BDS Brazilian Dental Science
Ciência Odontológica Brasileira
unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE
doi: 10.14295/bds.2013.v16i4.924

In vitro evaluation of marginal leakage at enamel and dentin treated by different laser densities

Avaliação in vitro da infiltração marginal no esmalte e dentina tratada por diferentes densidades de laser

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ABSTRACT

Objectives: To evaluate "in vitro" the degree of marginal leakage in class V cavities irradiated with Nd:YAG laser onto enamel and dentin pretreated with bonding system (Single Bond, 3M ESPE) without light-curing. **Material & Methods:** Class V cavities were performed and standardized in 60 bovine incisors. After the acid etching, the teeth were divided into three groups: G1 – Application of bonding system, irradiation with Nd:YAG laser at 140 mJ/pulse without light-curing. G2 – Application of bonding system, irradiation with Nd:YAG laser at 200 mJ/pulse without light-curing. Group C - Application of bonding system and light-curing. Next, resin composite restorations were executed and thermo-cycled at alternated baths from 2 to 50 ºC (± 2 ºC), totalizing 500 cycles. To assess the microleakage, the teeth were submersed into 50% silver nitrate solution for 24 h, cut at 1 mm of thickness and analyzed through stereoscopy. The following leakage criterion scores were employed: 0 – none leakage; 1 – gingival or incisal wall; 2 – axial wall towards the pulp. **Results :** It was observed a high leakage frequency with score 1 among the experimental conditions. There was a higher predominance of score 2 leakage for control group at cementum. Concerning to enamel and cementum, Group C was similar to G1. By comparing control group with G2, and G1 with G2, it was verified statistically significant differences. **Conclusion:** The laser energy provided a reduction of the marginal leakage at enamel and dentin/cementum; however, the energy density increase resulted in smaller marginal leakage at enamel and dentin/cementum.

KEYWORDS

Nd-YAG Laser; Energy; Marginal leakage; Enamel; Dentin.

RESUMO

Objetivo: A presença de infiltração marginal é considerada a principal causa de fracasso clínico para restaurações em resina composta. Devido a isso alternativas para promover melhor vedação das margens foram desenvolvidas, como a utilização da energia do Nd:YAG laser. Portanto o objetivo deste estudo foi avaliar "in vitro" o grau de infiltração marginal em cavidades classe V irradiadas com Nd:YAG laser sobre esmalte e dentina bovinos pré-tratados com sistema adesivo Single Bond (3M ESPE) não-polimerizado. **Material e métodos:** Cavidades classe V padronizadas foram realizadas em 60 incisivos bovinos. Após realizar o condicionamento ácido, lavagem e secagem os dentes foram aleatoriamente divididos em 3 grupos: G1 – Aplicação do sistema adesivo, irradiação com Nd:YAG laser a 140 mJ/pulso em seguida a fotopolimerização. G2 – Aplicação do sistema adesivo, irradiação do Nd:YAG laser a 200 mJ/pulso e fotopolimerização. Grupo C (Controle) - Aplicação do sistema adesivo, seguido da fotopolimerização. Restaurações em resina composta foram realizadas e em seguida submetidas a termociclagem em banhos alternados de 2º a 50ºC(±2ºC), totalizando 500 ciclos. Para a avaliação da microinfiltração, os dentes foram submersos em solução de nitrato de prata 50% por 24 h, os dentes foram cortados com 1 mm de espessura e então analisados os níveis de infiltração marginal ocorrida na interface dente-restauração, por meio de de lupa estereoscópica. Os critérios para a análise de infiltração foram: grau 0 – nenhuma infiltração; grau 1 – parede gengival ou incisal; grau 2 – parede axial em direção a polpa. **Resultados:** Observou-se alta frequência de infiltração marginal grau 1 entre as condições experimentais. Houve predomínio da infiltração grau 2 somente para o grupo controle em cemento. Nos grupos esmalte e cemento, o Grupo C não apresentou diferença estatística entre o G1. Entretanto, ao se comparar o grupo Controle com o G2, e o G1 com G2, verificou-se que diferem estatisticamente entre si. **Conclusão:** Diante desses resultados, foi possível concluir que a energia laser proporcionou uma redução significativa da infiltração marginal tanto em esmalte quanto em dentina/cemento; e o aumento da densidade de energia resultou numa menor infiltração marginal, tanto em esmalte, como em dentina/cemento.

