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ORIGINAL ARTICLE

Qualitative evaluation of hybrid layer formation using Er:YAG laser in QSP mode for tooth cavity preparations

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Abstract

The purpose of this study was to evaluate the thickness and qualitative characteristics of the hybrid layer after two cavity preparation methods, using Er:YAG laser in QSP mode and conventional carbide burs. Additionally, two different adhesive techniques were investigated using etch-and-rinse and self-etch adhesive systems. Sixty sound human third molars were used and were randomly divided into four groups ($n = 15$). In the first two groups, large (4 mm length, 3 mm wide, and 3 mm deep) class I cavities were prepared using Er:YAG laser (2.94 μm) in QSP mode, while in the other two groups, the cavities were prepared using carbide burs. After cavity preparations, two different adhesive techniques with GLUMA® 2 Bond (etch-and-rinse) and Clearfil™ Universal Bond Quick (self-etch) were applied. For the qualitative evaluation of the formed hybrid layer, photomicrographs were taken using SEM, and elemental semi-quantitative analysis was performed using EDS to confirm the extent of the hybrid layer. One-way ANOVA was applied to verify the existence of statistically significant differences, followed by Tukey test for post hoc comparisons (Bonferroni corrected), and the level of significance was set at $a = 0.05$. The laser-treated groups exhibited higher hybrid layer thickness than bur-treated groups ($p < 0.001$). Between the laser-treated groups, etch-and-rinse technique presented higher hybrid layer thickness than self-etch technique ($p < 0.001$), while between the bur-treated groups, no significant differences were detected ($p = 0.366$). Er:YAG laser cavity preparations in QSP mode may be advantageous for adhesion of composite restorations, but more data are necessary to confirm its clinical effectiveness.

Keywords Quantum square pulse mode · Er:YAG · Hybrid layer · Scanning electron microscopy · Energy dispersive X-ray spectroscopy

Introduction

Nowadays, there is a consensus regarding the importance of hybrid layer formation between the adhesive agent and the dentin substrate in composite restorations. The correlation of the quality of the forming hybrid layer with the longevity of the bond between a composite restorative and dentin has been documented in previous investigations [1, 2]. Hybrid layer is formed in two stages. The first stage involves etching, which removes calcium phosphates from the substrate, formation of micropores on dentin surface, and exposure of the collagen scaffold. The second stage includes penetration of the adhesive monomers into the exposed network of collagen fibrils providing micromechanical retention. These monomers, which contain both hydrophilic and hydrophobic chemical groups, penetrate into dentin and being photo-polymerized by using a dental light-curing unit [1]. Consequently, the adhesion of a composite restorative to dentin is achieved through a combination of mechanical and chemical mechanisms.

Research in adhesive dentistry is mainly focused on two developments: the investigation of novel and more effective adhesive systems and the replacement of conventional rotating burs (diamonds or carbides) with laser irradiation for cavity preparations. Both advancements are promising for the future of clinical dentistry. In particular, new adhesive systems aim to improve bonding to dentin, which is often unreliable due to increased water and organic content of dentin. Bonding failure attributed to hydrolysis and proteolytic breakdown of dentin collagen, as well as the possibility of hydrolytic degradation of the adhesive, needs to be addressed [3–7]. It is important to mention that immediate high bond strength of a

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composite restoration to dentin does not necessarily ensure its longevity and prognosis [8]. Durability of a forming bond requires additional chemical bonds between the functional groups of the adhesive monomers and the remnant hydroxyapatite of dentin. However, the formation of chemical bonds is difficult to occur since there is only a weak affinity between the functional groups of the monomers and the hydroxyapatite of depleted collagen [9].

Both thickness and functionality of a hybrid layer are influenced by the composition and morphology of dentin surface, the method of cavity preparation (laser or bur treatment), and the type and composition of the adhesive system used [10–12]. Since there is a tendency for utilization of laser technology in tooth cavity preparation in order to replace classical bur treatments, characterization of dental surface after cavity preparation with a laser system is crucial. Erbium (Er) family lasers including erbium/yttrium-aluminum-garnet—Er:YAG ($\lambda = 2.94$ μm) and erbium, chromium/yttrium-scandium-gallium-garnet—Er,Cr:YSGG ($\lambda = 2.78$ μm) are now widely used in daily clinical practice for removing hard dental tissues due to their high absorption in hydroxyapatite and water [13]. Laser irradiation creates micro-structural alterations on dentin surface leaving a relatively rough surface with wide open and protruding dentinal orifices, a characteristic lack of smear layer and minor demineralization, which contribute to a better substrate for adhesion of a composite [14]. The laser parameters that affect the characteristics of the prepared dentinal surface are wavelength, pulse energy, pulse frequency, pulse duration, time of irradiation, type of the tip of the device, distance between the tip and the surface, water irrigation, and air flow [15].

So far, there is only sparse literature regarding the comparison of hybrid layer characteristics and bond strength between composite restorations prepared with burs and erbium lasers [16]. In addition, it is interesting to mention that the existing literature is controversial with some studies reporting same or higher bond strength with the use of lasers [14, 16], while others found lower [12, 17]. More perplexing are the results concerning the thickness and quality of the forming hybrid layer on laser-irradiated dentin [18].

Recently, a novel laser irradiation mode has been introduced for tooth cavity preparations, which seems advantageous compared to conventional irradiation modes. The novelty of Quantum Square Pulse (QSP) mode is that a 600-μs macro-pulse is chopped into five micro-pulses of 50 μs with an intervening temporal spacing of 85 μs between two micro-pulses in order to avoid the formation of the ablation cloud that inevitably absorbs part of the energy and hinders correct focusing of the laser beam. As a result, when QSP mode is employed, almost 90% of the applied energy is delivered to the target. Furthermore, due to the succession of micro-pulses and pauses, there is enough time for thermal relaxation of the dental tissues, allowing only minimal heat dissipation to the substrate. Thus, the possibility of thermal damage to the adjacent dental tissues is limited [19].

