Effect of Er:YAG laser energy densities on thermally affected dentin layer: Morphological study

P. Nahas [1], T. Zeinoun [2], M. Namour [3], T. Ayach [3], S. Nammour [3]

1: Lebanese University, Department of Restorative and Esthetic Dentistry, Beirut, Lebanon.
2: Lebanese University, Department of Oral and Maxillo-Facial Surgery, Beirut, Lebanon.
3: University of Liege, Department of Dental Sciences, Belgium

Background and aims: Physical and chemical composition of dentin is subject to modification when irradiated with Er:YAG laser. Temperature rise causes water evaporation and micro-mechanical ablation of dentin. The misuse of laser parameters could affect negatively dentin collagen fibers leading to failure in bonded composite restorations.
The aim of this *in vitro* study was to evaluate the effect of Er:YAG laser radiation at different levels of energy on the morphology of thermally affected dentin layer.
Materials and methods: Forty-eight freshly extracted human third molars were randomly divided into six groups (n = 8). In all groups, except for the control groups, dentin was subject to irradiation with H02 handpiece Er:YAG laser in non-contact mode (SSP mode = 50 µs; 10 Hz; speed of 1 mm/second; air 6 mL/min; and water 4 mL/min) with the following levels of energy (40, 60, 80, 100, and 120 mJ) respectively. Teeth were sliced longitudinally. Photo-ablated cavities were observed. The cavity depth and dentin fiber collagen deterioration were measured.
Results: Laser irradiation increased the depth of dentinal crater from 46.57 µm to 178.2 µm, when energy level increased from 40 mJ to 120 mJ. A superficial black layer, representing dentinal affected collagen fibers, was present in all groups except for control group. When comparing the thickness of the black layer, there was no significant difference between groups. It increased at 40 mJ to 28.17 µm then decreased to 15.19 µm at 60 mJ and then increased again for 80 mJ to 19.93 µm, 100 mJ to 22.87 µm and 120 mJ to 28.53 µm. Only one group (60 mJ) showed low values and significant difference as compared to the other irradiated groups, when multiple comparisons tests (ANOVA) were made using Newman-Keuls test.
Conclusion: Dentin organic matrix presented the minimum alteration when Er:YAG laser is used specifically at an appropriate level of energy (60 mJ).

Key words: Dentistry • Dentin • Collagen fibers • Laser • Er-YAG.

Introduction

Used to cut and prepare dentin hard tissues, Er:YAG laser has a large spectrum of parameters when irradiating teeth. It starts from energy level, to action mode (peak time), frequency, and cooling with air/water. All of these factors are combined with different types of handpieces and fiber tips [1)]. When laser light hits dental hard tissue, whether for carious removal, cutting or modifying enamel or dentin surfaces [2-4)], part of the energy is converted into heat which may cause alterations in the physical and chemical composition [5)], while the other part is transformed into micro-mechanical ablation [6-9)]. Thus, treatment should be set in a way to proceed with minimal damage to dental tissues as well as for pulp vitality [10)]. Heat transmitted inside the tissue is considered to be the most dangerous fraction of the treatment. Several studies agreed that erbium lasers' effect is superficial and acts by dental hard tissue ablation as well as eliminating dentinal smear layer [11, 12)].

Lasers used on vital teeth, for cavity preparations, are practically followed by composite restoration. Unfortu-

Addressee for Correspondence:
Dr Paul Nahas
Department of Restorative and Esthetic Dentistry, Lebanese University, Campus Rafic Hariri, Hadath, Beirut, Lebanon
Mobile: 961-3-742459
E-mail: paulnahas@yahoo.com

Received date: January 31st, 2018
Accepted date: February 21st, 2018

nately, research has proven the inefficiency of lasers to enhance bond strength (micro-tensile or shear bond strength) without the use of acid-etch before applying bonding and composite [1, 13-18)]. Moreover, structural changes from shallow to deep irradiated dentin have impact on the success of bonded restorations. It has been demonstrated that shallow dentin can affect negatively bond strength when compared to deep dentin [19, 20)]. In other studies, success was reported especially when using short-pulsed Er:YAG laser [21-24)]. Moosavi *et al.* (2016) demonstrated an enhancement in the mechanical and compositional properties of dentin exposed to laser [25)]. Cavities prepared with laser followed by conventional composite restoration were reported to have the best clinical performance over a period of 12 months [26)]. However, only a small number of studies have discussed the origin behind failure or success in bonding to tooth structure, especially on dentin.

