Lasers in Medical Science (2019) 34:1391–1399
https://doi.org/10.1007/s10103-019-02739-y

ORIGINAL ARTICLE

Influence of Er:YAG laser pulse duration on the long-term stability of organic matrix and resin-dentin interface

Livia Tosi Trevelin[1,2] · Beatriz Togoro Ferreira da Silva[1] · Patrícia Moreira de Freitas[1] · Adriana Bona Matos[1]

Received: 2 October 2018 / Accepted: 25 January 2019 / Published online: 14 February 2019

Abstract

The purpose of this study was to explore the influence of Er:YAG laser irradiation with different pulse durations on the organic matrix, micromorphology of the hybrid layer (HL), and bond strength over time. Sixty caries-free human molars were cut to obtain flat dentin surfaces which were randomly divided into 4 groups: control (not irradiated–G1) and laser groups (80 mJ/2 Hz) with pulse duration ranging between 50 (G2), 300 (G3), and 600 μs (G4). A self-etch adhesive system (Universal 3M ESPE) was applied on pre-treated dentin surfaces and cylinders of resin composite were built up and stressed in a universal testing machine (μSBS) at 24 h and after12 months ($n = 12$). In addition, 3 other dentin-bonded specimens were prepared as previously described for each group with the adhesive doped with 0.1 wt% Rhodamine B to analyze hybrid layer morphology under Confocal Laser Microscope Scanning (CLMS). Organic matrix and collagen fibrils were analyzed by second harmonic generation (SGH). Two-way ANOVA and Tukey's test detected significantly higher μSBS values for the control group, whereas the lower values were observed in all laser groups at 24 h ($p < 0.05$). Storage in artificial saliva did not reduce μSBS in all groups. The low signal emitted by SHG images below the irradiated area demonstrated thermal damage of the collagen matrix. CLMS images of laser groups exhibited thicker and irregular resin-dentin interfaces than the control group. Regardless of the pulse duration, Er:YAG laser pre-treatment altered the organic matrix and HL formation which resulted in low μSBS values at 24 h. The alterations on dentin's organic structure did not jeopardize the μSBS after 1 year of saliva storage.

Keywords Er:YAG laser · Bond strength · Dentin · Pulse duration · Long term

Introduction

Adhesion process is obtained through an exchange process in which minerals removed from the hard dental tissue are replaced by resin monomers, forming chemical bonds or micromechanical interlocking, called hybrid layer [1, 2]. This inter-diffusion zone is essential to guarantee the retention of tooth resin-composition fillings. Also, stability and durability are the most desirable features of the hybrid layer.

The dissolution of hydroxyapatite crystals is, therefore, a prerequisite for hybrid layer formation, usually achieved by acid etching, via the etch-rinse technique (ER). Because enamel is a highly mineralized tissue composed of 96 wt% mineral, ~3 wt% water, and only ~1 wt% residual biomacromolecules [3]. On the other hand, dentin presents an intricate mineralized organic matrix, and the adhesion process is based on a complex and technique sensitive mechanism which contributes to the short lifespan of resin composite restorations [1, 4]. The heterogeneity of the structure and composition of bulk dentin makes the adhesion a challenging task.

It is well known that the dentin conditioning with phosphoric acid completely removes the smear layer and leads to a deep demineralization of dentin matrix, which compromises the complete infiltration of resin monomers into the collagen-rich matrix, resulting in a more rapid interface degradation [5, 6]. Also, the use of acidic components, such as phosphoric acid, can expose and activate the host-derived matrix metalloproteinases (MMPs) [6, 7] that might degrade the exposed collagen fibrils [6, 7] also leading to a poor adhesive interface.

✉ Livia Tosi Trevelin
livia.trevelin@usp.br; liviattrevelin@gmail.com

[1] Department of Operative Dentistry, School of Dentistry, University of Sao Paulo, Av. Prof. Lineu Prestes, 2227, Cidade Universitária, São Paulo, SP 05508-000, Brazil

[2] Department of Operative Dentistry, School of Dentistry, University of Cruzeiro do Sul, Av. Rua Galvão Bueno, 868, São Paulo, SP 01506-000, Brazil

Furthermore, other critical factors associated with the use of phosphoric acid are the sensitivity of the technique and the incidence of post-operative tooth sensitivity [1, 8].

To overcome these limitations and aligning them with the technological development of bonding systems, self-etch adhesives (SE) were introduced as a strategy to simplify the clinical practice. SE adhesives interact with the smear layer and underlying dentin without removing the former [8]. This bonding strategy combines acidic monomers that simultaneously etch and prime dental substrates, followed by the infiltration of a hydrophobic adhesive layer. Overall, SE approach could be subdivided as "two-step" and "one-step" adhesives. The two-step, the etching and priming occur simultaneously, and then the bonding is done in a separate step, and one-step simplified adhesive systems combine "conditioning," "priming" and "application of the adhesive resin" incorporated into a single bottle that forms the hybrid layer [8, 9].

The morphological features of the hybrid layer produced by SE adhesives depend to a great extent on the ability of their functional monomers (acidic monomers) to demineralize the dental substrate. Therefore, according to their acidity or etching aggressiveness, SE adhesives can be classified as strong (pH $\leq$ 1), intermediate (pH ~ 1.5) and mild (pH $\geq$ 2) [10]. Briefly, strong SE adhesives produce deep demineralization zone, which interfacial ultra-morphological features resemble those of etch and rinse systems. On the other hand, mild self-etch demineralize dentine partially, leaving substantial amounts of hydroxyapatite around collagen fibrils. These remains allow additional chemical interactions with calcium from hydroxyapatite that contributes for durability and stability of interfaces [8, 9].