PALAVRAS-CHAVE

Nd-Yag Laser; Energia; Infiltração marginal; Esmalte; Dentina.

INTRODUCTION

The actions provided by laser technology meet their main barrier into tooth substrate. Many researches have been conducted to know the best parameters to be used inside the different Dentistry specialties, such as Surgery, Periodontics, Endodontics, Prosthodontics and Operative Dentistry [1].

Among the several laser devices, the following ones have been employed in Dentistry: Nd:YAG, Er:YAG, Ho:YAG and CO2 [2-7]. These have shown different wavelengths for the treatment of the tooth tissues (caries removal, sterilization of cavities and root canals, and hypersensitivity treatment) and bonding purposes [7,8].

Neodymium laser has been applied for remodeling the soft tissues and blood coagulation [9], modifying the enamel and dentin surface [9], and desensitizing the dentin [10]. Depending on the energy delivered by this laser, the physical structure of the dentinal substrate can be altered by promoting the sealing of the tubules and formation of the fungiform projections that may help in either the mechanical bonding or even the dentin vitrification [11,12]. The first neodymium laser applications were executed prior to the application of the bonding system, so that most of the studies exhibited a reduction of the bond strength and increasing of the acid resistance in comparison with the non-irradiated cavities. This occurred because of the property of this laser type which provokes the denaturation of the organic components of dentin by generation of heating and the obliteration of the dentinal tubules by melting and resolidification the inorganic content [4].

Considering this laser effects on dentinal morphology, Gonçalves (1997) [8] developed a technique for neodymium laser application as pre-treatment of dentin. This technique comprised the irradiation of Nd:YLF laser onto dentin previously etched with phosphoric acid and impregnated with bonding system without light-curing. Nd:YLF laser, at the parameters used, provoked the melting and resolidification of the dentinal hydroxyapatite in the presence of resinous monomers, resulting in an increasing of bond strength. According to the author, the pre-treatment of dentin with laser irradiation could result in a more stable interface with smaller marginal leakage degree, therefore extending the useful life of the restoration,.

According to Dayem (2010) [13], by treating the dentin with Nd:YAG laser, the bonding agent penetrates more deeply, once the laser removes the collagen net more effectively than the treatment with 10% NaOCl, making the dentinal substrate more receptive to the bonding agent penetration. Additionally, this laser generates a significantly better penetration of the bonding agent than that achieved by 37% phosphoric acid etching for 15 s.

The phenomenon so-called marginal microleakage occurs by the formation of marginal cracks because of the lack of effective sealing of the restoration margins to the dentinal structure [14]. The oral environment (occlusal forces and temperature) and the different physical properties of the teeth and restorative materials (thermal expansion coefficient, modulus of elasticity and polymerization shrinkage) may contribute for the leakage at the preparation margins [15]. The presence of leakage is considered the main cause of the clinical failure of resin composite restorations because the cracks enable the transference of fluids between the dentin-pulp complex and the oral environment, leading to post-operative sensitivity, marginal discoloration, secondary caries, and pulp inflammation [16]. Moreover, the marginal leakage is one of the most employed in vitro methods to study the restorations durability, mainly class V preparations through the bonding interface [17]. Considering this aforementioned information and based on the "bonding laser" approach by Gonçalves

(1997) [8], Araújo et al. (2001) [2] evaluated the microleakage and nanoleakage in class V restorations with cavity preparation and dentin pre-treatment with neodymium laser. The authors observed that the dentin pre-treatment with irradiation of 60 mJ/pulse of Nd:YAG laser onto the bonding system prior to the polymerization promoted the best sealing of the gingival margins. Additionally, Ribeiro et al. (2005) [3] found that 140mJ/pulse was the best energy parameter for irradiation onto dentin/cementum, among the three parameters evaluated (120, 140 and 160 mJ/pulse). The authors reported that the laser energy increasing could also reduce the marginal microleakage in enamel. Franke et al. (2006) [4] obtained good results of bond strength with 5J/cm2 of energy density.

However, there exist still controversies in literature regarding the use of this bonding technique. Kawaguchi et al. (2003) [18], based on the study of Ferreira et al. [5], verified that Nd:YAG laser at 40 mJ/pulse did not influence on the marginal leakage of adhesive restorations regardless of the moment of the laser irradiation onto the dentinal substrate. Accordingly, Castro et al. (2012) [6] did not observed significant influence on the improvement of the bond strength with the technique.