Bonded restorations should infiltrate dentinal tubules, as well as create hybrid layer. Erbium lasers can demineralize dentin, eliminate smear layer from the surface of irradiated teeth, and open dentinal tubules for bonding infiltration [27)], as well as remove hydroxyapatite from inter and peri-tubular dentin and affect the exposed dentinal organic component. Thus, collagen fiber web also seems to be thermally affected by laser ablation. A significant increase in micro-tensile bond strength was observed when 90 s acid-etch was applied on dentin after Er:YAG laser irradiation [28)]. Moreover, hybrid layer formation has never been proven or shown in latest published reports when skipping acid-etch step and applying bonding systems immediately after laser irradiation [15, 29, 30)].

Collagen fibers, (type I in majority), are the main component of dentin organic matrix (90%), while the other 10% are non-collagenous proteins [31, 32)]. Collagen fibers support apatite crystals. They therefore, play an important role in dentin strength and biochemical properties [33)]. Rise in temperature during dentin irradiation could damage the above-mentioned fibers. Reports have shown that when using laser at high level of energy, the superficial layer of dentin undergoes melting and cracking condition of its organic and inorganic components [5, 34-37)]. Also, a major decrease in both dentin components has been reported [14)]. Therefore, low level of energy was recommended [11, 35, 38, 39)]. The purpose behind the use of low level of energy is to decrease the rise of temperature in dentin, hence to decrease the risk of organic, partial or total alteration.

Considering these conflicting facts, the aim of this *in vitro* study was to evaluate the effect of Er:YAG laser radiation at different levels of energy on the morphology of thermally affected dentin layer.

The null hypothesis supposes that there will be no negative effect of laser on collagen fibers and no significant differences between all irradiated groups with Er:YAG laser at different levels of energies.

Materials and Methods

Forty-eight, human third molar, extracted for dental treatment reasons, were collected in accordance with the recommendations of our University Ethics Committee. Only teeth free from caries, enamel and dentin pathology were selected. They were washed, cleaned with a scaler and stored in 0.1% thymol solution at 4°C to prevent germ growth. Specimens were embedded in acrylic resin 2 mm above the cementum-enamel junction. Then, they were cut horizontally with a low speed saw (IsoMet 2000, Buehler®, Ltd., IL, USA) 4 mm from the occlusal plane exposing areas of middle-depth dentin. To standardize smear layer, the cut surfaces were grounded with silicone carbide papers 320, then 600 grit, respectively. Resulting surfaces were examined under stereomicroscope (AmScope SM-1TSZZ-144S, Irvine, CA, USA) to assure absence of exposed pulp and enamel, except for the margins.

Samples were divided randomly into 6 groups (n = 8). Dentin of the first group G0 (Control group) did not receive any laser irradiation. The other groups received irradiation with Er:YAG laser wavelength 2.94 µm (Fidelis; Medical Laser, Ljubljana, Slovenia) using the following parameters: Super-short pulse mode (SSP, pulse duration: 50 µs), frequency of 10 Hz, under air/water spray (air, 6 mL/min, and water, 4 mL/min), with a non-contact mode handpiece H02 (at a distance of 5 mm from dentin). Only energy levels and fluency differ between groups, respectively, as follows: 40 mJ for G1 (13.846 J/cm^2), G2 corresponds to 60 mJ (20.769 J/cm^2), G3-80 mJ (25.385 J/cm^2), G4-100 mJ (31.769 J/cm^2), and G5-120 mJ (38.123 J/cm^2). Laser irradiation was standardized using a 2-dimensional custom-made computer numerical control (CNC) machine at a speed of 1 mm/sec.

Specimens were sliced perpendicularly to the irradiated surface using IsoMet 2000, to the thickness of 200 µm. Samples were decalcified for 10 minutes in 37% volume of ortho-phosphoric acid to eliminate smear layer and isolate dentin organic material (collagen fibers). Samples were stored in a humid atmosphere in order to avoid dentinal collagen collapse. Optical microscopic observation was carried by means of transmitted light under 100 x magnifications (AmScope ME580TA-PZ-2L-16M3, Irvine, CA, USA) with an integrated 16 megapixels digital camera (AmScope MU1603, Irvine, CA, USA). Five measures of the most superficial black layer of decalcified dentin localized in the center of the irradiated dentin crater, representing thermally affected dentin, were registered. One-way ANOVA was used for multiple group (G1 to G5) comparisons within each measured layer with p value < 0.05, for statistical significance.

