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SEM Evaluation of the Hybrid Layer After Cavity Preparation with Er:YAG Laser

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Clinical Relevance

The thinner, irregular hybrid layer found when a cavity is prepared with the Er:YAG laser may have a negative effect on bonding.

SUMMARY

This study compared the thickness of the hybrid layer formed using Scotchbond Multi-Purpose Plus, Single Bond 2, Prime & Bond 2.1 and Xeno III on a dentin surface prepared with a diamond bur in a high speed handpiece or prepared with an Er:YAG laser used with two parameters of pulse energy (200 and 400 mJ) and two parameters of frequency (4 and 6 Hz). Flat dentin surfaces obtained from 20 human third molars were treated with the two methods and were then prepared with the dentin adhesive systems according to the manufacturers' instructions. After a layer of composite was applied, the specimens were sectioned, flattened, polished and prepared for Scanning Electronic Microscopy observation. Five different measurements of the hybrid layer thickness were obtained along the bonded surface in each specimen. The results were statistically analyzed using Analysis of Variance and Student-Newman-Keuls tests (*p*≤0.05). When analyzing the hybrid layer thickness and comparing the cavity preparation method, four groups were formed: Group I (diamond bur) > Group II (Laser 200 mJ/4 Hz) = Group III (Laser 200 mJ/6 Hz) > Group IV (Laser 400 mJ/4 Hz) > Group V (Laser 400 mJ/6 Hz). When comparing the dentin adhesive systems, there were no statistically significant differences. These results showed that the four tested dentin adhesive systems produced a 2.90 ± 1.71 µm hybrid layer in dentin prepared with a diamond bur. This hybrid layer was regu-

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lar and routinely found. In the laser groups, the dentin adhesive systems produced hybrid layers ranging from 0.41 ± 1.00 µm to 2.06 ± 2.49 µm, which were very irregular and not routinely found. It was also concluded that the Er:YAG laser, with the parameters used in this experiment, has a negative influence on the formation of a hybrid layer and cavity preparation methods influence formation of the hybrid layer.

INTRODUCTION

The hybrid layer appears to play a critical role in adhesive dentistry. In a study by Nakabayashi and Saimi,[1] a good quality hybrid layer is very important as a barrier against demineralization caused by the action of cariogenic agents. Krejci and others[2] stated that an adhesive system should promote perfect marginal sealing, exhibit stability under occlusal load and provide protection against secondary caries, marginal staining and post-treatment sensitivity. According to Krejci and others,[2] there is a close relationship between the hybrid layer morphology and obtaining excellent marginal sealing. Therefore, tests evaluating the junction layer micromorphology and the hybrid layer thickness should be carried out in order to evaluate an adhesive system. A significant amount of literature and research dealing with morphology evaluation and the hybrid layer thickness is available.[3-6]

Perdigão and others[7] stated that the dentinal substrate type could influence the adhesion mechanism and formation of the hybrid layer. If this is true, the manner in which the substrate for adhesion is prepared becomes critical. In other words, the preparation technique may influence the final restorative result.

Since the usefulness of the Er:YAG laser in the removal of dental hard tissues has been recognized[8-9] and its safety proven,[10-11] mainly after its approval by the US Food and Drug Administration,[12] the laser has been widely applied in the treatment of dental caries. In studies evaluating preparation effectiveness, the degree of microleakage or bond strength, the laser has compared favorably with conventional preparation methods that use a high-speed turbine handpiece.[13-16] In general, the authors concluded that there was no significant statistical difference among the studied groups, indicating that the use of the Er:YAG laser had no significant influence on microleakage or bond strength in the adhesive filling procedures.

On the other hand, other studies[10,16-21] have shown that dentinal surfaces prepared with the Er:YAG laser present very different characteristics from those prepared with conventional rotary instruments. The morphological aspect of irradiated dentin after cavity preparation with the Er:YAG laser has been reported as presenting an irregular surface with open dentinal tubules and lack of a smear layer, which were considered favorable aspects for dentin bonding. These studies have also shown that different parameters will produce different tissue characteristics. Hossain and others[18] showed that there is a linear relation between ablation rate and energy used.

Some authors have discussed the possible denaturation of collagen fibrils.[19-22] Ishizaka and others[21] demonstrated that the number of odontoblastic processes markedly decreased in the laser-treated area and concluded that Er:YAG laser irradiation might have denatured the organic material of dentin.