Contemplating these two strategies of adhesives, ER and SE, a recent formulation was introduced known as multimode universal adhesives, which is designed to bond to tooth structures via the ER or the SE technique using the same single bottle of adhesive solution [11]. Collectively, these universal adhesives also contain functional resin monomers blends with specific components which create a miscible and stable adhesive cocktail that creates strong bonds with a variety of tooth substrates and dental restorative materials [12].

Moreover, new dentin pre-treatment methods, such as the Erbium laser irradiation, have been widely investigated in the field of adhesive dentistry. The Er:YAG laser has been widely reported as the most effective wavelength for dentin surface pre-treatment [13, 14] and for preserving the healthy dental structure [15, 16]. Its wavelength coincides with the peak of both water and hydroxyl group of hydroxyapatite. During irradiation, the incident energy is highly absorbed at the dentin, inducing sudden heating and water evaporation, which leads to microexplosions and ejection of the particles [17, 18]. The preferential removal of the intertubular dentin (more organic content) creates a scaly and flaky surface with open dentinal tubules and smear layer—free [19]. The micromorphology pattern created should be helpful for bonding to dentin [19, 20].

However, inconclusive results concerning laser pre-treatment and adhesion efficacy are reported in the literature [2, 20–22]. The lack of clarity is associated with different bonding results obtained due to different adhesive systems used [22], a lack of longitudinal study designs, and a great variation on laser parameters settings [22].

The effect of Er:YAG laser pulse duration on the target tissues seems to play a significant role in ablative ability, surface morphology [14, 23], and thermal effects [23, 24]. Shorter pulses are more efficient once all pulse energy is used up for ablation, instead of being converted to heat [25, 26].

To the best of our knowledge, investigating the influence of different pulse durations on bonding effectiveness is considered crucial to grasp the general features of adhesion process over time. Besides, the hybrid layer characteristics, the architecture of organic matrix, and the collagen fibers are valuable structural information necessary for adhesion process and its stability. Multiphoton excitation microscopy constitutes a new method, minimally invasive imaging technique, which provides useful information on the structure of dentin and collagen fibers [27].

Therefore, the purpose of this in vitro study was to investigate the structural modifications on dentin, following the pre-treatment with Er:YAG laser at different pulse durations. Moreover, to verify the impact of this pre-treatment associated with universal adhesives on hybrid layer formation and resin-dentin bond strength over time. The null hypothesis tested were (1) Er:YAG laser irradiation with different pulse durations do not lead to organic alterations on dentin substrate, (2) there are no differences on immediate resin-dentin bond strength (24 h) between laser-irradiated and non-lased group, and (3) the storage in artificial saliva does not affect the long-term stability of the resin-dentin interface of the tested groups.

Materials and methods

Ethical aspects

Sixty caries-free human molars were collected after being approved by the Ethical Research Committee of the School of Dentistry, University of São Paulo, Brazil (Protocol: 124143/2015). The teeth were stored in distilled water at 4 °C and used within 3 months after extraction.

Sample preparation and surface pre-treatment

The crowns were removed to expose the mid-coronal dentin and the roots were cut 3 mm below the cement-enamel-junction (CEJ) with a diamond saw (Isomet 1000, Buehler Ltd., Lake Buff, IL, USA) under water cooling. Specimens

Table 1 Description of the experimental groups

Group ($n = 12$)	G1	G2	G3	G4
Energy (mJ)	_____	80	80	80
Hertz (Hz)	_____	2	2	2
Pulse width (μs)	_____	50	300	600
Peak power density (W/cm^2)	_____	251,572	41,823	20,911
Peak Power (W)	_____	1600	266	133
ED (J/cm^2)	_____	12.58	12.58	12.58

The output power of the Er:YAG laser system was measured every three irradiated samples using a power meter for high-power lasers

were embedded in auto-polymerizing acrylic resin (JET, Clássico, São Paulo, Brazil) using polyethylene molds (15 mm in diameter) with the mid-dentin surface facing upwards. Subsequently, the dentin surface was wet ground (Ecomet 6 / AUTOMET 2 - Buehler Ltd., Lake Bluff, IL, USA) with #320, #400 silicon carbide sandpapers until no enamel remained on the surface and finalized with 600-grit (Buehler Ltd., Lake Bluff, IL, USA), for 60 s to standardize the smear layer.

Specimens were randomly divided into 4 experimental groups. In group 1, no laser pre-treatment was performed (G1: control). In groups 2, 3, and 4, the dentin surface was irradiated using an Er:YAG laser (Fidelis ERIII 1000 model, Fotona, Slovenia) working at a wavelength of 2.94 μm and with a beam diameter of 0.9 mm. The parameters considered for irradiation were 2 pulses s, 80 mJ/pulse. With movement of the handpiece and no overlap of pulses, each pulse produced 12.58 J cm2 at the dentin surface. Continuous air-water spray (40% of air/60% of water = 21 ml/min) was used throughout the study. The pulse durations studied were 50 μs (G2), 300 μs (G3), and 600 μs (G4) (Table 1). The handpiece (R02) was fixed at a designed apparatus holder which delivered the laser beam perpendicular to the dentin surface at a focal distance of 7 mm from the target point (focused mode). For irradiation, a translate motor device XYZ (ESP301, Newport Corporation, Invine, CA, USA) was used to guarantee a standard of irradiation for all groups by previously commands established through a computer connected to the scanning device. The motor device was automatically moved with speed (0.8 mm/s) and distance (600 um) of specimen displacement between laser pulses, thereby avoiding the creation of any gaps between the laser pulses.