Results

Descriptive results

Depth of laser impact

Cross sections of dentin after laser irradiation show the depth of ablated dentin. When air/water cooling was constant, values increased with the increase of laser energy densities **(Table 1, Figure 1)**.

Impact of laser on dentin is shown in **Figure 2**. Deepest ablation was visible for the highest level of energy used (120 mJ).

Structural dentin modifications

Light Microscopic Observation. Longitudinally cut specimens after laser irradiation, decalcification, and light microscopic observation showed a black affected layer representing the remaining component of dentin after

Table 1: Mean with standard deviation values of crater depth ablated dentin with Er:YAG laser

Experimental group	Minimum	25% Percentile	Median	75% Percentile	Maximum	Mean	Std. Deviation	Std. Error of Mean	Lower 95% CI	Upper 95% CI
G1	22.42	29.69	48.48	61.55	63.33	46.57	15.86	5.609	33.31	59.84
G2	43.76	56.95	64.13	70.23	79.69	63.33	10.66	3.768	54.42	72.24
G3	54.45	70.84	72.85	78.04	94.62	73.97	11.04	3.904	64.74	83.21
G4	78.59	87.88	132.2	141.8	177.1	123.8	33.06	11.69	96.17	151.4
G5	148.1	161.4	179.1	197	202	178.2	18.9	6.684	162.4	194

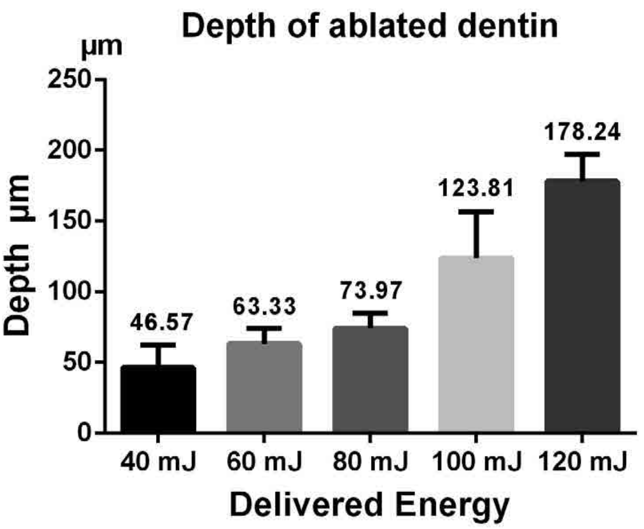

Fig. 1: Bar graph showing the mean values of crater depth ablated dentin with Er:YAG laser.

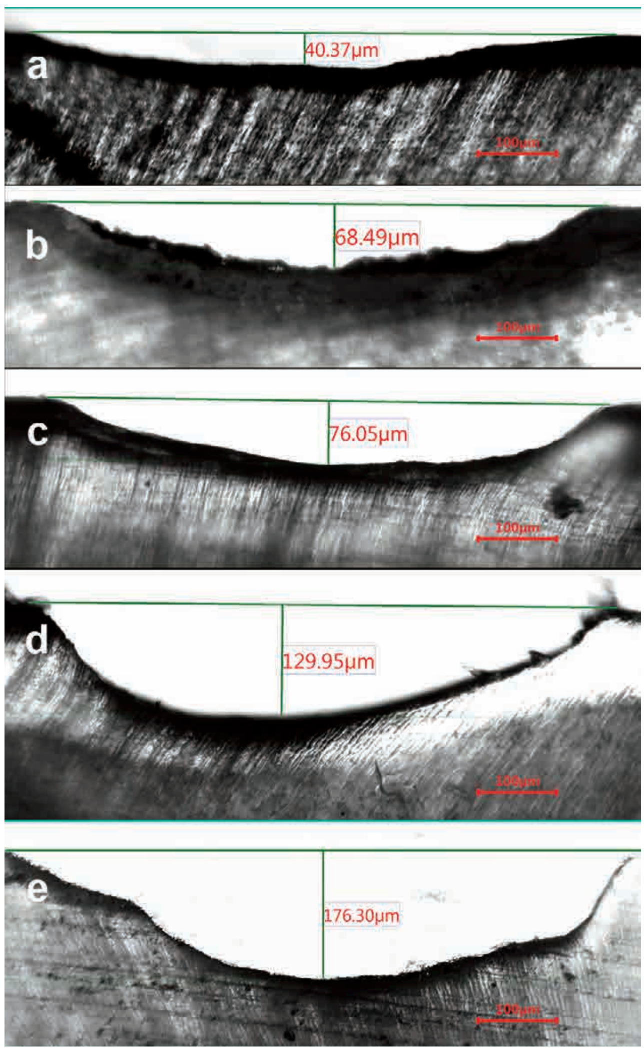

Fig. 2: Depth of ablated dentin with Er:YAG laser respectively with a-40 mJ, b-60 mJ, c-80 mJ, d-100 mJ, e-120 mJ.