Therefore, the aim of this study was to evaluate "in vitro" the marginal leakage degree in class V cavities irradiated with com Nd:YAG laser onto bovine enamel and dentin pretreated with bonding system without light-curing.

MATERIAL AND METHODS

The materials employed in this study, their composition and manufacturers are specified in Chart 1.

Sixty sound bovine teeth were extracted immediately after slaughter. They were cleaned and stored into distilled water at - 18 °C, for a maximum period of 14 days [19]. According to Watanabe et al. (1994) [20], the freezing process maintain the dentinal collagen as closest as the in vivo condition thus avoiding its denaturation or fixation, which occurs in the conventional methods of storage (water, saline solution, formalin, among others).

Then, the roots were cut at the medium third to remove the remaining pulp tissues with the aid of endodontic files - (size 20 to 40 Hedströem instruments – Maillefer, according to the opening size). The pulp chambers were carefully washed with distilled water and dried with gentle air jets. The apical sealing was accomplished with Araldite (Ciba-Geigy Química), to avoid the penetration of the acrylic resin within the teeth during their embedding.

Following, the teeth were embedded with the aid of a silicone mold for dental purposes

Chart 1 – Composition and manufacturers of the materials

Materials	Composition/ Characteristic	Manufacturer
Bonding system - Single Bond	Acid + Primer/Bonding Agent	3M ESPE
Laser Nd:YAG	No contact; energy of 140 and 200 mJ/pulse; density of energy/pulse of 43.75 and 62.5 J/cm^2; power of 1.4 and 2.0 W; frequency up to 10 Hz; fiber of 320 µm, scanning time of 1 min.	Pulse Master 600 IQ American Dental Technologies,Inc.
Resin Composite Z100	Hybrid	3M ESPE

(Rodhorsil, Artigos Odontológicos Clássico, Campo Limpo Paulista, SP, Brazil) and inserted into colorless fast-curing acrylic resin (Jet, Artigos Odontológicos Clássico Campo Limpo Paulista, SP, Brazil). The teeth were embedded by leaving their labial/buccal surface exposed at the acrylic block surface.

Standardized class V preparations were executed with the aid of n. 3053 diamond drills (KG Sorensen) at the cervical-buccal/labial surfaces with 4.0 mm of diameter and 2.0 mm of deepness. Then, the preparation surfaces were cleaned with detergent solution (Tergensol, INODON), to eliminate the oily residues from the handpiece. All teeth received acid etching (30 s for enamel and 15 s for dentin/cementum) according to the manufacturers' instructions; were washed by water jet for 10 s; and dried with absorbent papers to avoid the dryness of the dentin exposed.

The 60 teeth were randomly divided into three groups (n = 20), according to the treatment employed:

• **Group 1** – Application of the bonding system (Single Bond, 3M ESPE), without light-curing. After that, the surfaces were irradiated with Nd:YAG laser at 140 mJ/pulse for 1 min, without cooling and contact, scanning all the area prepared and including the margins. Then, the bonding system was light-cured for 20 s.

• **Group 2** – Application of the bonding system (Single Bond, 3M ESPE), without light-curing. After that, the surfaces were irradiated with Nd:YAG laser at 200 mJ/pulse for 1 min, without cooling and contact, scanning all the area prepared and including the margins. Then, the bonding system was light-cured for 20 s.

• **Group C (Control)** - Application of the bonding system (Single Bond, 3M ESPE), followed by light-curing for 20 s, without laser irradiation.

After the pre-treatment of the tooth surfaces, the restorations were executed with resin composite (Z100, 3M ESPE), by incremental technique at two portions, and each one was light-cured for 40 s. Following, the teeth were stored into distilled water at 37 °C for 24 h. After that period, finishing and polishing of the restorations were carried out.

The specimens underwent thermocycling (Ética Equipamentos Científicos S. A) in alternated baths from 2 °C to 50 °C (± 2 °C), totalizing 500 cycles [21]. The specimens were dried and their surfaces were waterproofed with red nail polish at 1 mm below the restorations, and submersed into dye solution of 50% silver nitrate for 24 h.