Microshear bond strength (μSBS)

Following the completion of the surface pre-treatment, a universal adhesive system (3M ESP batch #15119000505; 3M ESPE) was applied on the dentin specimens, in a self-etch mode and according to manufacturer's instructions (Table 2). After, a total of eight composite resin (Z350- Filtek batch#1511900505; 3M ESPE) cylinders per tooth were prepared by filling composite resin into Tygon tubes (1.0-mm internal diameter and 1.0-mm thickness). The cylinders were then light cured (Radii Plus, SDI Limited, Australia) for 20 s each, with the intensity of 500 mW/cm^2, measured by the radiometer coupled to the light curing unit.

After 24 h of storage in distilled water, four cylinders per tooth were immediately submitted to microshear bond strength test while the other four cylinders were kept stored in artificial saliva for 12 months. Artificial saliva was replaced every 14 days. The shear bond strength was measured by a universal testing machine (Instron 5942; Canton) at a cross-head speed of 1.0 mm/min. Shear bond strength (MPa) was calculated from the peak load at failure divided by the specimen surface area. One operator evaluated the fracture surfaces with a stereomicroscope at × 50 magnification. Failure modes were classified according to the predominant mode of fracture: cohesive, adhesive, or mixed.

Table 2 Adhesive system (batch number), composition, and application mode, according to the manufacturer's instructions

Adhesive	Composition	Self-Ecth
Scotchbond Universal Adhesive	Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, and silane	1. Apply the adhesive to the entire dental surface using a disposable applicator for 20 s. If necessary, rewet the disposable applicator during the treatment. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely. 3. Light polymerize for 10 s.

Micromorphology of the hybrid layer and the dentin organic matrix

Twelve additional dentin-resin bonded specimens ($n = 3$) were prepared, as previously described, for each experimental group using the same adhesive system but doped with 0.1 wt% of Rhodamine B. Prior to the application of the adhesive with Rhodamine B on the dentin surface, the bottle was kept under agitation for 2 h. The composite increment was placed over the adhesive system and light cured for 20 s. After 24 h storage at 37 °C, the specimens were serially mesio-distal sectioned into several 1-mm thick slabs using a diamond saw, under refrigeration. The specimens were cleaned with sonication and simultaneously examined under Confocal Laser Scanning Microscopy (CLSM) (Confocal Zeiss LSM 780-NLO EC Plan – Neo-fluar 40x/1.30 oil DIC M27, at 543 nm excitation with HeNe laser) for the analyses of the micromorphology of the hybrid layer and under Second Harmonic Generation (SHG) (at 380 nm excitation with a chroma filter of 400/40M 2P) to analyze the organic matrix and collagen fibrils. After then, the specimens were further incubated at 37° with artificial saliva for 12 months for long-term assessment. During the aging, the artificial saliva was replaced every 2 weeks. The long-term analyses were performed with the same microscopes parameters above described.

Statistical analysis

The statistical analyses were performed with SPSS-22 (IBM Corp., NY, US). Normal distribution of the microtensile bond strength data was confirmed by Kolmogorov–Smirnov test. Data were analyzed by two-way ANOVA and post-hoc Tukey tests ($p < 0.05$). Microscope Confocal laser Scanning and Second Harmonic Generation have consisted of an observational evaluation only, so no statistical analysis was performed and only visual differences among experimental groups were considered findings.

Results

Microshear bond strength

Mean values and standard deviations of the microshear bond strength and failure modes are depicted in Table 3. Two-way ANOVA revealed significant difference for the factor "treatment" ($p = 0.000$) but no statistical significance difference for the factor "time" ($p = 0.294$) neither for the interaction between these two factors ($p = 0.299$).

The control group (G1) exhibited immediate higher values of μSBS with statistical significance difference ($p < 0.001$), whereas the lower values were exhibited by the laser-treated groups (G2–G4). No differences were found among laser groups, regardless of the pulse duration investigated. For all the experimental groups, storage in artificial saliva for 12 months did not reduce the bond strength values ($p = 0.29$) when compared with the immediate bond strength (24 h).

Table 3 Microshear bond strength (μSBS) values (means ± standard deviations) of the different experimental groups

Group	24 h	12 months
G1	26.17 ± 3.78 A	24.97 ± 4.70 A,C
G2	22.14 ± 2.86 B	22.85 ± 2.95 B,C
G3	21.25 ± 3.04 B	22.13 ± 2.99 B,C
G4	20.62 ± 1.86 B	23.01 ± 2.94 B,C

* Similar capital letter means there is no statistically significant difference between lines ($p < 0.05$)

The groups G1 and G2 exhibited only mixed failures whereas the groups G3 and G4 exhibited mixed failures of 90% and 95%, respectively, followed by adhesive failures.