The question then exists, how it can be possible to have similar adhesion results[13-16] in different dentinal substrates?[10,17-19] Based on this question, and using the statement by Krejci and others[2] that tests evaluating the junction layer micro-morphology and the hybrid layer thickness should be carried out in order to evaluate an adhesive system, Barceleiro and others[23] compared the hybrid layers formed between an adhesive system and dentin prepared with a diamond bur in a high speed handpiece or with the Er:YAG laser using high energy parameters. In their study, Barceleiro and others demonstrated that, in the laser group, the dentin adhesive produced a very irregular, inconsistent, thin hybrid layer. These authors suggested that additional studies were in order, including alterations in parameters used by the laser device or in the adhesive systems used in order to find a better hybrid layer quality formation over dentin prepared with the Er:YAG laser.

Based on this suggestion, the objective of this study was to compare the thickness of the hybrid layer that was formed using four different adhesive systems on a dentin surface prepared with a diamond bur in a high speed handpiece or with an Er:YAG laser using two parameters of pulse energy and two parameters of frequency.

METHODS AND MATERIALS

Twenty extracted human mandibular third molars were selected for this study. All the teeth were free from caries and previous restorations. The samples were cleaned with a periodontal curette and a fine flour of pumice using a rubber cup in a low-speed hand piece for 30 seconds. The samples were then stored in distilled water, changed every seven days, at 37°C for 28 days. The teeth were longitudinally sectioned into four parts with a mesio-distal cut and a facio-lingual cut using a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water irrigation. After the initial cuts, the occlusal surface was removed with a horizontal cut 1 mm below the DEJ using the low speed diamond saw.

After the fragments were obtained, they were randomly separated into five groups, with four subgroups:

Group I—High speed turbine (Control) (Group B)

One section from each tooth was placed in an individual container with distilled water and assigned a group number from BMP1 to BMP4 (Scotchbond Multi-Purpose Plus), BSB1 to BSB4 (Adper Single Bond 2), BPB1 to BPB4 (Prime & Bond 2.1) and BXE1 to BXE4 (Xeno III). Throughout the study, the numbering of each section corresponded to the same tooth in each group. For example, the section numbered BMP1 in the MP subgroup came from the same tooth used in the BSB1 in the SB subgroup, respectively. A #1013 diamond bur (KG Sorensen, Alphavile, Barueri, São Paulo, Brazil) was placed in a high-speed handpiece (KaVo, Biberach, Germany) and was used on the flattened occlusal surface of each section under abundant water spray. The surface was prepared using random movements for 10 seconds, simulating the bottom of an occlusal cavity. One diamond bur was used for each dentin section.

Group II—Er:YAG laser (200 mJ, 4 Hz) (Group LA)

One section from each tooth was placed in an individual container with distilled water and assigned a group number (LAMP1 to LAMP4, LASB1 to LASB4, LAPB1 to LAPB4 and LAXE1 to LAXE4) using the same criteria as in the B group. The Kavo Key II Er:YAG laser (KaVo, ET 603 A) device was used to irradiate the previously flattened occlusal dentin sections. The laser was applied using a handpiece kept at a standardized distance and fixed with an orthodontic thread fastened to the handpiece. Scanning movements were carried out at random for 10 seconds, simulating the bottom of an occlusal cavity prepared with the laser according to the parameters described in Table 1.

Group III—Er:YAG laser (200 mJ, 6 Hz) (Group LB)

This group was similar to Group II, except for the frequency (6 Hz). In this group, the LA code was changed to LB.

Group IV—Er:YAG laser (400 mJ, 4 Hz) (Group LC)

This group was similar to Group II, except for the pulse energy (400 mJ). In this group, the LA code was changed to LC.

Group V—Er:YAG laser (400 mJ, 6 Hz) (Group LD)

This group was similar to Group II, except for the pulse energy (400 mJ) and frequency (6 Hz). In this group, the LA code was changed to LD.