decalcification: altered collagen fibers. Thickness of the affected layer of collagen fibers are shown in **Figure 3**. **Figure 3-b** (60 mJ) showed a discontinued and thin layer of altered collagen fibers while **Figure 3-a** (40 mJ) presented a thick and well delimited black layer. Only the control group that did not receive any irradiation showed total absence of superficial black layer **Figure 4**.

Measurement of dentin layer modified by laser irradiation is shown in **Table 2**. Mean value in group G2 was lower than all other irradiated groups, while groups G1 and G5 appeared to be almost identical and showed the highest values **(Figure 5)**.

Analytical results
One-way ANOVA repeated measures, followed by Newman-Keuls multiple comparisons test, were performed on irradiated groups (G1 to G5) using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla California USA), and showed statistical difference for $p < 0.05$. In sum, multiple comparisons between groups, demonstrated a significant difference between group 2 and all other irradiated groups same as between G3 - G5 and G4 - G5 **(Table 3)**.

Discussion

The effect of different low level Er:YAG laser energy on standardized middle-depth dentin surfaces was tested in this study. Based on earlier study, on shear bond strength, of self-adhering flowable composite, using the same different low level of energy on dentin, it was concluded that, 60 mJ (20.769 J/cm^2) gave the optimum values, under the same working conditions [22)]. Therefore, a more advanced and specific investigation regarding dentin modifications by laser seemed essential.

Irradiated samples were observed microscopically. Depth of crater formed at the impact was measured. Also, depth of the most superficial black (collagen affected) layer was measured. At 40 mJ, a shallow crater was observed (mean = 46.57 µm) in comparison to 120 mJ, where maximum depth of crater was recorded (mean = 178.24 µm). Values demonstrated that the more energy is decreased, the less dentin ablation is visible. These findings correlate with the study of Bakry *et al*. (2009), proving that low level laser energy decreases dentin surface roughness [40)]. Laser ablation is related to energy transformation into micro-physical dentin ablation and heat absorption. As observed in previous studies, lower laser settings, due to photo-ablation volume, would lengthen treatment time unnecessarily [41)]. Therefore, maximum efficiency with least heat effect or damage of dental hard tissues is considered to be the main objective for researchers, especially when it comes to preparing dentin surface to adhere or bond to resin composite restorations.

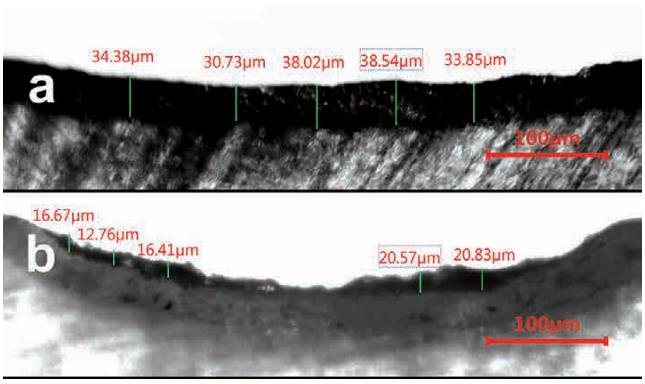

Fig. 3: ltered layer of dentin respectively irradiated with Er:YAG laser with a-40 mJ, b-60 mJ.

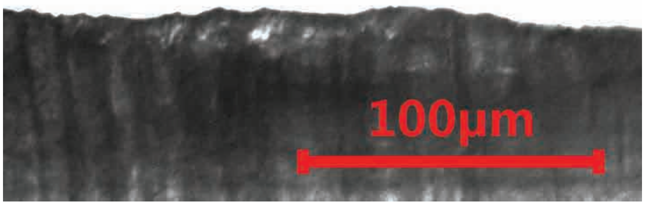

Fig. 4: bsence of altered dentin layer in the control group.