Micromorphology of the hybrid layer and the dentin organic matrix

In the immediate assay, the group G1 (control) exhibited a flat dentin surface with a thin hybrid layer as well as a tiny adhesive layer, which were regular and uniform through the entire surface. The resin tags were short and equally distributed through the surface (Fig. 1a).

The G1 reveal an organized organic matrix through the contrast provided by surrounding collagen and dentin tubules with strong signal of SGH. The intertubular dentin is characterized by a strong signal of SGH. The collagen signal is well visible through the entire area of the dentin surface. The tubules appear as dark canals and could be observed due to the strong SHG signal of the surrounding collagen fibers (Fig. 1a). Regardless of the storage in saliva for 12 months, significant alterations into organic matrix and hybrid layer were not observed in the control group (Fig. 1a, e).

In group G2, the hybrid zone showed a non-uniform pattern due to the irregularities created by the Er:YAG laser irradiation (Fig. 1b). Also, the adhesive layer is thicker than in group G1. The resin tags are more evident and longer when compared with group G1 (Fig. 1a, b). Saliva storage increased the Rhodamine B porosities filled, which is represented by high Rhodamine B intensity in the hybridization zone (Fig. 1f). Below the irradiated dentin surface, the SGH signal disappeared, indicating changes in the organic matrix after the laser irradiation in G2 group (Fig. 1b, f). Toward deeper dentin, the SGH emission provides information about the microarchitecture of the dentin (dentin tubules, peritubular,

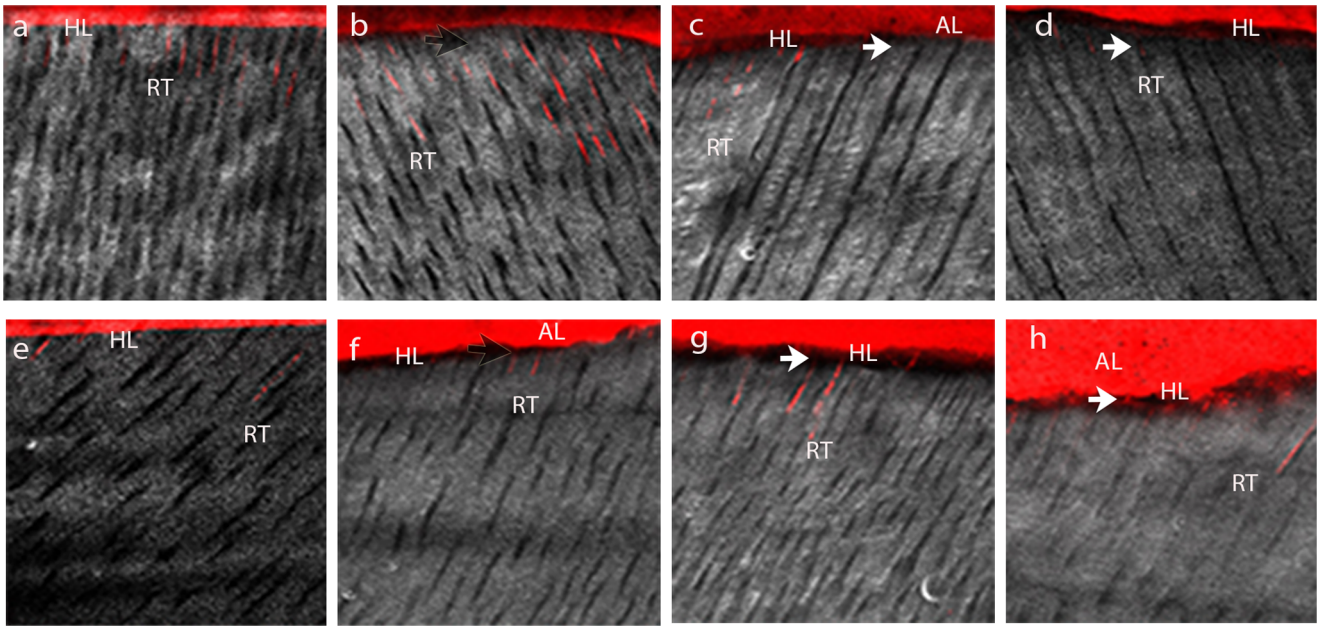

Fig. 1 Combined multiphotin CLSM and SHG images of the resin-dentin interfaces for control (G1) and laser groups (G2–G4). The adhesive was stained with 0.1 wt% Rhodamine B showing red fluorescence color. For the SHG images, a gray signal is emitted for the intertubular dentin and collagen fibers, producing a white signal. Control group (**a**, **e**) and laser groups (80 mJ/2 Hz), varying pulse duration between 50 μs (**b** and **f**), 300 μs (c and g), and 600 μs (d and h). The images (**a**, **b**, **c**, and **d**) represent the experimental groups at 24 h and the images (**e**, **f**, **g**, and **h**) were obtained after 12 months of saliva storage. Uniform and thin hybrid layer was created on a flat dentin surface with short resin tags (**a**, **d**). In laser groups, an irregular adhesive layer was observed with a non-uniform hybrid layer (**b**, **c**, **f**, and **g**). Long resin tags were observed only in specimens irradiated with 50 μs pulse duration (**b**) while 300 μs and 600 μs pulse durations exhibited short and sparsely resin tags distributed through the images. (HL, hybrid layer; AL, adhesive layer; RT, resin tags). SHG signal showed the structure of dentin; the lumen of the tubules appears in black; and the intertubular dentin in gray, collagen fibrils. Control group maintained the typical configuration of the dentin organic matrix and collagen fibers through the strong signal of SGH (**a**, **d**). A narrow zone of the altered organic matrix was observed below the irradiated dentin represented by low SHG signal (black arrow) (**b**, **f**). For long pulses, the organic matrix degradation compromised a thicker zone, which exhibited black voids underneath the irradiated area, represented by the absence of SHG signal (white arrow) (**c**, **g**, **d**, and **h**). Magnification × 40