After all the occlusal surfaces were prepared, each section was treated as follows:

MP Subgroup: Each section was conditioned with 37% phosphoric acid (Vigodent, Rio de Janeiro, Brazil, Lot 001-04) for 15 seconds. After application of the conditioning gel, the surfaces were rinsed with distilled water for 15 seconds, then gently dried with oil- and dust-free air for two seconds. The Scotchbond Multi-Purpose Plus (3M ESPE, St Paul, MN, USA) adhesive system was applied according to the manufacturer's instructions. A summary of the instructions follow: A thin layer of the Primer (3M ESPE, Lot 5AW), applied with a brush, was left undisturbed on the conditioned surfaces for 30 seconds. The solvent was removed from the surface with oil- and dust-free air jets for 5 seconds and a thin layer of the adhesive (3M ESPE, Lot 5PB) was applied. The adhesive layer was light-cured for 20 seconds with a 3M light source (intensity = 400 mW/cm^2 and evaluated by means of a radiometer after every 10 uses).

SB Subgroup: Each section was conditioned with 37% phosphoric acid (Vigodent, Lot 001-04) for 15 seconds. After application of the conditioning gel, the surfaces were rinsed with distilled water for 15 seconds and gently dried with oil- and dust-free air for 2 seconds. The Adper Single Bond 2 (3M ESPE, Lot 5CU) adhesive system was applied according to the manufacturer's instructions. A summary of the instructions follow: A thin layer of the adhesive, applied with a brush, was left undisturbed on the conditioned surfaces for 30 seconds. The solvent was removed from the surface with oil- and dust-free air jets for five seconds, then another thin layer of the adhesive was applied. The second adhesive layer was light-cured for 10 seconds.

PB Subgroup: Each section was conditioned with 37% phosphoric acid (Vigodent, Lot 001-04) for 15 seconds. After application of the conditioning gel, the surfaces were rinsed with distilled water for 15 seconds, then gently dried with oil- and dust-free air for two seconds. The Prime & Bond 2.1 (Dentsply, Petropolis, RJ, Brazil, Lot 259152) adhesive system was applied according to

Table 1: *Kavo Key II Er:YAG Laser Parameters*

	Group II	Group III	Group IV	Group V
Wave Length	2.940 ηm	2.940 ηm	2.940 ηm	2.940 ηm
Pulse Energy	200 mJ	200 mJ	400 mJ	400 mJ
Frequency	4 Hz	6 Hz	4 Hz	6 Hz
Operation Mode	Pulse	Pulse	Pulse	Pulse
Operation Distance	10 mm	10 mm	10 mm	10 mm
Coolant	Spray air/water	Spray air/water	Spray air/water	Spray air/water

the manufacturer's instructions. A summary of the instructions follow: A thin layer of the adhesive was applied with a brush and was left undisturbed on the conditioned surfaces for 20 seconds. The solvent was removed from the surface with oil- and dust-free air jets for five seconds and the first layer was light-cured for 20 seconds. After light curing, a second layer of the adhesive was applied. This second adhesive layer was immediately light-cured for 10 seconds.

XE Subgroup: The self-etching Xeno III (Dentsply, Lot 0502000204 [Liquid A] and 0502000203 [Liquid B]) adhesive system was applied according to the manufacturer's instructions. A summary of the instructions follow: One drop of liquid A and one drop of liquid B were mixed, applied with a brush and left undisturbed on the surfaces for 30 seconds. The solvent was removed from the surface with oil- and dust-free air jets for 5 seconds, then the surface was light-cured for 20 seconds.

The next step was to apply a 1 mm thick micro-hybrid composite layer, Fill Magic (Vigodent, Lot FM 04898) A1 shade in a unique increment that covered the entire occlusal surface and was light-cured for 40 seconds.[24] Upon completion of the procedures, the samples were kept in distilled water for seven days.

After seven days, a transverse section was made 5 mm below the tooth/composite interface using a diamond saw (Isomet); the roots of the sections were set apart and the remaining portion was sectioned along the long axis through the middle of the composite using the same diamond saw under abundant water irrigation. Two sections, which were formed from the enamel and dentin, adhesive system and microhybrid composite, were obtained. The two sections were hand-polished on wet 600 grit silicon carbide paper (Norton Abrasivos, São Paulo, Brazil); they were then polished with a felt wheel placed in a polishing device (Prazis). An alumina polishing paste with 0.5 µm particles (AP-Paste SQ, Struers) was used until no grooves were observed with a 50x magnifying glass. Upon completion, the sections were again conditioned in distilled water.