Therefore, the purpose of this in vitro study was to evaluate the thickness and qualitative characteristics of the forming hybrid layer after two cavity preparation methods, using Er:YAG laser in QSP mode and conventional carbide burs. Additionally, two different adhesive techniques were investigated for each method using etch-and-rinse and self-etch adhesive systems. The novelty of the study was that this protocol for tooth cavity preparation using QSP mode has never been investigated before regarding the hybrid layer characteristics. Moreover, the methodology of evaluating the thickness of the hybrid layer was novel using elemental analysis for more precise measurements.

Two null hypotheses were formulated prior to this study: the first null hypothesis ($H_0 1$) was that there were no significant differences in thickness of the hybrid layer between laser-treated and bur-treated tooth specimens of the same adhesive procedure and the second null hypothesis ($H_0 2$) was that there were no significant differences in thickness of the hybrid layer between etch-and-rinse and self-etch adhesive groups of the same cavity preparation method.

Materials and methods

Preparation of the teeth

Sixty sound freshly extracted human third molars were used in the present investigation. The teeth were extracted for orthodontic reasons with the informed consent of the donors and stored immediately in normal saline solution (0.9% *w*/*v* NaCl) at 4 °C for up to 2 months. The crowns were separated from the roots using a water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The 60 teeth were not allowed to be dehydrated, examined by means of optical microscope under × 10 magnification for any surface structural damage or defection, immersed in an ultrasonic bath (Euronda Spa, Montecchio Precalcino, Vicenza, Italy) to remove any impurities, and were randomly divided into four groups ($n = 15$). During the experimental period, the teeth were stored in fresh solution of normal saline at 37 ± 1 °C.

Experimental groups of the study

In group 1, large (approximately 4 mm length, 3 mm wide, and 3 mm deep) class I cavities were prepared on the occlusal surfaces of the teeth using an Er:YAG ($\lambda = 2.94$ μm) laser device (LightWalker AT, Fotona, Ljubljana, Slovenia) in QSP mode (a 600-μs macro-pulse is chopped into five micro-pulses of 50 μs with an intervening temporal spacing of 85 μs between two micro-pulses). The entire procedure of cavity preparation was performed with the HO2 handpiece of the device. To ensure consistent spot size (1.2 mm^2) with the hand irradiation, an endodontic file was fixed at the handpiece and kept a distance of 6 mm from the surface during irradiation. The handpiece was positioned perpendicularly to tooth surfaces, and the samples were irradiated by hand by the same researcher. The laser parameters were set as follows: pulse repetition rate = 15 Hz, average power = 3.75 W, and pulse energy = 250 mJ, while during the procedure, air flow and water flow were used. The total water flow directed onto the samples was 20 mL/min, with approximately 10% falling directly on the point of laser incidence. After the cavity preparations, an etch-and-rinse adhesive system (GLUMA® 2 Bond, Heraeus Kulzer GmbH, Hanau, Germany) was applied to dentin surfaces (Table 1). Initially, the dentin surfaces were etched with 35% phosphoric acid gel (Scotchbond™ Etchant, 3M ESPE, St. Paul, MN, USA) for 15 s, thoroughly rinsed, and air-dried for 5 s with a dental air-syringe at a distance of 15 cm and air pressure of 3.8 kg/cm^2. Subsequently, the adhesive agent was applied to the etched dentin according to manufacturer's instructions, gently air-dried for 5 s, and light-cured for 10 s using a dental LED unit (Valo, Ultradent, South Jordan, UT, USA) operating at 1400 mW/cm^2 with standard curing mode and wavelength range 395–480 nm.

In group 2, the same procedure was followed for cavity preparations as group 1. After cavity preparations, a universal adhesive system (Clearfil™ Universal Bond Quick, Kuraray Noritake Dental Inc., Okayama, Japan) was applied to dentin surfaces using self-etch adhesive technique (Table 1). More specifically, the adhesive was applied to the dentin surfaces with rubbing motion, air-dried for 5 s, and light-cured for 10 s according to manufacturer's instructions.

In goup 3, the same size of cavities was prepared as groups 1 and 2 by using straight flat cylinder end plain carbide burs (HP #57, SS WHITE®, Lakewood, NY, USA) on a high-speed handpiece (Bora, Bien-Air Dental, Bienne, Switzerland) at approximately 320,000 rpm with air/water coolant. Subsequently, the same etch-and-rinse adhesive technique was followed as group 1 using GLUMA® 2 Bond.

In group 4, the same cavity preparation procedure was followed as group 3. After cavity preparations, the same self-etch adhesive procedure was followed as group 2 using Clearfil™ Universal Bond Quick. The experimental groups of the study are presented in Table 2.

Evaluation of hybrid layer thickness by SEM and EDS analysis

After adhesive procedures, the specimens were stored in saline solution for 24 h at 37 ± 1 °C. Then, the crowns of the teeth were cut perpendicularly in the middle using a chisel in order to avoid the formation of smear layer on the cut surfaces if rotating burs or disks were used. The half of each tooth crown was mounted on aluminum stubs with the cut surface facing up, carbon sputter-coated to a thickness of approximately 200 Å in a vacuum evaporator (at low vacuum), and examined using a scanning electron microscope (JEOL, JSM-840, Tokyo, Japan) at 19 kV accelerated voltage. For the qualitative evaluation of the formed hybrid layer, photomicrographs were taken at × 1000 magnification at three different areas of the hybrid layer of the pulpal wall, one in the center and the other two at least 500 μm apart. For each image, five measurements of hybrid layer thickness were conducted under SEM microscope resulting in 15 measurements for each specimen. The average thickness of the formed hybrid layer of each experimental group was calculated in μm and the outcomes were statistically analyzed. Additionally, the morphology of the tested area was examined and elemental semi-quantitative analysis was performed using energy dispersive X-ray spectroscopy (EDS) to confirm the extent of the hybrid layer. In particular, images in backscattered mode were performed to chemically confirm the limits of hybrid layer and to be separated from the adhesive layer above and the partially dentin demineralization (PDD) zone below. Moreover, the images were line-scanned to identify the fluctuations of Ca, P, C, and Si elements which indicate the penetration of the adhesive resin into the dentin. More specifically, concentrations of Si and C indicated resin while Ca and P concentrations indicated