and intertubular dentin). As observed in the control group (G1), the dentinal tubules appeared as dark canals. After 12 months of saliva storage, the SHG emission decreases below the irradiated surface, pointing out the deterioration of both the organic matrix and the collagen fibers in group G2 (Fig. 1f).

In groups G3 and G4 (300 and 600 μs, respectively), the micromorphology of the hybrid layer is more irregular and inconsistent when compared with groups G1 and G2 (Fig. 1a–d). Resin tags were present, but were short and sparsely distributed through the surface and were poorly infiltrated by resin (Fig. 1c, d). After storage in saliva for 12 months, the degradation process of the hybrid layer became worse (Fig. 1g, h). The porous adhesive interfaces were evident due to the higher concentration of Rhodamine B intensity, where the entrapped water occurred within the resin-dentin interface (Fig. 1g, h). In both groups (G3 and G4), the absent signal of SHG below the irradiated dentin provides precise information on the degradation of the dentin organic matrix and modifications of the collagen matrix structure. Also, the storage time decreased the SHG contrast, pointing toward the destruction of the collagen matrix (Fig. 1c, d, g and h).

Discussion

Dental irradiation with the Er:YAG laser is considered a safe procedure with little increase in the temperature (< 5.5 °C) [28], causing minimal thermal damage for pulp and for surrounding tissues [29]. Even though little temperature changes are expected, the present data showed that dentin irradiation with the Er:YAG laser resulted in damage to this tissue structures, regardless of the pulse duration used (50, 300, or 600 μs) for the pre-treatment protocol. Laser-irradiated dentin surfaces were characterized by an alteration of the organic matrix, rejecting the first null hypothesis given in the current study.

The interactions of erbium lasers with target dental tissues depend on several parameters. Energy per pulse and pulse frequency are directly associated with the ablation capacities of erbium lasers. Also, it is pivotal to note that uncontrolled energy density deposition can produce undesirable thermal damage, though.

An accurate choice of the laser pulse duration and an adequate air-water tissue cooling system are required in order to limit undesired effects throughout dentin irradiation [13]. Shorter pulse durations are expected to increase the ablation

rate of hard dental tissues with less heat generation [30, 31]. The ablation promoted by short pulses occurs at a higher speed of ablation than the diffusion of heat into the tissue, while long pulses do not allow the tissue relaxation time, resulting in deeper thermal damage [30–32].

The long pulses are considered more harmful for surrounding tissues than short pulses [23, 31, 33]. Although, our results do not corroborate with the literature, since all pulse widths investigated showed similar structural modifications in the organic matrix and collagen fibrils, clearly observed by a reduction of SGH signal intensity below the irradiated area. Alterations of the organic matrix with fused collagen fibers were previously described in irradiated dentin [20]. In contrast, the microarchitecture of the intact dentin revealed their organization and the presence of collagens fibers perpendicular to the tubule, which exhibited a strong SGH signal through the entire surface of the control group.

The Er:YAG laser irradiation promoted a significant reduction on the organic compounds of dentin and chemical assessment revealed deformation of the organic matrix [13, 34]. The laser irradiation yields a high steam pressure and vaporization of the water entrapped into the tissue, that overheated, caused micro-explosions of the hard tissue [15]. Previous findings showed that irradiation with the Er:YAG laser does not affect the gradient in mineral content in dentin surface, but promoted the greatest change in collagen content with a decomposition of proteins as demonstrated by spectroscopy analyses [34]. The chemical analyses showed an absence of Amide I, Amide III, and protein intensity, which can indicate damage or removal of collagen fibrils [34]. Previous findings are in accordance with our study, since we demonstrated an absence of SGH signal underneath the irradiated area in all pulse durations investigated.

Moreover, previous studies reported that the Er:YAG laser irradiation produces changes in the composition and conformation of the organic matrix at the dentin surface, which results in partial collagen degradation and 3–5 μm of denatured subsurface [20, 21, 35] while others showed a range from 1.5 to 2.5 μm of denatured subsurface when the same protocol of pre-treatment was used [32]. Investigations on the nanohardness properties and elastic modulus of irradiated dentin showed that thermal damages could affect the underlying dentin up to 10 μm depth, whereas no difference was detected between irradiated dentin and non-irradiated dentin from 15 to 50 μm depth [34]. Even though the analysis considered for the present study does not allow precise measurement of the altered depth zone, the results are in agreement with previous one [34]. Likewise, from the irradiated dentin toward the deep dentin zone, the organic matrix showed a strong SGH signal, as observed in the control group, indicating an organized organic matrix and the arrangement of the intertubular collagen fibrils.