After seven additional days, one section of each previously formed pair was gently decalcified with 37% phosphoric acid for 10 seconds, rinsed with distilled water and deproteinized with 3% sodium hypochlorite for 60 seconds.[24] The sections were then rinsed with distilled water, placed on aluminum stubs and sputter-coated with gold (Edwards Coater S150B).

After sputter-coating, the samples were evaluated under DSM 960 (Zeiss Electron Microscopy LTD, Germany) Scanning Electron Microscopy (SEM). Microphotographs of the hybrid layers were taken at standard magnifications (1000x, 10 KV, 80 mA). Five measurements of the hybrid layer thickness were taken for each sample as follows: Two measurements were made in the outer part of the hybrid layer, one on each side, and three measurements were made in the central part of the same layer. These measurements were performed using Adobe Photoshop software (Version 6.0 Adobe Systems Brazil, SP, Brazil). This software allows for measurements of distance between two points on an image with a 2% margin of error

RESULTS

Table 2 shows a sample distribution of the five groups in relation to the cavity preparation modes, adhesive systems and hybrid layer thickness measurements.

The data derived from the five groups are indicated in Tables 3 to 7. In these tables, the code nf means that a hybrid layer was not found.

The measurement data were statistically analyzed with ANOVA using the SPSS for Windows release 5.0 program (SPSS Brazil, SP, Brazil). The variance analysis ($p \leq 0.05$) showed that there was a significant statistical difference among the combined effects of the five treatments. The Student-Newman-Keuls test ($p \leq 0.05$) separated the treatments into four homogeneous and distinct groups: Group I > Group II = Group III > Group IV > Group V. Regarding the adhesive systems, there were no statistically significant differences.

In Table 8, the average results obtained in the five groups can be observed after they had been statistically treated.

Figures 1 and 2 show the differences between the hybrid layer thickness and morphology. These figures were obtained from fragments of the same tooth (Samples B1 and L1).

DISCUSSION

The high-speed handpiece is the primary method for cavity preparation in dentistry. Alternative methods for cavity preparation, such as the Er:YAG laser, still present many deficiencies, while preparations are not standardized. A number of authors[25-26] have recommended techniques and have noted some advantages when using the Er:YAG laser, with an emphasis on a reduction in pain, noise and pressure. These authors have considered the possible use of such preparation procedures in Class I, II, III, IV and V direct restorations. Experiments comparing the microleakage data of adhesive fillings carried out in cavities prepared with different preparation methods are quite abundant in the literature and, in general, all works have shown that the studied methods present statistically similar results.[13-16,27] Nonetheless, according to the description by Krejci and others,[2] tests that only evaluate bonding forces would be important for an adhesive system quality evaluation. However, these studies should not be used as the sole or primary parame-

Table 2: *Samples Distribution*

Group	Subgroup	Surface Treatment	Adhesive Systems	# of Samples	# of Measurements
I	BMP	Diamond Bur	SBMP Plus	4	20
	BSB		Single Bond 2	4	20
	BPB		Prime & Bond 2.1	4	20
	BXE		Xeno III	4	20
II	LAMP	Laser 200 mJ/4 Hz	SBMP Plus	4	20
	LASB		Single Bond 2	4	20
	LAPB		Prime & Bond 2.1	4	20
	LAXE		Xeno III	4	20
III	LBMP	Laser 200 mJ/6 Hz	SBMP Plus	4	20
	LBSB		Single Bond 2	4	20
	LBPB		Prime & Bond 2.1	4	20
	LBXE		Xeno III	4	20
IV	LCMP	Laser 400 mJ/4 Hz	SBMP Plus	4	20
	LCSB		Single Bond 2	4	20
	LCPB		Prime & Bond 2.1	4	20
	LCXE		Xeno III	4	20
V	LDMP	Laser 400 mJ/6 Hz	SBMP Plus	4	20
	LDSB		Single Bond 2	4	20
	LDPB		Prime & Bond 2.1	4	20
	LDXE		Xeno III	4	20
Total				80	400

