF) elemental distribution images of calcium (Ca) (a, c) and phosphorus (P) (b, d) from one dentin specimen before (a, b) and after (c, d) phosphoric acid etching (control group). The gradient in the intensity of the color scale indicates variation in Ca and P component concentration in percentage, thus, a high component concentration showed as red and white. A regular distribution of inorganic elements throughout the dentin surface was found after acid etching.

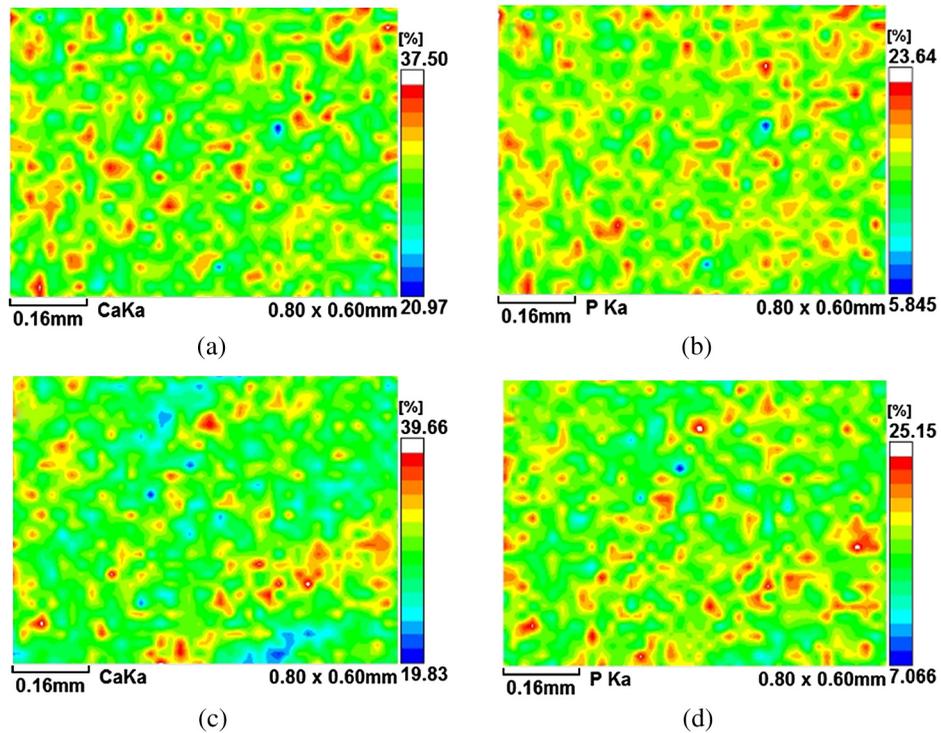


Fig. 3 μ -EDXRF elemental distribution images of calcium (Ca) (a, c) and phosphorus (P) (b, d) from one dentin specimen before (a, b) and after (c, d) erbium:yttrium–aluminum–garnet (Er:YAG) laser irradiation with 100 mJ (GI). The gradient in the intensity of the color scale indicates variation in Ca and P component concentration in percentage, thus, a high component concentration showed as red and white.