Table 1 The adhesive systems used in this study

Dental adhesive system	Adhesive technique	Manufacturer	Composition	Lot number
GLUMA® 2 Bond	Etch-and-rinse	Heraeus Kulzer GmbH, Hanau, Germany	Methacrylate, ethanol, fillers, photoinitiators glutaraldehyde	
Clearfil™ Universal Bond Quick	Self-etch (mild)	Kuraray Noritake Dental Inc., Okayama, Japan	10-Methacryloyloxydecyl dihydrogen phosphate (MDP), Bisphenol A diglycidylmethacrylate (Bis-GMA), 2-Hydroxyethyl methacrylate (HEMA), Hydrophilic amide monomers, Colloidal silica fillers (10 wt%), Silane coupling agent, Sodium fluoride, dl-Camphorquinone, Ethanol, Water, pH = 2.3	2F0021

Table 2 The experimental groups of the study

Group	Cavity preparation technique	Adhesive technique
Group 1	Er:YAG laser	Etch-and-rinse
Group 2	Er:YAG laser	Self-etch
Group 3	Carbide burs	Etch-and-rinse
Group 4	Carbide burs	Self-etch

dentin in the line-scanned areas. The average thickness of the formed adhesive layer and PDD zone was also calculated.

Statistical analysis

The thickness of the hybrid layer of the experimental groups of the study was statistically analyzed using SPSS statistics 20.0 software (IBM Corp, ILL, Chicago, USA). For the determination of the sample size of the method, a statistical power test (G*Power software) was performed. The sample size was calculated considering 80% power and a significance level of 0.05. The normality and homogeneity of the data were checked using Kolmogorov–Smirnov test and Levene test, respectively. One-way ANOVA was applied to verify the existence of statistically significant differences, followed by the Tukey test for post hoc comparisons (Bonferroni corrected). Additionally, the data were analyzed by two-way ANOVA to define how cavity preparation method and adhesive technique affected the thickness of the hybrid layer. In all the analyses, the level of significance was set at $a = 0.05$.

Results

Mean values and standard deviations of hybrid layer, adhesive layer, and PDD zone thickness in μm of the experimental groups of the study are presented in Table 3. Two-way ANOVA revealed that both cavity preparation method and adhesive technique affected hybrid layer thickness individually ($p_{prep} < 0.001$, $p_{adhes} < 0.01$) but there was no evidence of a synergistic (interaction) effect of the two ($p_{prep*adhes} = 0.264$).

The laser-treated groups exhibited significant higher hybrid layer thickness than bur-treated groups ($p < 0.001$). Between the laser-treated groups, etch-and-rinse adhesive technique presented significant higher hybrid layer thickness than self-etch adhesive technique ($p < 0.001$), while between the bur-treated groups, no statistically significant differences were detected ($p = 0.366$). Moreover, the laser-treated groups presented higher adhesive layer thickness ($p < 0.01$) as well as PDD zone thickness ($p < 0.001$) than bur-treated groups. PDD zone thickness was higher for etch-and-rinse adhesive technique in both laser-treated and bur-treated groups compared to self-etch adhesive technique ($p < 0.05$).

Table 3 Mean values and standard deviations of hybrid layer, adhesive layer, and partially demineralized dentin zone thickness (μm) of the experimental groups of the study. Same uppercase superscripts in rows indicate no significant differences between cavity preparation methods ($p > 0.05$). Same lowercase superscripts in columns indicate no significant differences between adhesive techniques ($p > 0.05$)

Hybrid layer thickness	Laser-treated	Bur-treated
Etch-and-rinse	11.2 ± 2.3^{Aa}	3.5 ± 1.9^{Ba}
Self-etch	9.2 ± 2.3^{Ab}	3.6 ± 2.2^{Ba}
Adhesive layer thickness	Laser-treated	Bur-treated
Etch-and-rinse	5.3 ± 1.7^{Aa}	1.6 ± 0.9^{Ba}
Self-etch	3.6 ± 1.1^{Ab}	2.3 ± 1.2^{Ba}
Demineralized dentin zone thickness	Laser-treated	Bur-treated
Etch-and-rinse	7.1 ± 1.3^{Aa}	3.7 ± 1.4^{Ba}
Self-etch	5.3 ± 1.4^{Ab}	1.3 ± 1.0^{Bb}

Representative SEM images, showing the adhesive layer, hybrid layer, and PDD zone of specimens of each experimental group, are shown in Figs. 1a, 2, 3, and 4a. Figures 1b, 2, 3, and 4b present the same areas as Figs. 1a, 2, 3, and 4a at backscattered mode, where the limit between hybrid layer and PDD zone is more clearly shown. Backscattered electrons consist of high-energy electrons originating in the electron beam that are reflected out of the specimen interaction volume by elastic scattering interactions with specimen atoms. Since heavy elements backscatter electrons more strongly than light elements, and as a result appear brighter in the image, this method is used to detect contrast between areas with different chemical compositions [20]. In Figs. 1c, 2, 3, and 4c, elemental analysis of the same areas is illustrated using EDS method (line-scanned images) showing the differences in chemical composition of each morphological layer.

Representative SEM images of laser-treated (a) and bur-treated (b) dentin surfaces after cavity preparations and before adhesive procedures are illustrated in Fig. 5a, b. The laser-treated dentin surface is rougher with open dentinal tubules, while the bur-treated dentin surface is smoother, more uniform, and is covered with smear layer.