After that period, the specimens were washed and positioned into a cutting machine (Labcut 1010, Extec Technologies Inc., Enfield, CT, USA) at low speed under refrigeration, with the diamond disc displaced sagittally to obtain parallel cuts of 1 mm thick of the restorations.

The degrees of marginal leakage at the tooth-restoration interface were analyzed through stereoscopy (Tecnival Carl Zeiss), both for the gingival (cementum) and incisal margin (enamel). with the following scores: 0 – none leakage; 1 – gingival or incisal wall; 2 –axial wall towards the pulp.

RESULTS

The distributions of the obtained marginal leakage values are seen in Figure 1. A high frequency of score 1 marginal leakage was obtained among the experimental conditions. There was a higher predominance of score 2 only for the control group at cementum.

Taking into account the groups in enamel, a comparison between scores 0 and scores different from 0 (1 and 2) were executed thorugh Chi-square test (Table 1).

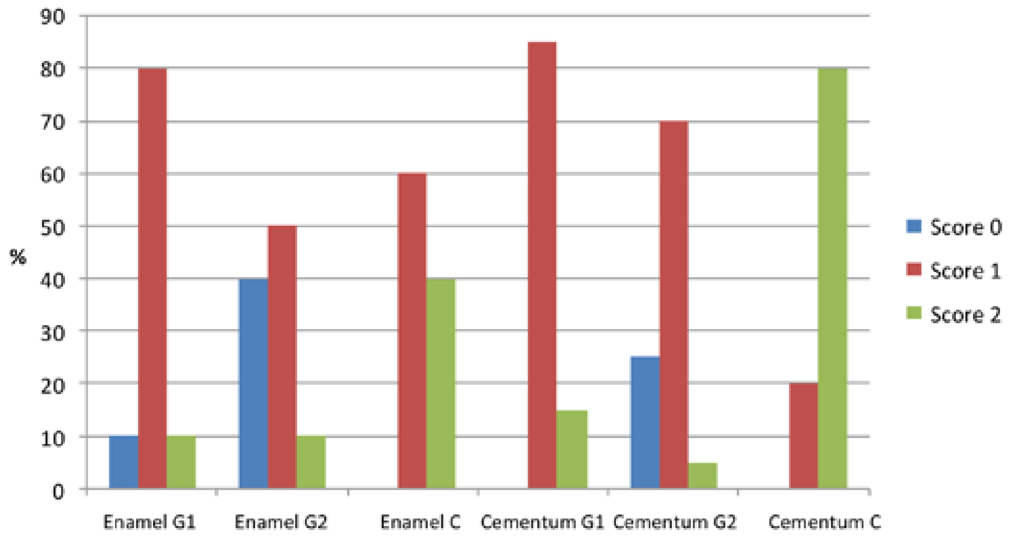

Figure 1 – Distribution of the relative frequency (%) of the marginal leakage degrees in each group at enamel and cementum, according to the groups (G1, G2 and C). Score 0 = none leakage; Score 1 = gingival or incisal wall; Score 2 = axial wall towards the pulp.

Table 1 – Distribution of the data obtained for enamel, according to the group division (n = 20).

Scores	Enamel		
	Control	G1	G2
0	0	2	8
1	12	16	10
2	8	2	2

Concerning to enamel, the control group did not exhibit statistically significant differences between the score 0 and the score different from 0 (scores 1 and 2) by applying the Chi-square test (p-value = 0.147 > 0.05) when compared with G1. Notwithstanding, when the control groups was compared with G2, there were statistically significant differences by applying the Chi-square test (p-value = 0.002 < 0.05). Also, the comparison between G1 and G2 showed statistically significant differences by applying the Chi-square test (p-value = 0.028 < 0.05).

In G2, eight specimens did not show any leakage and statistically differed from control group and G1. Therefore, it was observed that the greater the energy parameters (G2 = 200 mJ/pulse) the smaller the marginal leakage at enamel.

Table 2 – Distribution of the data obtained for cementum, according to the group division (n = 20)

Scores	Cementum		
	Control	G1	G2
0	0	0	5
1	4	17	14
2	16	3	1

The groups in cementum were evaluated similarly to those in enamel (Table 2).