Table 2: Mean with standard deviation of the modified dentin layer when using Er:YAG laser.

Experimental group	Minimum	25% Percentile	Median	75% Percentile	Maximum	Mean	Std. Deviation	Std. Error of Mean	Lower 95% CI	Upper 95% CI
G1	13.80	20.38	28.23	35.05	39.79	28.17	8.759	3.0970	20.84	35.49
G2	12.03	12.94	15.31	17.40	17.81	15.19	2.283	0.8072	13.28	17.10
G3	16.77	17.62	20.21	21.85	23.44	19.93	2.456	0.8685	17.87	21.98
G4	17.92	19.04	23.13	24.71	31.25	22.87	4.336	1.5330	19.24	26.49
G5	22.14	27.16	28.26	31.81	32.60	28.53	3.357	1.1870	25.72	31.33

When temperature rises above 175°C, dentinal collagen fibers start to denature, while temperature above 310°C resulted in a total elimination of organic material [36] leaving an unusable and fragile layer of collagen. Also, when organic matrix fusion occurs, a lack of inter- fibrillary space would limit bonding system diffusion to tubular dentin [42]. Moreover, Bachmann *et al*. (2005) have proven that temperature below 225°C denatures partially collagen fibers [36], such layer can be the main cause of the weak bond strength. Moreover, Bachmann *et al*. (2005) have demonstrated that dentin collagen degradation is higher at the surface and lower at the sub-surface [5], which has been proven in a large number of publications, since it would act against hybridization of dentin [14, 19, 22], and could even induce micro-cracks in dentin sub-surface layer [34]. Practically, when low-level laser energy is deployed, a decrease in photo-ablation efficiency is clear, as it was shown in our results. Hence, less damage should be observed to the superficial collagen layer. Unfortunately, at very low level of energy 40 mJ, where laser ablation values are minimum (crater depth = 46.57 µm), superficial layer of damaged collagen increased dramatically (mean = 29.70 µm), and appeared to be equivalent to 120 mJ (mean = 28.53 µm). By analyzing the concept of laser cutting (photo-ablation and heat), and from the thickness of the damaged layer, we can suppose that Er:YAG laser beam at 40 mJ, was mainly transformed into heat with dramatic reduction in ablation efficiency. When 60 mJ was used, collagen damaged layer decreased to the lowest value (mean = 15.19 µm) and then increased progressively from 80 mJ (mean = 19.93 µm), to 100 mJ (mean = 22.87 µm).

Previous studies showed the importance of the elimination or the minimizing of affected collagen layer in order to improve the bonding quality of composites. Lahmouzi *et al*. (2012) demonstrated that the application of 0.5% sodium hypochlorite for 30 s on irradiated dentin, would improve restoration adaptation [43]. Ceballo *et al*. (2002) showed that affected collagen fibers, referred to as laser-modified layer, whether minimized or eliminated, have an impact on the quality of bonding to dentin after Er:YAG laser irradiation [44]. Such a modified superficial layer was found in our study **(Figure 3)** and we have described it as black layer. It's nature and morphology are the major causes behind weak bonding. Our results also correlate with He *et al*. (2017) showing a dark shade subsurface dentin layer after laser irradiation. Those authors also concluded that this subsurface layer has micro-cracks which might be one cause for the decreased mechanical properties of irradiated dentin [14].

It is still impossible, to compare our results with published data due to the multitude of parameters used within the same type of laser: cooling system (air/water), frequency, mode of action (SSP and type of handpiece and tips). Whereas, further investigations would be interesting, when it comes to calculating the hardness of the remaining dentin after Er:YAG laser irradiation at different level of energies.

Conclusion

Within the limitation of this study, it can be concluded that the importance of dentinal photo-ablation is proportional to the level of energy delivered by Er:YAG laser. Also, dentin organic matrix presented the least alteration, when Er:YAG laser is used at an appropriate level of energy (60 mJ, 10 Hz in SSP non-contact mode) under air/water cooling.