Discussion

According to the results of the present study, H_01, stating that there were no significant differences in thickness of the hybrid layer between laser-treated and bur-treated tooth specimens of the same adhesive procedure, was rejected. Previous investigations also found significant differences in dentin-resin interface between laser-treated and bur-treated dentin specimens [14, 21, 22]. The advantages of laser cavity preparation include an acquired rough dentin surface, not demineralized but presenting patent dentin tubules, which may improve micromechanical retention [23], while in bur-prepared dentin, the surface is smoother and

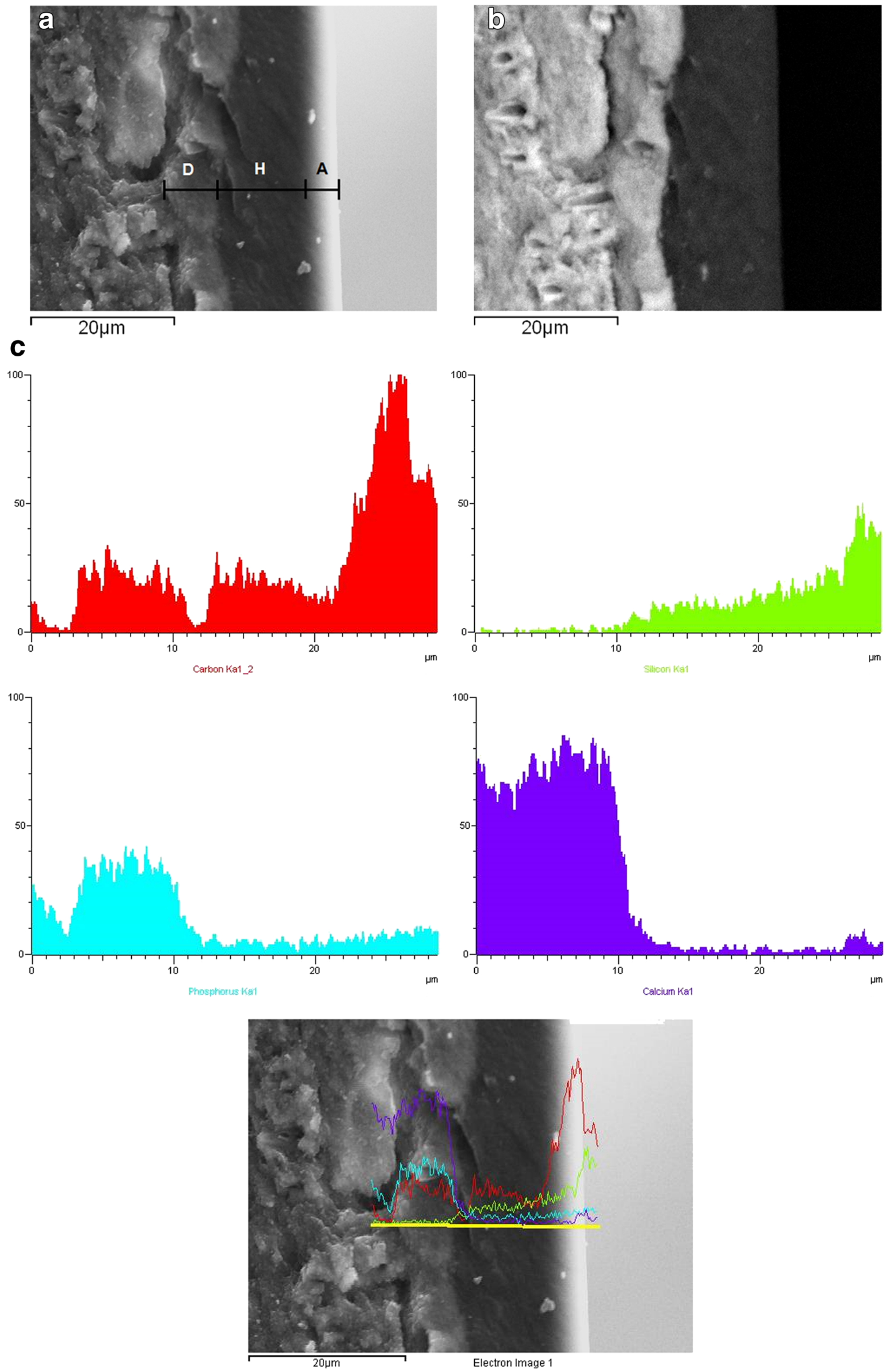

Fig. 1–4 **a–c** Representative SEM images (× 1000 magnification) of groups 1–4, respectively, showing adhesive layer (A), hybrid layer (H), and PDD (D) zone (**a**), the same image at backscattered mode where the limit between hybrid layer and PDD zone is clearly shown (**b**), and elemental analysis of the same area using EDS method (line-scanned images) showing the fluctuations in chemical composition of each morphological layer. Red color: carbon wt% (indicates resin monomers), green color: silica wt% (indicates resin fillers), light blue color: phosphorus wt% (indicates dentin), purple color: calcium wt% (indicates dentin)

dentin tubules are covered with smear layer which prevent resin infiltration. These morphological characteristics may explain the higher thickness of the hybrid layers formed in laser-treated groups. Comparing laser-treated with bur-treated dentin, in some studies, favorable outcomes were obtained for laser-prepared dentin as regards to bond strength [22, 24, 25] and microleakage [11, 26]. On the other hand, various studies reported poor effectiveness of laser-treated dentin surfaces [27, 28]. These discrepancies may be attributed to various parameters such as different laser irradiation parameters, dentinal substrates, experimental design, methodology etc.