There were statistically significant differences between the control group and G2 by comparing the score zero (none leakage) and the scores different from (scores 1 and 2) and applying the chi-square test (p-value = 0.017 < 0.05). By comparing G1 and G2, the same differences among the leakage scores were observed. Thus, it was noted an improvement in the marginal leakage values for cementum when the laser energy parameter was increased.

DISCUSSION

This present study aimed to assess the influence of the bonding system/laser association on the reduction of the marginal leakage. For this purpose, bovine teeth were used instead of human teeth because they are of easy obtainment, storage and standardization of the age range [22,23].

Still considering the morphological aspect of the dentinal tissue, it was observed that the use of bovine teeth in laboratorial tests of bonding systems are supported by the difficulty in obtaining human teeth free of either caries or restorations, the scientific proof that they can replace the human teeth in the evaluation of the first models of performance for the bonding systems prior to the clinical tests [23], and the fact of the diameter, orientation and concentration of tubules per unit of area are similar to that of human teeth [21].

To execute the preparations onto the teeth, a microscope was adapted aiming to obtain their accuracy and homogeneity. The cavities were performed onto the buccal/labial surface because it exhibits a smaller variation in the pattern of the tubules so that it can provide better standardization conditions of the measurements in research. A mean thickness of 2 mm was standardized to obtain a smaller coefficient of variation of the results [23]. Many authors reported the significance of the deepness in the studies on adhesion and stated the patterns of density, diameter and orientation of the dentinal tubules in relation to this deepness at the different areas of the human dentin [24]. The use of the diamond drill also aimed to a greater similarity among the specimens because they had dimensions that allowed to reach the cementoenamel junction and to provide an adequate number of cuts for further microleakage assessment.

Despite the criticisms of the most recent studies, the microleakage test provides important subside to the researcher regarding to the sealing and consequently the longitudinal predictability.

Many studies in the literature [1,15] assessed the influence of many adhesive systems on the microleakage of resin composite restorations. They have employed either Scotchbond Mult-Purpose Plus (SMPP) or Single Bond, both manufactured by 3M ESPE, with the latter being an improvement of the former. Accordingly, Single Bond system was chosen to enable the comparison with the literature.

All teeth underwent acid etching (30 s for enamel and 15 s for dentin/cementum) according to the manufacturers' specifications. The acid etching assures the smear layer removal and provides the formation of an adequate hybridization, providing an efficient sealing of the adhesive restorations. However, this sealing may vary according to the adhesive system, technique used by the operator and variables of the clinical conditions [12].

The smear layer reduces the surface energy by decreasing the superficial activity; exhibits low bond strength values by turning the systems bonding to it more vulnerable to microleakage; denatures over time; and may present viable microorganisms within it thus constituting a source of constant risk of pulp irritation. Therefore, its removal can provide a substrate physically strong and chemically opportune to the adhesive purposes [1]. Admittedly, the smear layer removal leads to an markedly increase of the permeability mainly at deep dentin, and consequently to an increase of the superficial humidity, in vivo [25] Notwithstanding, in vitro, the problem is the dentin dryness after the acid etching, during the drying step of the bonding system [26].

In the presence of marked humidity, the resinous monomers do not completely reach polymerization and are constant sources of pulp irritation [25]. Factors as the molecular weight and size, among others, influences on the potential of penetrability and consequently cytotoxicity of these molecules, such as: its concentration at the primer or bonding agent; the deepness of the cavity or dentinal remnants; patient's age; presence of sclerotic dentin; contact time of the material with the substrate; positive pressure of the fluid; and the associations with other monomers within the resin composites and adhesive systems (TEGDMA and BIS-GMA).

The drying must be executed carefully because in vitro there is not the dentinal fluid flow at the absence of simulated pulp pressure, which would generate collagen collapse and reduction of the adhesion. This fact is similar to that of endodontically treated teeth in vivo [12].

In an attempt to reduce the large variability among the results from the factors inherent to the dentinal substrate, the pre-treatment by hypochlorite [27] and laser association has gained space in the researches [6,7,12]. This tendency has aimed to modify the dentin in order to make it a substrate closer to enamel in terms of the physical features, since the bonding to enamel is more reliable longitudinally.

Kimyai [28] evidenced a significantly greater marginal leakage at the gingival margin than that of the occlusal margin of class V preparations where Single Bond was employed.