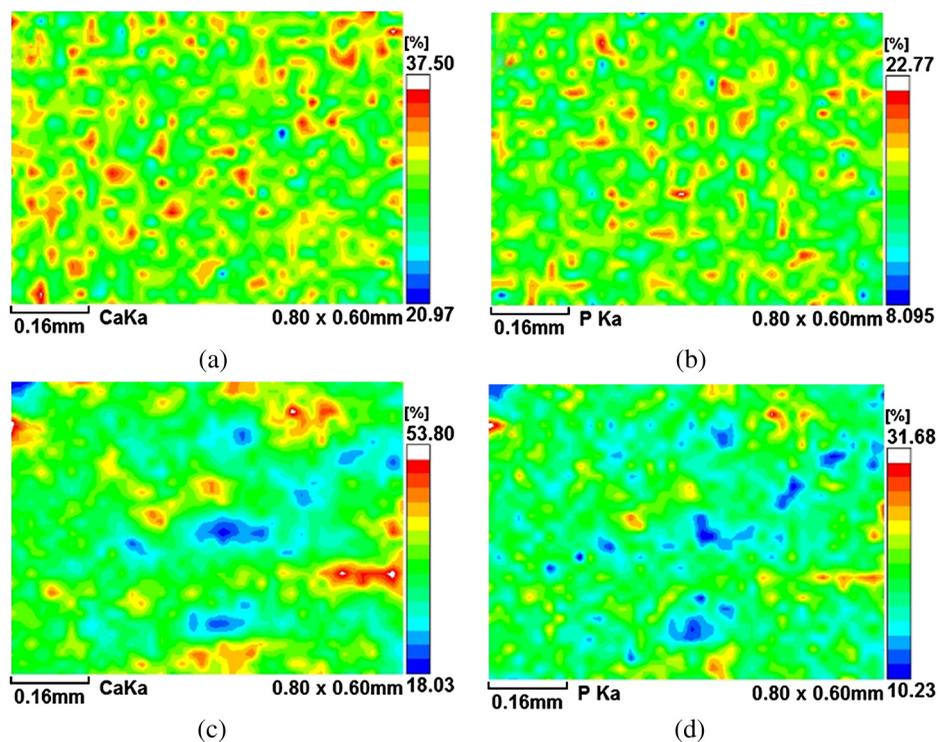


Fig. 4 μ -EDXRF elemental distribution images of calcium (Ca) (a, c) and phosphorus (P) (b, d) from one dentin specimen before (a, b) and after (c, d) Er:YAG laser irradiation with 160 mJ (GI). The gradient in the intensity of the color scale indicates variation in Ca and P component concentration in percentage, thus, a high component concentration showed as red and white. Isolated regions with blue spots are shown after irradiation indicating low Ca and P concentration.

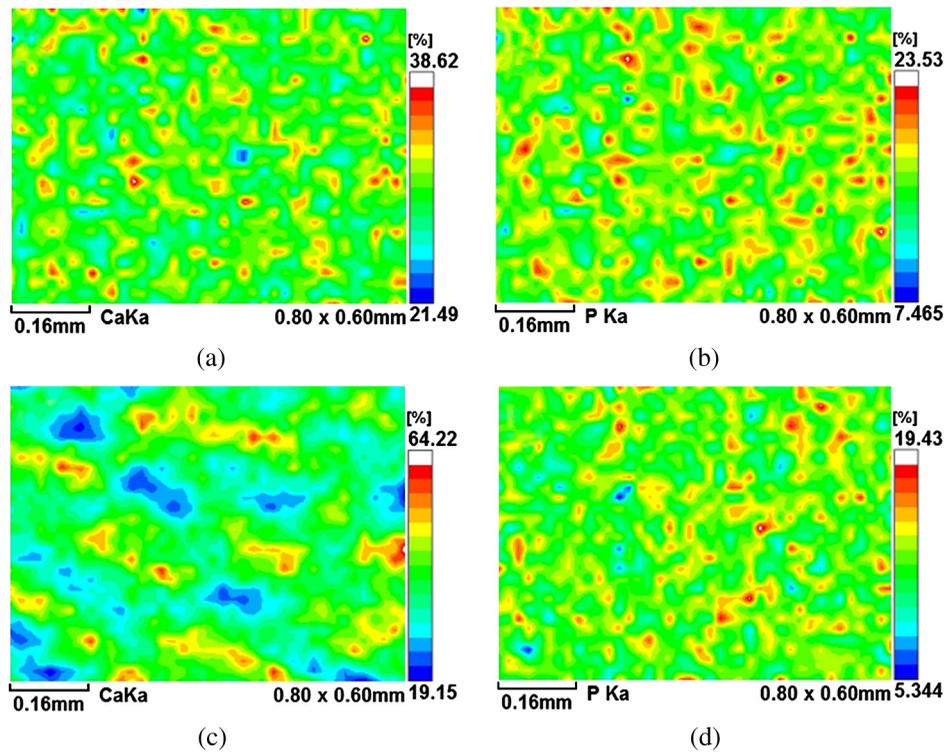


Fig. 5 μ -EDXRF elemental distribution images of calcium (Ca) (a, c) and phosphorus (P) (b, d) from one dentin specimen before (a, b) and after (c, d) Er:YAG laser irradiation with 220 mJ (GIII). The gradient in the intensity of the color scale indicates variation in Ca and P component concentration in percentage, thus, a high component concentration showed as red and white. Isolated regions with blue and red spots are shown after irradiation indicating low and high Ca and P concentration, respectively. A significant demineralization (dark blue areas) was found for Ca element (C).