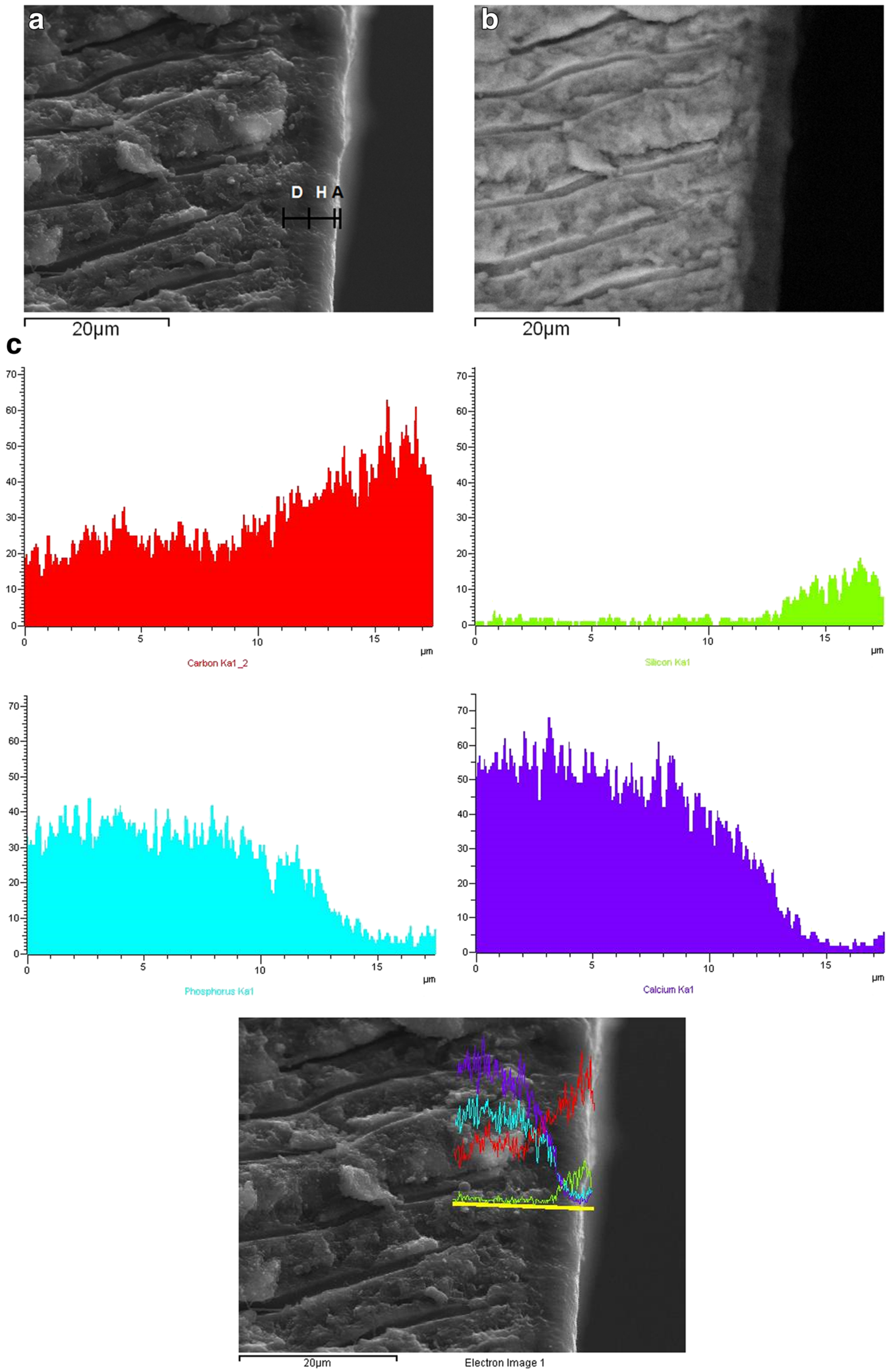

Fig. 1–4 (continued)

Hybrid layer thickness affects bond strength between dentin and resin and, as a consequence, is a crucial factor for the longevity of a composite restoration. The importance of the hybrid layer in adhesion of a composite restorative material to dentin is threefold: firstly, via hybrid layer, an imperative micro-mechanical and chemical adhesion is achieved [1, 2, 6, 7]; secondly, it has been demonstrated that marginal sealing and stability of a restoration under occlusal load are also achieved through the hybrid layer, which also serves as a barrier against demineralization of the dentin by cariogenic