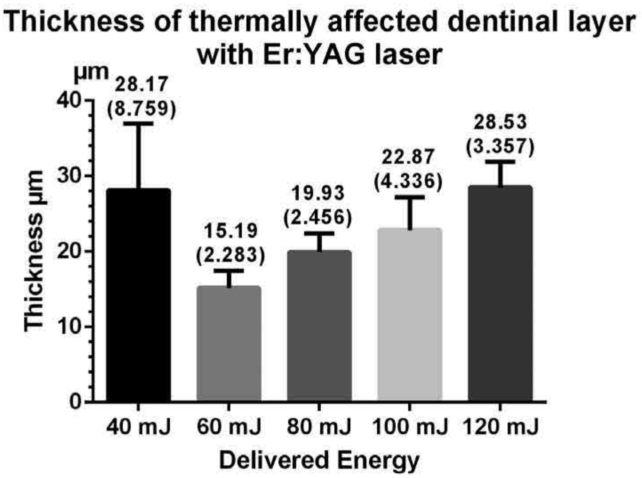

Fig. 5: Bar graph showing the mean values with the standard deviations.

Table 3: Statistical comparison for the experimental groups

Groups	Mean Diff.	SE of diff.
40 mJ vs. 60 mJ	12.98*	3.138
40 mJ vs. 80 mJ	8.237	3.517
40 mJ vs. 100 mJ	5.3	3.986
40 mJ vs. 120 mJ	-0.3601	3.775
60 mJ vs. 80 mJ	-4.739*	1.351
60 mJ vs. 100 mJ	-7.676*	1.975
60 mJ vs. 120 mJ	-13.34*	1.587
80 mJ vs. 100 mJ	-2.937	1.453
80 mJ vs. 120 mJ	-8.597*	0.7717
100 mJ vs. 120 mJ	-5.66*	1.589

*The mean difference was significant at the 0.05 level.

References

1: de Oliveira MT, Arrais CA, Aranha AC, de Paula Eduardo C, Miyake K, Rueggeberg FA, *et al*. Micromorphology of resin-dentin interfaces using one-bottle etch&rinse and self-etching adhesive systems on laser-treated dentin surfaces: a confocal laser scanning microscope analysis. Lasers in Surgery and Medicine. 2010;42(7):662-70.

2: Atsu SS, Kilicarslan MA, Kucukesmen HC, Aka PS. Effect of zirconium-oxide ceramic surface treatments on the bond strength to adhesive resin. Journal of Prosthetic Dentistry. 2006;95(6):430-6.

3: Gokce B, Ozpinar B, Dundar M, Comlekoglu E, Sen BH, Gungor MA. Bond strengths of all-ceramics: acid vs laser etching. Operative Dentistry. 2007;32(2):173-8.

4: Harashima T, Kinoshita J, Kimura Y, Brugnera A, Zanin F, Pecora JD, *et al*. Morphological comparative study on ablation of dental hard tissues at cavity preparation by Er:YAG and Er,Cr:YSGG lasers. Photomedicine and Laser Surgery. 2005;23(1):52-5.

5: Bachmann L, Diebolder R, Hibst R, Zezell DM. Changes in chemical composition and collagen structure of dentine tissue after erbium laser irradiation. Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy. 2005;61(11-12):2634-9.

6: Cardoso MV, Coutinho E, Ermis RB, Poitevin A, Van Landuyt K, De Munck J, *et al*. Influence of Er,Cr:YSGG laser treatment on the microtensile bond strength of adhesives to dentin. Journal of Adhesive Dentistry. 2008;10(1):25-33.

7: Esteves-Oliveira M, Zezell DM, Apel C, Turbino ML, Aranha AC, Eduardo Cde P, *et al*. Bond strength of self-etching primer to bur cut, Er,Cr:YSGG, and Er:YAG lased dental surfaces. Photomedicine and Laser Surgery. 2007;25(5):373-80.

8: Tachibana A, Marques MM, Soler JM, Matos AB. Erbium, chromium:yttrium scandium gallium garnet laser for caries removal: influence on bonding of a self-etching adhesive system. Lasers in Medical Science. 2008;23(4):435-41.

9: Lee BS, Lin PY, Chen MH, Hsieh TT, Lin CP, Lai JY, *et al*. Tensile bond strength of Er,Cr:YSGG laser-irradiated human dentin and analysis of dentin-resin interface. Dental Materials : official publication of the Academy of Dental Materials. 2007;23(5):570-8.

10: Raucci-Neto W, Raquel Dos Santos C, Augusto de Lima F, Pecora JD, Bachmann L, Palma-Dibb RG. Thermal effects and morphological aspects of varying Er:YAG laser energy on demineralized dentin removal: an *in vitro* study. Lasers in Medical Science. 2015;30(4):1231-6.