The “bonding-laser” technique, introduced by Gonçalves et al. [14], states the formation of a new hybridization modality. The tissue previously treated with acid and bonding system without light-curing is irradiated with neodymium laser. Accordingly, the tissue would be modified by the laser in the presence of the bonding agent, therefore providing after the polymerization, a chemical-mechanic interaction between the laser-modified substrate and the bonding system. The authors observed a significant improvement in the bond strength results with this aforementioned technique. However, further studies have shown that this technique is much sensitive to the variations in the laser device’s parameters, the application modes (with or without contact of the optical fiber and scanning time), and bonding agent types [4,7].

Araújo et al. [20] aimed to verify whether the application of Nd:YAG laser (60 mJ) after the dentinal pre-treatment with bonding systems without light-curing in class V preparations with Er:YAG laser (350 mJ) would promote a better sealing of the gingival margins than that obtained by the conventional method. It was concluded that the bonding-laser technique promoted a better sealing and smaller nanoleakage than those of the conventional method, which was in agreement with the studies of Ribeiro et al. [3] and Youngson et al. [14].

Studies conducted by Navarro et al. [29], Franke et al. [4], by employing the bonding-laser technique introduced by Gonçalves et al. [14], obtained a significant improvement of bond strength, but with smaller energy parameters (60 mJ and 100 mJ, respectively). According to authors such as Kawaguchi et al. [18] and Castro et al. [6] there was no significant improvement in both microleakage reduction and bond strength by using bonding-laser technique, with parameters from 0.75 to 1 W of power.

Thus, based on the aforementioned discussion, it is necessary to find laser parameters that assure the technique effectiveness, since these account for the variations in the results of these studies.

In this present study, it was observed that the best results in terms of microleakage reduction were obtained with greater energy parameters, which disagrees with the studies of Castro et al. [6] on bond strength, but corroborates with the studies of Ribeiro et al. [3] and Marimoto et al. [7].

The probably rationale behind the quality improvement of the sealing of restorations by bonding-laser technique is the evaporation of the solvents in addition to the substrate modification. According to Batista et al. (2013) [30], the technique allows a greater evaporation of the solvents within the adhesive systems by optimizing the degree of conversion of the monomers into polymers, that is, by improving polymerization.

Notwithstanding, despite of the good results with the irradiation of 200 mJ of laser energy obtained in this study for enamel and dentin/cementum, it is highlighted that this energy may result in overheating of the tissues by considering an uninterrupted scanning of 60 seconds, which could lead to pulp impairment as well as to superficial collagen deterioration by denaturation or loss of the primary structure of this protein. However, in clinical conditions, the variable temperature undergoes the action of the internal perfusion of fluids and the presence of the periodontal and bone tissues, which may contribute for regulating this undesirable temperature increasing [24].

It was observed that the enamel and dentin behaved similarly after the laser irradiation, suggesting an approximation of their response, with many specimens showing total absence of microleakage, as seen in tables 1 and 2. The goal of obtaining a dentin value close to that of enamel may have been reached and further morphological studies using SEM and TEM should be conducted.

The literature controversy on the best parameters of energy to be employed in the studies supports the reflection on the available devices and their energy delivery. Optical fibers of small size (200, 320 and 400 μm) and the requirement for the scanning of large adhesive areas may lead to overheating due to the current Nd:YAG laser devices. New application modes need further investigation such as how to unfocus the device to scan large areas with shorter application time.

The results of this present study agree with the tendencies suggested by Gonçalves (1997) [8], regarding to the formation of a substrate constituted by fused hydroxyapatite when the laser was applied after the bonding system by providing an increasing of the bond strength and consequently by decreasing marginal leakage [7,9]. Accordingly, Cooper et al. [11], obtained favorable results regarding to the laser application with an increasing of about 300% of the bond strength when compared with the group not receiving laser application, although these authors used CO2 laser prior to the 2nd generation bonding system. Therefore, it can be suggested that the use of bonding system/laser is a promising association for the marginal sealing of the adhesive restorations.

CONCLUSION

Based on the results of this study, it can be concluded that:

a) The laser energy provided a significant reduction of the marginal leakage both at enamel and dentin/cementum;

b) The increasing of the energy density resulted in a smaller marginal leakage both at enamel and dentin/cementum.

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Date submitted: 2013 Sep 11
Accept Submission: 2013 Oct 07