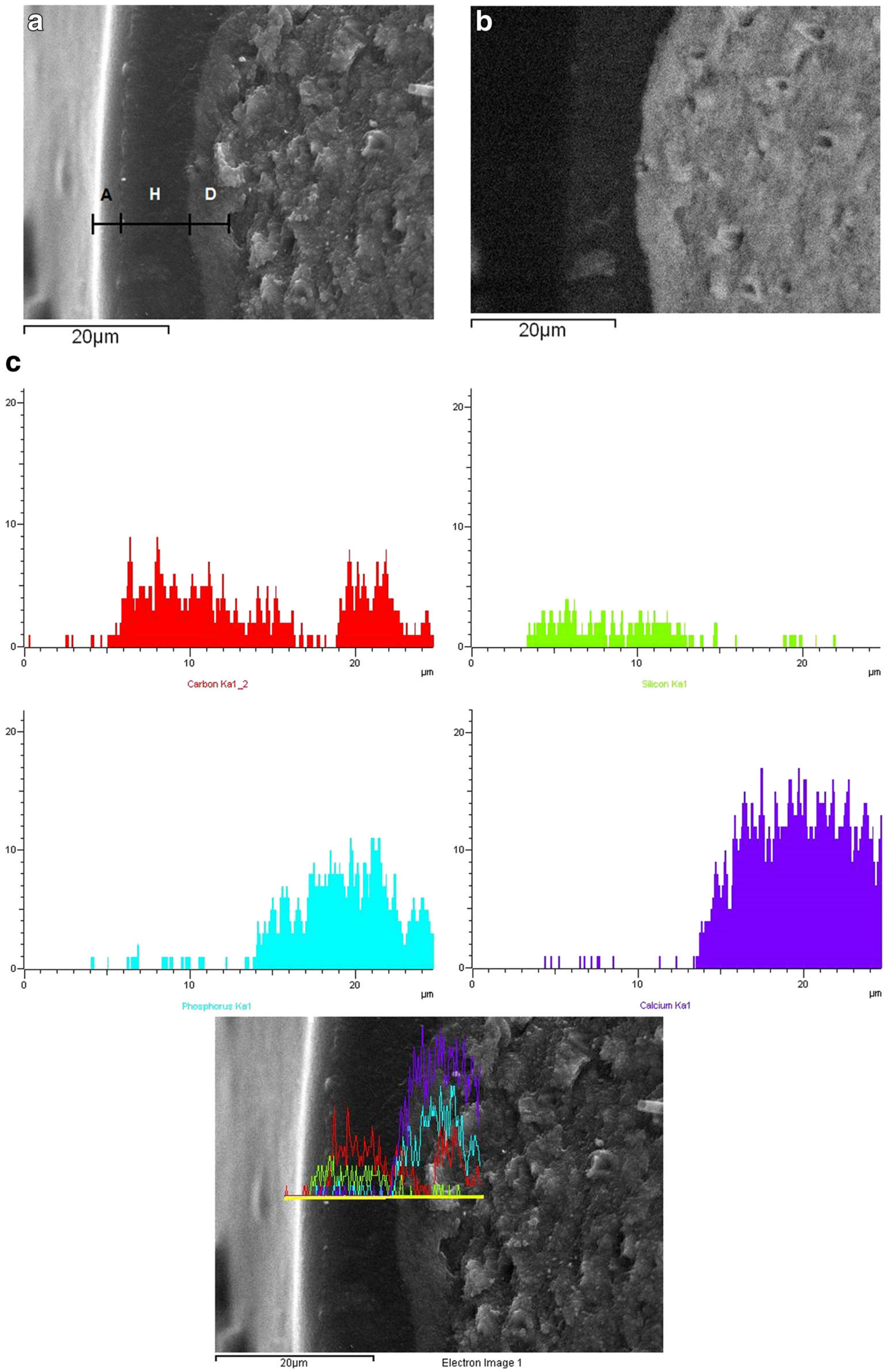

Fig. 1–4 (continued)

agents [29]; and thirdly, it has been proposed that hybrid layer could play a stress-absorbing role after resin-dentin bond formation [30]. Although higher hybrid layer thickness provides better adhesion to dentin, a remarkable increase in the thickness may lead to decreased bond strength [31].

Average hybrid layer thickness for etch-and-rinse and strong self-etch adhesive systems usually ranges between 3 and 5 µm [32, 33]. In the present investigation, laser-treated groups exhibited almost three times higher hybrid layer thickness compared to bur-treated groups in both adhesive

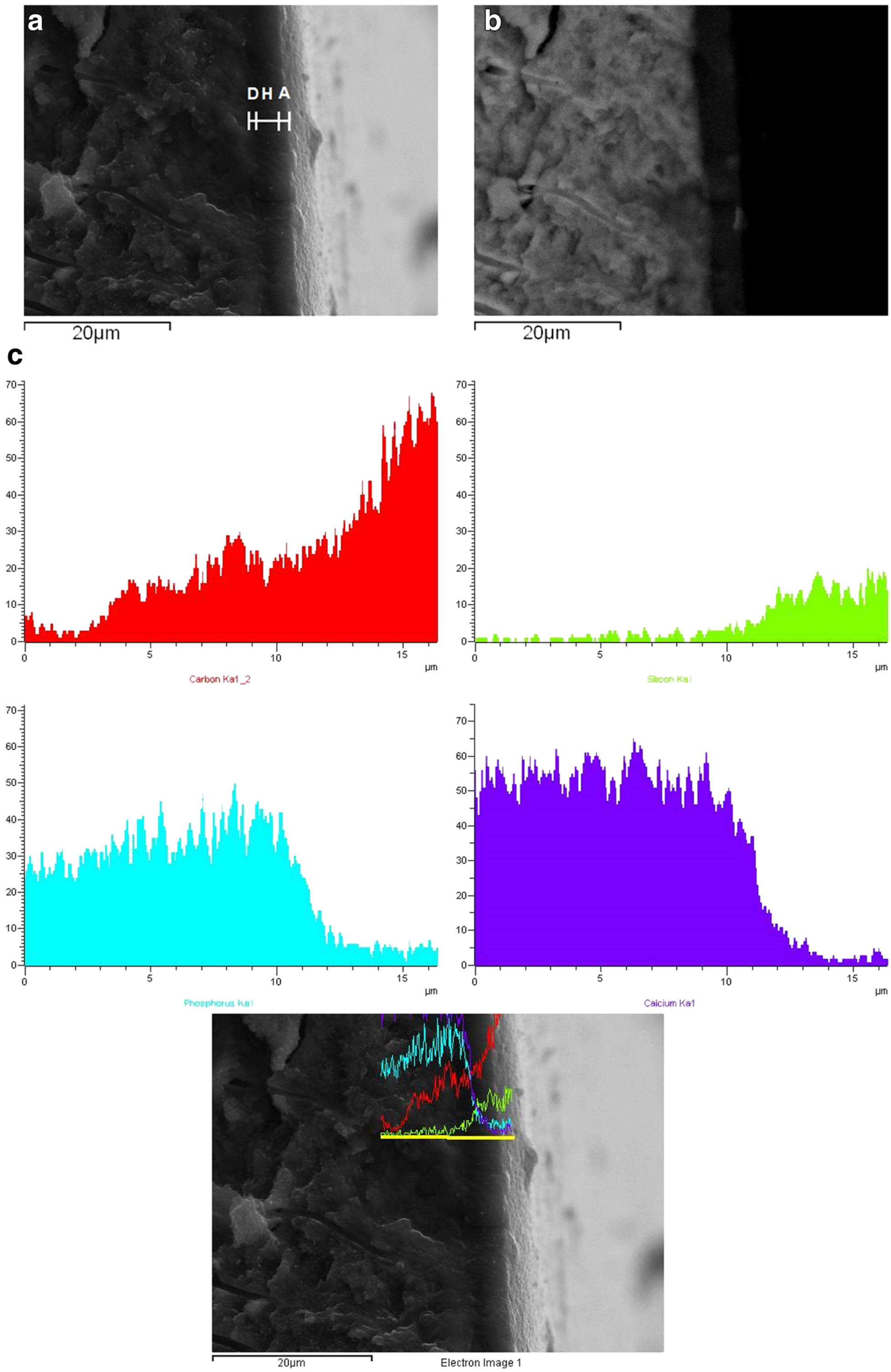

Fig. 1–4 (continued)