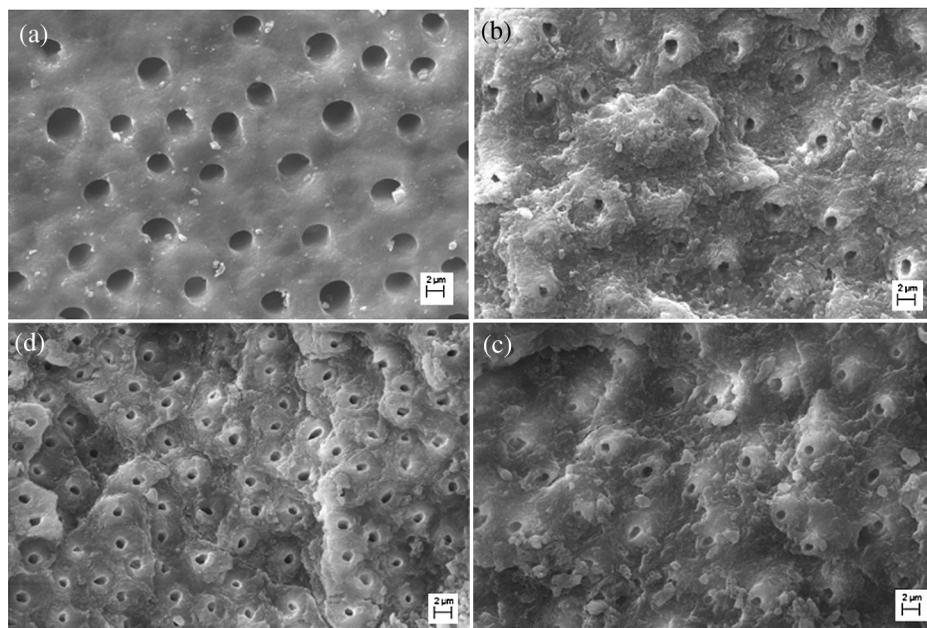


Fig. 6 Representative scanning electron microphotographs of treated dentin surfaces. (a) The acid-treated dentin surface (control group) is flat, and the dentinal tubules are enlarged. (b–d) Er:YAG laser-irradiated dentin surfaces shows a scaly, rough surface with partially opened dentinal tubules, no smear layer, and the presence of holes of dentin tubules and mounds. (d) Group III dentin surfaces showed higher number of partially opened dentin tubules than in other laser groups (5000 \times).

dentin was significantly removed enabling, thus, an increase in the permeability which could favor the adhesive penetration.

The significant decrease in the Ca/P ratio after Er:YAG irradiation with 100 mJ (Table 2) may be explained by the higher organic content in the dentin, which adds to the higher phosphorus values and, therefore, the lower Ca/P values.²⁴ The significant increase found in the Ca and P content after Er:YAG laser irradiation on dentin with 220 mJ is a result of the evaporation of organic components during laser irradiation due to an increase in temperature in the irradiated area,²⁵ which may well lead to an increase in Ca or P content of superficial dentin. Clinically, this increase in Ca and P levels may result in acquired acid resistance of the dentin surface.^{11,26}