The denatured collagen fibrils found for the irradiated dentin were fused and were devoid of interfibrillar spaces which compromised the adequate diffusion of the resin monomer into the interfibrillar space [20], thus compromising resin adhesion bond when compared with the non-irradiated group (control). The presence of the remnant denatured collagen fibrils had a deleterious effect on the immediate bond strength, which also leads to the rejection of the second null hypothesis tested. It was verified that there was a difference between the non-lased and laser-irradiated groups, regardless of pulse durations tested.

The absence of smear layer, a roughness pattern, and open dentinal tubules created after laser ablation are expected to promote suitable bonding [19]. The laser protocol used in the present study, however, did not improve the immediate bond strength of resin to dentin when compared to the control group. The organic matrix and collagen fibers were modified by the ablation phenomenon and specimens showed a non-uniform thickness of the adhesive layer [36]. This fact jeopardizes the proper evaporation of the solvent and decreases the bond strength.

In this study, a multi-mode universal adhesive was applied in a self-etch approach, which is based on the use of non-rinse acidic monomers that simultaneously condition, prime, and bond tooth tissues creating hybrid layers that are approximately 0.5 μm thick (pH 2; 0.5–1 μm interaction depth) [8, 9] with the advantage of avoiding excessive decalcification, which is a characteristic of the etch-rinse technique [37]. As observed in the CLSM images, the self-etch adhesive displayed a specific pattern on dentin. The self-etch adhesive system showed short resin tags, less widening of the tubules, and did not show the funnel shape appearing, commonly achieved with the use of the phosphoric acid. However, in specimens irradiated with the 50 μs pulse width the intratubular permeability of dentin increased, as shown by long resin tags that diffused through the dentin surface. Some authors [19] have also reported that resin tags were more pronounced in dentin prepared using a laser than in that prepared using a bur, regardless of the adhesives applied. Nevertheless, the long resin tags had no effect on the immediate bond strength, corroborating with the idea that micromechanical interaction is not the factor responsible for bonding to dentin, but the entire resin-dentin interactions and integrity of this substrate [35, 38]. Our results clearly indicate that 12 months of storage in artificial saliva was not sufficient to cause severe degradation of adhesives or their respective hybrid layers, regardless of the pre-treatment performed. Based on these findings, the third null hypothesis tested was accepted. These findings could be related to the composition of the universal adhesive, which contained the acidic monomer 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP), which provides ionic bond with the calcium of the hydroxyapatite crystal. Overall, 10-MDP is the most popular and highly stable acidic monomer; its stability is attributed to the long carbonyl chain (space) between the functional and the polymerizable groups in the monomer structure [39]. Also, the MDP-Ca salt deposition along the interfaces

might explain the high bond stability [40, 41] according to their low dissolution rate in water [42].

It has been reported that chemical interaction performed by 10-MDP did not increase the immediate bond strength to dentin, but biodegradation resistance studies of adhesive interfaces have shown that it enhanced the bond durability [42, 43] by protecting the hybrid layer from hydrolytic degradation [8]. Although these findings were performed in non-irradiated dentin, these results can explain the findings of the present study.

Regarding irradiated dentin, only a few studies investigated the laser-irradiated dentin adhesive bond stability. Karadas et al [2] compared the bond stability between lased (energy densities of 19.9 J/cm2 and 15.7 J/cm2) with non-lased dentin and reported no significant difference in bond strength values after aging methods (thermocycling–15,000 cycles) when self-etch adhesive agent was used. Their findings are consistent with the current study since we used a lower energy density than the previous report. Our study used an energy density of 12.58 J/ cm2 and as expected, did not show a significant difference in the comparison of bond strength between control and irradiated groups after 1 year of storage in artificial saliva. Akin et al. [44] examined the performance of all-in-one bonding agents applied to an occlusal surface irradiated with the Er:YAG laser (200 mJ, 10 Hz, 2 W) after water storage for 6 months and thermocycling (10,000 cycles). In their study, the performance of the respective bonding agents was also not affected by the aging methods.

The final dentin surface morphology will be a result of the techniques used to prepare the tissue as well as the constituents of this tissue and the adhesive system used. Also, when the cavity is performed with an Er:YAG laser, the parameters must influence the morphology and the thermal conditions of the remnant tissue. The lack of longitudinal studies involving lased dentin and considering the heterogeneity in parameter's settings/methodologies hinder the establishment of a reliable comparison of among the literature on this subject.

Although the thermal damage of the ablation process could be observed in this in vitro study, these permanent alterations were not enough to impair the bond strength values after 1 year of artificial saliva storage. Further studies investigating the effect of laser irradiation should be performed in order to determine a gold standard protocol for clinical applicability.

Conclusions

Based on the results of this in vitro study, the following can be concluded:

- The pre-treatment of the dentin surface with the Er:YAG laser, regardless of the pulse duration, did not improve the immediate bond strength of resin-dentin when compared to the control group.
- The Er:YAG laser irradiation altered the organic matrix and denatured collagen fibers below the irradiated area.
- The permanent alterations within organic structures of dentin following Er:YAG laser irradiation were not enough to decrease the bond strength values after 12 months of saliva storage.

Acknowledgments The authors would like to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support given for the purchase of the laser equipment (Fidelis III-Fotona), Grant # 2015/12651-1.

Funding This study was funded by FAPESP (2015/12651-1 and 2015/13571-1).