techniques. More specifically, mean values of hybrid layer thickness of laser-treated groups were around 10 μm, while for bur-treated groups were around 3.5 μm. These significant discrepancies may be due to different mechanism of tooth tissue removal during cavity preparation.

The mechanism of tooth cavity preparation procedure of Er:YAG laser is the removal of tooth substrate effectively and precisely by means of a thermo-mechanical ablation process involving microexplosions [34]. Water flow is necessary to prevent cracking and melting of enamel and dentin, as well

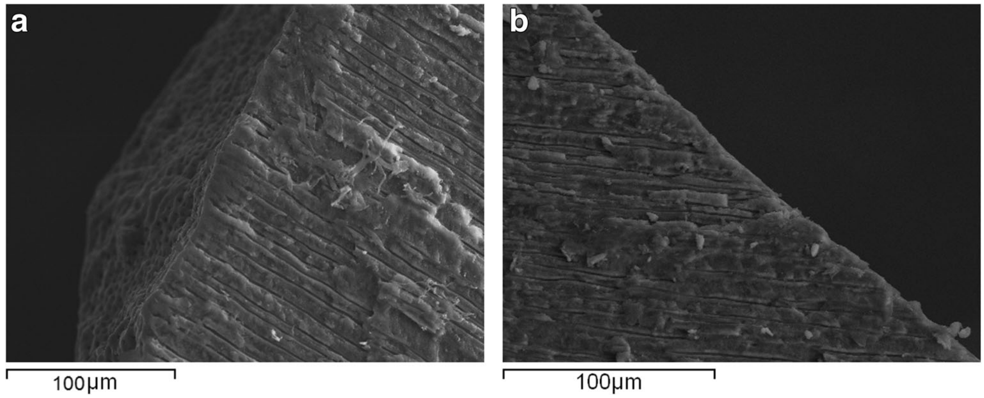

Fig. 5 **a**, **b** Representative SEM images (× 200 magnification) of laser-treated (**a**) and bur-treated (**b**) dentin surfaces after cavity preparations and before adhesive procedures

as thermal damage to dental pulp [35]. A very important difference compared to bur-treated preparation is that after laser treatment, there is no smear layer on dentinal substrate [34]. As a consequence, the entire acid action on the laser-prepared surface was concentrated to the dentin substrate due to the lack of smear layer, which may partially explain the increased porosity on the laser-treated dentin surface.

Morphological characteristics of the dentin surface after Er:YAG laser ablation include a typical scaly, coarse, and irregular surface which are attributed to the microexplosions and volatilization of tooth tissues during laser operation [21, 35, 36]. In the present investigation, the laser-treated groups exhibited rougher and more irregular interface between adhesive agent and dentin substrate describing a dentin surface as rough, relatively inhomogeneous, without smear layer, and with wide open orifices of dentin tubules.

As mentioned before, this study has documented a consistently thicker hybrid layer for the laser-treated samples. The mechanical burs that are mainly used in cavity preparation lead to formation of smear layer of considerable thickness. However, the laser-prepared cavities lack smear layer [37], and the acidity of the etching procedure produces a thicker demineralized zone with exposed collagen fibers. This coincides with the outcomes of the present study. As a result, the adhesive monomers infiltrate deeper the porous dentin forming a rather thick hybrid layer. In this particular case, QSP mode is theoretically anticipated to cause very minimal thermal damage leaving more intact collagen fibrils and possibly more remnant hydroxyapatite crystals for chemical adhesion [19].

Laser cavity preparation requires fast, sharp, precise, and quiet cutting of the dental tissues, as well as minimal amount of residual heat that remains in the tissues following laser irradiation. It has been demonstrated that ablation thresholds of dental tissues are reduced at laser pulses shorter than the thermal relaxation time of the ablated surface [38]. In particular, at shorter pulses, the energy has shorter time to escape from the ablated area, and as a consequence, less heat is transferred into the surrounding tissues, leading to faster ablation and smaller amount of residual heat deposition [39, 40]. However, short laser pulses induce higher-frequency vibrations to tissues, which cause discomfort to patients [41]. Moreover, at longer pulses, an undesirable interaction of the laser beam with the debris cloud takes place, so the influence of debris screening is less pronounced [39, 40]. At short pulses, the absorption of the laser beam is higher in the debris cloud, which is formed above the irradiated tooth surface, resulting in a decreased ablation rate and a less precise cutting. Furthermore, this reheated debris cloud, which falls back to the laser-treated area, provides an additional residual heat deposition [19].