11: Brulat N, Rocca JP, Leforestier E, Fiorucci G, Nammour S, Bertrand MF. Shear bond strength of self-etching adhesive systems to Er:YAG-laser-prepared dentin. Lasers in Medical Science. 2009;24(1):53-7.

12: Akkus A, Akkus O, Souza-Gabriel AE, Sousa-Neto MD, Contreras-Arriaga B, Rodriguez-Vilchis LE, *et al*. Chemical and morphological changes in human dentin after Er:YAG laser irradiation: EDS and SEM analysis. Lasers in Medical Science. 2015;78(11):1019-25.

13: Mozaffari HR, Ehteshami A, Zallaghi F, Chiniforush N, Moradi Z. Microleakage in class V composite restorations after desensitizing surface treatment with Er:YAG and CO_2 lasers. Laser Therapy. 2016;25(4):259-66.

14: He Z, Chen L, Hu X, Shimada Y, Otsuki M, Tagami J, *et al*. Mechanical properties and molecular structure analysis of subsurface dentin after Er:YAG laser irradiation. Journal of the Mechanical Behavior of Biomedical Materials. 2017;74:274-82.

15: de Oliveira MT, Reis AF, Arrais CA, Cavalcanti AN, Aranha AC, de Paula Eduardo C, *et al*. Analysis of the interfacial micromorphology and bond strength of adhesive systems to Er:YAG laser-irradiated dentin. Lasers in Medical Science. 2013;28(4):1069-76.

16: Moretto SG, Azambuja N, Jr., Arana-Chavez VE, Reis AF, Giannini M, Eduardo Cde P, *et al*. Effects of ultramorphological changes on adhesion to lased dentin-scanning electron microscopy and transmission electron microscopy analysis. Microscopy Research and Technique. 2011;74(8):720-6.

17: Ramos AC, Esteves-Oliveira M, Arana-Chavez VE, de Paula Eduardo C. Adhesives bonded to erbium:yttrium-aluminum-garnet laser-irradiated dentin: transmission electron microscopy, scanning electron microscopy and tensile bond strength analyses. Lasers in Medical Science. 2010;25(2):181-9.

18: Carvalho AO, Reis AF, de Oliveira MT, de Freitas PC, Aranha AC, Eduardo Cde P, *et al*. Bond strength of adhesive systems to Er,Cr:YSGG laser-irradiated dentin. Photomedicine and Laser Surgery. 2011;29(11):747-52.

19: Alaghehmand H, Nezhad Nasrollah F, Nokhbatolfoghahaei H, Fekrazad R. An *in vitro* comparison of the bond strength of composite to superficial and deep dentin, treated with Er:YAG laser irradiation or acid-etching. Lasers in Medical Sciences. 2016;7(3):167-71.

20: He Z, Chen L, Shimada Y, Tagami J, Ruan S. Evaluation of sub-surface penetration and bonding durability of self-etching primer systems to Er:YAG laser treated cervical dentin. Dental Materials Journal. 2017;36(2):174-81.

21: Cebe F, Bulbul M, Simsek I, Cebe MA, Ozturk B. Effect of erbium:yttrium aluminum garnet laser on bond strength of a total-etch adhesive system to caries-affected dentin on gingival wall. Nigerian Journal of Clinical Practice. 2017;20(6):734-40.

22: Nahas P, Zeinoun T, Majzoub Z, Corbani K, Nammour S. The effect of energy densities on the shear bond strength of self-adhering flowable composite to Er:YAG pretreated dentin. BioMed Research International. 2016;2016:6507924.

23: Yazici AR, Agarwal I, Campillo-Funollet M, Munoz-Viveros C, Antonson SA, Antonson DE, *et al*. Effect of laser preparation on bond strength of a self-adhesive flowable resin. Lasers in Medical Science. 2013;28(1):343-7.

24: Cersosimo MC, Matos AB, Couto RS, Marques MM, de Freitas PM. Short-pulse Er:YAG laser increases bond strength of composite resin to sound and eroded dentin. Journal of Biomedical Optics. 2016;21(4):48001.

25: Moosavi H, Ghorbanzadeh S, Ahrari F. Structural and morphological changes in human dentin after ablative and subablative Er:YAG laser irradiation. Lasers in Medical Sciences. 2016;7(2):86-91.

26: Galafassi D, Scatena C, Galo R, Curylofo-Zotti FA, Corona SAM, Borsatto MC. Clinical evaluation of composite restorations in Er:YAG laser-prepared cavities re-wetting with chlorhexidine. Clinical Oral Investigations. 2017;21(4):1231-41.