Compliance with ethical standards

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

Ethics approval Human third molars used in this in vitro study followed the approval of the Human Research Ethics Committee of the School of Dentistry of the University of São Paulo, Brazil (Protocol n. 124143/2015).

References

1. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P et al (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 28:215–235
2. Karadas M, Çağlar İ (2017) The effect of Er:YAG laser irradiation on the bond stability of self-etch adhesives at diferente dentin depths. Lasers Med Sci 32(5):967–974. https://doi.org/10.1007/s10103-017-2194-x
3. Gordon LM, Cohen MJ, MacRenaris KW, Pasteris JD, Seda T, Joester D (2015) Dental materials. Amorphous intergranular phases control the properties of rodent tooth enamel. Science 13(347):746–750
4. Agee KA, Prakki A, Abu-Haimed T, Naguib GH, Nawareg MA, Tezvergil-Mutluay A, Scheffel DL, Chen C, Jang SS, Hwang H, Brackett M, Grégoire G, Tay FR, Breschi L, Pashley DH (2015) Water distribution in dentin matrices: bound vs. unbound water. Dent Mater 31(3):205–116
5. Li B, Zhu X, Ma L, Wang F, Liu X, Yang X, Zhou J, Tan J, Pashley DH, Tay FR (2016) Selective demineralisation of dentine extrafibrillar minerals-a potential method to eliminate water-wet bonding in the etch-and-rinse technique. J Dent 52:55–62
6. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, Pashley DH, Cadenaro M (2016) Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability –a literature review. Dent Mater 32(2):e41–e53. https://doi.org/10.1016/j.dental.2015.11.007
7. Carrilho MR, Carvalho RM, De Goes MF, Di Hipolito V, Geraldeli S, Tay FR et al (2007) Chlorhexidine preserves dentinbond in vitro. J Dent Res 86:90–94
8. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL (2011) State of the art of self-etch adhesives. Dent Mater 27:17–28