Considering the above shortcomings of the use of laser systems for tooth cavity preparations, it seems that the standard Er:YAG laser protocols for this indication may be insufficient. As a matter of fact, previous studies reported substructural cracks in dentin [27] and significantly lower bonding effectiveness of composite restorations to laser-treated dentin [28]. This structural damage does not only appear to superficial dentin, but also in deeper layers over a thickness of 3–5 μm. For this reason, QSP laser irradiation mode has been suggested as a solution for the residual side effects of Er:YAG laser beam scattering and absorption in the debris cloud [40]. QSP mode ablation is implemented with the efficacy of short pulses, with the advantage of decreased debris screening at longer pulses. This phenomenon is attributed to the duration of each of the pulse (~ 50 μs) which is shorter than the rise time of the debris cloud, while the separation between the pulses (~ 85 μs) is longer than the decay time of the debris cloud [19]. In a recent investigation, Lukac et al. [19] reported that QSP mode presented higher ablation drilling efficacy with lower heat deposition and reduced vibrations compared to standard Er:YAG laser pulse duration modes. This lower heat deposition may be advantageous for the formation of hybrid layer due to lower thermal damage of the collagen fibrils of dentin substrate. In the present study, the thickness of hybrid layer was high for laser-treated groups. Nevertheless, a bond strength test is necessary to confirm the possible beneficial function of QSP mode in adhesion to dentin.

Notwithstanding the thickness of hybrid layer is very important for the quality and longevity of resin bonding to dentin, this is not the only morphological characteristic of resin-dentin interface that affects bond strength [42]. A homogenous and continuous hybrid layer without voids or cracks is more necessary for sufficient adhesion [32]. In the current study, bur-treated specimens exhibited more homogenous and uniform hybrid layers than laser-treated specimens and agree with the results of previous studies [22, 42]. Moreover, it has been claimed that the length of the resin tags does not play an important role regarding the bond strength [42] and, for this reason in the present study, we did not evaluate their formation.

Another crucial parameter for the quality of the adhesion to dentin is the degree of resin impregnation of the collagen network after demineralization of dentin surface by the etching agent. Incomplete resin infiltration of the exposed collagen fibrils results in a partially demineralized zone of dentin beneath hybrid layer which may be deleterious for the longevity of the bond [43]. This zone may appear even when the resin fully impregnated the empty space of each collagen fibril within the exposed collagen network. It has been postulated that the top part of the exposed collagen network at the intertubular dentin is relatively nonporous, leading to collapse of the collagen fibrils due to the brief air drying of the dentin, which prevents the impregnation of the resin [44]. The anticipated porous region of the demineralized dentin may create a nanoleakage pathway at the resin-dentin interface which leads to degradation of the bond structure.

Anchieta et al. [33], who focused the attention on the role of PDD zone on biomechanically unfavorable scenarios for the dental restorations, concluded that PDD at the bottom of the hybrid layer is a critical part of the resin-dentin interface which concentrates high stress levels jeopardizing its mechanical integrity and possibly accelerating bond failure. Consequently, as the thickness of PDD zone increases, the bond strength may reduce. In the present investigation, although the laser-treated groups presented much thicker hybrid layer compared to bur-treated groups, the PDD zone thickness for the laser-treated specimens was also higher than bur-treated specimens.

The outcomes obtained from the current study require in part the rejection of the second null hypothesis ($H_0 2$) which stated that there were no significant differences in thickness of the hybrid layer between etch-and-rinse and self-etch adhesive groups of the same cavity preparation method. In particular, between laser-prepared groups, the specimens treated with the etch-and-rinse adhesive presented higher hybrid layer thickness than those treated with the self-etch adhesive, while between bur-prepared groups, there was no significant difference in hybrid layer thickness. In various previous studies, higher hybrid layer thickness was observed for etch-and-rinse adhesives [32, 45].

Etch-and-rinse adhesives include a separate etching step followed by a compulsory rinsing step. During the etching step, dentin is demineralized in order to remove smear layer and smear plugs and to achieve a porous surface for improved micromechanical retention. Demineralization of dentin surface by the phosphoric acid reaches over a depth of 3–5 μm, exposing a scaffold of collagen fibrils that is nearly totally depleted of hydroxyapatite [32]. After the etching step, resin monomers are applied to infiltrate the exposed collagen network and photopolymerization stabilizes the interlocking of the two phases.

On the other hand, self-etch adhesives contain acidic monomers that simultaneously etch and prime dentin. Consequently, the dissolved smear layer is not removed by rinsing but incorporated in the applied adhesive. The morphological characteristics of the formed hybrid layer by self-etch adhesives are affected by the aggressiveness of their functional monomers [46]. Mild self-etch adhesives such as Clearfil Universal Bond Quick demineralize dentin only shallowly, leaving hydroxyapatite crystals around the collagen fibrils available for possible chemical adhesion. In this case, the adhesive also takes advantage of the 10-methacryloxydecil dihydrogen phosphate (10-MDP) monomer and its high chemical bonding capacity to hydroxyapatite. This shallow hybrid layer may be attributed to incomplete removal of the smear plug from the dentin tubules due to lower acidity. Possibly, in the current study for the same reason, PDD zone was observed lower for the self-etch adhesive in both cavity preparation treatments. However, in the present investigation, the two tested adhesives exhibited similar hybrid layer thickness in bur-treated groups. Maybe the differences in composition of the two adhesives which possibly influence their effectiveness in hybrid layer formation were not so potential for their action in bur-treated groups. In contrast, in laser-treated groups, Gluma 2 bond presented significantly thicker hybrid layer, and as a result, it could be concluded that the modification of the dentin substrate may affect the functionality of the used adhesive systems.

Conclusions

Within the limitations of this in vitro study, it could be concluded that tooth cavity preparations using Er:YAG laser at QSP mode exhibited higher hybrid layer thickness compared to conventional bur-prepared specimens in both adhesive techniques. Moreover, the etch-and-rinse adhesive technique presented thicker hybrid layers but only for laser-treated groups. Although thickness and morphological characteristics of the hybrid layer affect bond strength of a composite restoration, further studies are needed using bond strength tests to confirm the prognosis of the dentin bonding. As a result, the present study indicates that QSP mode for Er:YAG laser cavity preparations may be advantageous for adhesion of composite restorations but more data are necessary to estimate the effectiveness of the method.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals There are no human participants or animals in this study.

Informed consent For this type of study, formal consent is not required.

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