The homogeneous demineralization pattern showed by μ -EDXRF mapping of Ca and P after acid etching at the dentin surface (Fig. 2) indicates a favorable substrate for adhesion procedures. In contrast, for the Er:YAG laser-irradiated dentin, the Ca and P distribution throughout the surface was inhomogeneous with some spots of high and others with low mineral contents (Figs. 3–5). This uneven distribution of minerals was more evident after laser irradiation with an energy of 220 mJ (Fig. 5). The high mineral content areas resulted from organic content evaporation²⁵ reducing the permeability of the laser treated dentin and increasing resistance of the superficial dentin to acid.²⁶ The low mineral content spots are caused by the ablative mechanism of this type of laser. During irradiation, the incident energy is readily and highly absorbed by water molecules present in the dentin's crystalline structures and organic components, thus causing sudden heating and water vaporization. The resulting high-stream pressure within the irradiated tissue leads to the occurrence of microexplosions in the surrounding structures, and thus, removal of the dental tissues.²⁶

Our μ -EDXRF mapping results are in agreement with the previous study of Moretto et al.,⁶ who found by SEM an irregular surface after Er:YAG laser irradiation exhibiting areas with valleys and peaks. In our study, μ -EDXRF analysis showed areas with low content of Ca and P (blue colors), which probably indicates regions of valleys (as result of laser ablative mechanism). Other areas were found with high content of minerals (with white and red colors), which probably indicates the peak regions found by SEM analysis. Those modifications in dentin after laser irradiation shown in previous reports raises concerns about chemical modifications of dentin by lasers and the interaction of adhesive restorations in substrates.⁶ A dentin substrate with chemical modifications alters the patterns of dentin bonding, and this indicates a necessity to develop new materials which result in effective bonding. The high mineral content after irradiation with 220 mJ, the significant changes in the Ca/P ratio after laser irradiation with 100 mJ, and the lower mineral content of etched surfaces than in irradiated dentin suggest that without chemical changes in dental adhesive the bonding will be compromised after laser irradiation.

SEM analysis of the laser-irradiated dentin showed a highly irregular surface, partially opened dentin tubules, and a scaly and flaky surface [Fig. 6(b)–6(d)], in contrast to the smoother surface and opened dentin tubules seen in acid-etched dentin [Fig. 6(a)]. These results agree with previous studies.^{8,27,28}

Er:YAG laser irradiation produces no demineralization of peritubular dentin [Fig. 6(b)–6(d)], so there are no funnel-shaped tubule orifices in dentin.²⁹ In this situation, resin bonding would form resin tags with parallel walls and with a diameter similar to that of the original tubule lumen. Since hybridization

of the lateral walls of dentinal tubules is lacking, lower shear bond strength is expected.²⁹

It is known that surface irregularity interferes with the EDXRF quantitative analysis with conventional fluorescence x-ray spectrometers, and corrections using Compton and Rayleigh scattering should be done.³⁰ Another possibility to reduce the surface irregularity effects is to reduce the excitation spot or to enlarge it, in order to excite the whole surface under analysis.³¹ In our case, the surface effect was reduced by the small excitation spot of the μ -EDXRF (50 μ m), and therefore, the intensity color's gradient scheme indicates the variation of the component's content. The holes found in SEM image (Fig. 6) are due to ablation of material produced by the laser irradiation resulting in a decrease of mineral content, which appear as a blue area in the μ -EDXRF images. In the opposite way, the mounds areas have a high mineral content, and appear in the μ -EDXRF images as a white and red area.

5 Conclusions

The results of μ -EDXRF mappings showed that the amounts of Ca and P (wt%) decreased significantly after acid etching enabling, thus, an increase in the permeability which could favor the adhesive penetration. The high mineral content after irradiation with 220 mJ, the significant changes in the Ca/P ratio after laser irradiation with 100 mJ, and the lower mineral content of acid etched surfaces than in irradiated dentin suggest that without chemical changes in dental adhesive the bonding will be compromised after laser irradiation. The laser energy of 220 mJ probably denatured the collagen of dentin, thus, changing the stoichiometric proportions of hydroxyapatite as shown by an increase of mineral. Data obtained with the μ -EDXRF images had correlation with features found in SEM images, where the decrease and increase of mineral content in the μ -EDXRF images were correlated to holes and mounds in micrographies, respectively. These findings help to elucidate the chemical structure of dentin after laser etching, and show the possibility of this association of analytical tools to make possible the development of effective guidelines for laser irradiation for dentin hybridization.

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