9. Tsujimoto A, Barkmeier WW, Takamizawa T, Watanabe H, Johnson WW, Latta MA, Miyazaki M (2017) Comparison between universal adhesives and twostep self-etch adhesives in terms of dentin bond fatigue durability in selfetch mode. Eur J Oral Sci 125:215–222
10. De Munck J, Van Meerbeek B, Vargas M et al (2005) One day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. Oper Dent 30:39–49
11. Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B et al (2012) Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine. J Dent 40:475–484
12. Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, Chen JH, Pashley DH, Tay FR (2015) Bonding of universal adhesives to dentine–old wine in new bottles? J Dent 43(5):525–536. https://doi.org/10.1016/j.jdent.2015.03.004
13. Camerlingo C, Lepore M, Gaeta GM, Riccio R, Riccio C, De Rosa A, De Rosa M (2004) Er:YAG laser treatments on dentine surface: micro-Raman spectroscopy and SEM analysis. J Dent 32(5):399–405
14. Trevelin LT, Marques MM, Aranha AC, Arana-Chavez VE, Matos AB (2015 Jun) Effect of super short pulse Er:YAG laser on human dentin-scanning eléctron microscopy analysis. Microsc Res Tech 78(6):472–478. https://doi.org/10.1002/jemt.22496
15. Hibst R, Keller U (1989) Experimental studies of the application of the Er: YAG laser on dental hard substances: I. Measurement of the ablation rate. Lasers Surg Med 9(4):338–344
16. Burkes Junior EJ, Hoke J, Gomes E, Wolbarsht M (1992) Wet versus dry enamel ablation by Er: YAG laser. J Prosthet Dent 67(6):847–851
17. Eberhard J, Eisenbeiss AK, Braun A, Hedderich J, Jepsen S (2005) Evaluation of selective caries removal by a fluorescence feedback-controlled Er:YAG laser in vitro. Caries Res 39(6):496–504
18. Kilinc E, Roshkind DM, Antonson SA, Antonson DE, Hardigan PC, Siegel SC, Thomas JW (2009) Thermal safety of Er:YAG and Er, Cr:YSGG lasers in hard tissue removal. Photomed Laser Surg 27(4):565–570
19. Aranha AC, De Paula EC, Gutknecht N, Marques MM, Ramalho KM, Apel C (2007) Analysis of the interfacial micromorphology of adhesive systems in cavities prepared with Er,Cr:YSGG, Er:YAG laser and bur. Microsc Res Tech 70(8):745–751
20. Ceballos L, Toledano M, Osorio R, Tay FR, Marshall GW (2002 Feb) Bonding to Er-YAG- laser-treated dentin. J Dent Res 81(2): 119–122
21. Jiang Q, Chen M, Ding J (2013) Comparison of tensile bond strengths of four one-bottle self-etching adhesive systems with Er: YAG laser irradiated dentin. Mol Biol Rep 40:7053–7059
22. Guven Y, Aktoren O (2015) Shear bond strength and ultrastructural interface analysis of different adhesive systems to Er:YAG laser-prepared dentin. Lasers Med Sci 30:769–778
23. Brandao CB, Contente MM, De Lima FA et al (2012) Thermal alteration and morphological changes of sound and demineralized primary dentin after Er:YAG laser ablation. Microsc Res Tech 75: 126–132
24. Lukac M, Marko M, Ladislav G (2004) Super VSP Er:YAG pulses for fast and precise cavity preparation. J Oral laser Applic 4:171–173. Medicine 9(4):338–344
25. Grgurevic J, Grgurevic L, Miletic I, Karlovic Z, Jukic Krmek S, Anic I (2005) In vitro study of the variable square pulse Er: YAG laser cutting efficacy for apicectomy. Lasers Surg Med 5:347–350
26. Baraba A, Kqiku L, Gabrić D, Verzak Ž, Hanscho K, Miletić I (2018) Efficacy of removal of cariogenic bacteria and carious dentin by ablation using different modes of Er:YAG lasers. Braz J Med Biol Res 51(3):e6872. https://doi.org/10.1590/1414-431X20176872.
27. Terrer E, Panayotov IV, Slimani A, Tardivo D, Gillet D, Levallois B, Fejerskov O, Gergely C, Cuisinier FJ, Tassery H, Cloitre T (2016) Laboratory studies of nonlinear optical signals for caries detection. J Dent Res 95(5):574–579. https://doi.org/10.1177/0022034516629400
28. Oelgiesser D, Blasbalg J, Ben-Amar A (2003) Cavity preparation by Er–YAG laser on pulpal temperature rise. Am J Dent 16:96–98
29. Hossain M, Nakamura Y, Yamada Y, Kimura Y, Nakamura G, Matsumoto K (1999) Ablation depths and morphological changes in human enamel and dentin after Er: YAG laser irradiation with or without water mist. J Clin Laser Med Surg 17(3):105–109
30. Majaron B, Lukac M, Sustercic D, Funduk N, Skaleric U (1996) Threshold and efficiency analysis in Er:YAG ablation of hard dental tissue. Proc SPIE 2922:233–242
31. Krmek SJ, Miletic I, Simeon P, Mehicić GP, Anić I, Radisić B (2009) The temperature changes in the pulp chamber during cavity preparation with the Er:YAG laser using a very short pulse. Photomed Laser Surg 27:351–355
32. Trevelin LT, Silva BT, Arana-Chavez VE, Matos AB (2018) Impact of Er:YAG laser pulse duration on ultrastructure of dentin collagen fibrils.2018. Lasers Dental Sci 15:1–7. https://doi.org/10.1007/s41547-017-0020-1
33. Firat E, Gurgan S, Gutknecht N (2012) Microtensile bond strength of an etch-and-rinse adhesive to enamel and dentin after Er:YAG laser pretreatment with different pulse durations. Lasers Med Sci 27(1):15–21
34. He Z, Chen L, Hu X, Shimada Y, Otsuki M, Tagami J, Ruan S (2017) Mechanical properties and molecular structure analysis of subsurface dentin after Er:YAG laser irradiation. J Mech Behav Biomed Mater 74:274–282. https://doi.org/10.1016/j.jmbbm.2017.05.036
35. Ramos AC, Esteves-Oliveira M, Arana-Chavez VE, de PaulaEduardo C (2010) Adhesives bonded to erbium:yttrium–aluminum– garnet laser-irradiated dentin: transmission electron microscopy, scanning electron microscopy and tensile bond strength analyses. Lasers Med Sci 25(2):181–189
36. Ferreira LS, Apel C, Francci C, Simoes A, Eduardo CP, Gutknecht N (2010) Influence of etching time on bond strength in dentin irradiated with erbium lasers. Lasers Med Sci 25(6):849–854
37. Arhun N, Arman A, Sesen C, Karabulut E, Korkmaz Y, Gokalp S (2006) Shear bond strength of orthodontic brackets with 3 self-etch adhesives. Am J Orthod Dentofac Orthop 129:547–550
38. Nakabayashi N, Pashley DH (1998) Hybridization of dental hard tissues, 1st edn. Quintessence, Tokyo
39. Bedran-Russo A, Leme-Kraus AA, Vidal CMP, Teixeira EC (2017) An overview of dental adhesives systems and the dynamics tooth-adhesive interface. Dent Clin N Am 61(4):713–731. https://doi.org/10.1016/j.cden.2017.06.001 **Review**
40. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T et al (2012) Self-assembled nano-layering at the adhesive interface. J Dent Res 91:376–381
41. Feitosa VP, Sauro S, Ogliari FA, Ogliari AO, Yoshihara K, Zanchi CH, Correr-Sobrinho L, Sinhoreti MA, Correr AB, Watson TF, Van Meerbeek B (2014 Dec) Impact of hydrophilicity and length of spacer chains on the bonding of functional monomers. Dent Mater 30(12): e317–e323. https://doi.org/10.1016/j.dental.2014.06.006
42. Tsuchiya K, Takamizawa T, Barkmeier WW, Tsubota K, Tsujimoto A, Berry TP, Erickson RL, Latta MA, Miyazaki M (2016) Effect of a functional monomer (MDP) on the enamel bond durability of single-step self-etch adhesives. Eur J Oral Sci 124(1):96–102

43. Erhardt MC, Rodrigues JA, Valentino TA, Ritter AV, Pimenta LA (2008a) In vitro microTBS of one-bottle adhesive systems: sound versus artificially-created caries- affected dentin. J Biomed Mater Res B Appl Biomater 86(1):181–187

44. Akin GE, Herguner-Siso S, Ozcan M, Ozel-Bektas O, Akin H (2012) Bond strengths of one-step self-etch adhesives to laser-irradiated and bur-cut dentin after water storage and thermocycling. Photomed Laser Surg 30(4):214–221