27: Baraba A, Nathanson D, Matijevic J, Gabric D, Miletic I. Ablative potential of Er:YAG laser in dentin: Quantum versus variable square pulse. Photomedicine and Laser Surgery. 2016;34(5):215-20.

28: Chousterman M, Heysselaer D, Dridi SM, Bayet F, Misset B, Lamard L, *et al*. Effect of acid etching duration on tensile bond strength of composite resin bonded to erbium:yttrium-aluminium-garnet laser-prepared dentine. Preliminary study. Lasers in Medical Science. 2010;25(6):855-9.

29: Aranha AC, De Paula Eduardo C, Gutknecht N, Marques MM, Ramalho KM, Apel C. Analysis of the interfacial micro-

morphology of adhesive systems in cavities prepared with Er,Cr:YSGG, Er:YAG laser and bur. Microscopy Research and Technique. 2007;70(8):745-51.
30: He Z, Otsuki M, Sadr A, Tagami J. Acid resistance of dentin after erbium:yttrium-aluminum-garnet laser irradiation. Lasers in Medical Science. 2009;24(4):507-13.
31: Zhang SC, Kern M. The role of host-derived dentinal matrix metalloproteinases in reducing dentin bonding of resin adhesives. International Journal of Oral Science. 2009;1(4):163-76.
32: Linde A. Dentin matrix proteins: composition and possible functions in calcification. The Anatomical Record. 1989;224(2):154-66.
33: S. V-C, P. G, PD. D. The role of collagen in bone strength. Osteoporosis International. 2006;17(3):319-36.
34: Cvikl B, Lilaj B, Franz A, Degendorfer D, Moritz A. Evaluation of the morphological characteristics of laser-irradiated dentin. Photomedicine and Laser Surgery. 2015;33(10):504-8.
35: Bahrami B, Askari N, Tielemans M, Heysselaer D, Lamard L, Peremans A, *et al*. Effect of low fluency dentin conditioning on tensile bond strength of composite bonded to Er:YAG laser-prepared dentin: a preliminary study. Lasers in Medical Science. 2011;26(2):187-91.
36: Bachmann L, Gomes AS, Zezell DM. Collagen absorption bands in heated and rehydrated dentine. Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy. 2005;62(4-5):1045-9.
37: Bakry AS, Sadr A, Takahashi H, Otsuki M, Tagami J. Analysis of Er:YAG lased dentin using attenuated total reflectance Fourier transform infrared and X-ray diffraction techniques. Dental Materials Journal. 2007;26(3):422-8.
38: Chen ML, Ding JF, He YJ, Chen Y, Jiang QZ. Effect of pretreatment on Er:YAG laser-irradiated dentin. Lasers in Medical Science. 2015;30(2):753-9.
39: Ekworapoj P, Sidhu SK, McCabe JF. Effect of different power parameters of Er,Cr:YSGG laser on human dentine. Lasers in Medical Science. 2007;22(3):175-82.
40: Bakry AS, Nakajima M, Otsuki M, Tagami J. Effect of Er:YAG laser on dentin bonding durability under simulated pulpal pressure. Journal of Adhesive Dentistry. 2009;11(5):361-8.
41: Jacobsen T, Norlund A, Englund GS, Tranaeus S. Application of laser technology for removal of caries: a systematic review of controlled clinical trials. Acta Odontologica Scandinavica. 2011;69(2):65-74.
42: Koliniotou-Koumpia E, Kouros P, Zafiriadis L, Koumpia E, Dionysopoulos P, Karagiannis V. Bonding of adhesives to Er:YAG laser-treated dentin. European Journal of Dentistry. 2012;6(1):16-23.
43: Lahmouzi J, Farache M, Umana M, Compere P, Nyssen-Behets C, Nammour S. Influence of sodium hypochlorite on Er:YAG laser-irradiated dentin and its effect on the quality of adaptation of the composite restoration margins. Photomedicine and Laser Surgery. 2012;30(11):655-62.
44: Ceballo L, Toledano M, Osorio R, Tay FR, Marshall GW. Bonding to Er-YAG-laser-treated dentin. Journal of Dental Research. 2002;81(2):119-22.

Authors
The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgments
The authors thank Mrs. Carol Barakat and Prof. Denise Davidson (Georgia State University, Atlanta, GA) for their valuable contribution in the revision and English correction of the manuscript.