Table 3: *Hybrid Layer Thickness in Group I (Diamond Bur)*

Sample		Hybrid Layer Thickness (µm)		
	SBMP Plus II	Single Bond 2	Prime & Bond 2.1	Xeno III
B 1	3.68	4.62	4.11	1.34
	6.84	3.67	3.73	1.00
	7.20	3.80	3.81	1.50
	4.80	3.30	4.52	1.53
	5.60	3.71	4.14	1.56
B 2	2.44	3.73	3.22	0
	2.50	3.57	3.19	1.86
	3.67	3.26	2.22	0.93
	2.25	2.21	2.53	1.30
	2.85	2.68	1.97	0.93
B 3	2.85	5.19	4.38	Nf
	3.20	6.93	2.73	Nf
	4.75	4.72	2.76	Nf
	3.28	4.63	3.78	Nf
	3.56	3.82	2.80	Nf
B 4	4.69	3.75	2.73	Nf
	3.53	3.81	2.27	Nf
	3.12	4.66	1.82	Nf
	3.14	4.21	2.68	Nf
	4.38	4.17	3.32	Nf

Table 4: *Hybrid Layer Thickness in Group II (Er:YAG Laser—200 mJ/4 Hz)*

Sample	Hybrid Layer Thickness (μm)			
	SBMP Plus II	Single Bond 2	Prime & Bond 2.1	Xeno III
LA 1	8.37	5.97	0	2.07
	6.51	4.41	4.78	2.96
	5.11	8.09	4.65	2.37
	5.02	5.42	0	2.96
	3.90	5.97	0	4.14
LA 2	nf	2.54	0	0
	nf	2.36	4.44	1.42
	nf	1.96	3.20	0
	nf	1.76	5.42	0
	nf	2.52	0	0
LA 3	nf	2.87	1.94	Nf
	nf	1.91	1.38	Nf
	nf	2.82	1.90	Nf
	nf	2.92	2.34	Nf
	nf	0	1.17	Nf
LA 4	nf	nf	nf	0
	nf	nf	nf	0
	nf	nf	nf	2.27
	nf	nf	nf	0
	nf	nf	nf	1.59

Table 5: *Hybrid Layer Thickness in Group III (Er:YAG Laser—200 mJ/6 Hz)*

Sample	Hybrid Layer Thickness (μm)			
	SBMP Plus II	Single Bond 2	Prime & Bond 2.1	Xeno III
LB 1	nf	2.94	nf	6.56
	nf	1.96	nf	4.25
	nf	0	nf	4.32
	nf	0	nf	4.40
	nf	0	nf	6.42
LB 2	1.84	1.56	1.46	6.81
	2.64	1.77	1.85	6.36
	2.60	0	2.40	0
	0	1.40	0	0
	2.99	1.19	0	6.86
LB 3	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
LB 4	5.93	0	6.95	1.28
	4.06	3.69	7.02	0.93
	5.06	5.12	6.74	1.25
	4.43	3.64	7.33	1.00
	5.09	3.87	7.30	1.50

ters for recommending one or another adhesive system. According to these authors, a perfect marginal seal would seem far more important; therefore, marginal adaptation tests would have a greater clinical value. There is a close relationship between the hybrid layer morphology and a perfect marginal seal; therefore, tests that evaluate the hybrid layer micromorphology and evaluate hybrid layer thickness should always be carried out to evaluate the quality of an adhesive system.

The hybrid layer is extremely dependent on the dentinal substrate over which the layer is being produced.[7] As a result, the manner in which the dentin is prepared may be important. Studies by Wigdor and others[10] and Tokonabe and others[19] indicate that preparations done with a laser exhibit a lack of the smear layer and present open dentinal tubules after preparation, which is quite different from preparations carried out with diamond points. These studies have also shown that different parameters will produce different tissue characteristics. Hossain and others[18] showed that there is a linear relation between the ablation rate and used energy. Some authors have discussed the possible denaturation of collagen fibrils.[19,22] Ishizaka and others[21] demonstrated that the number of odontoblastic processes markedly decreased in the laser-treated area and concluded that Er:YAG laser irradiation might have denatured the organic material of dentin. These findings suggest that the use of an Er:YAG laser would probably affect the hybrid layer formation.

Table 6: *Hybrid Layer Thickness in Group IV (Er:YAG Laser—400 mJ/4 Hz)*

Sample	Hybrid Layer Thickness (µm)			
	SBMP Plus II	Single Bond 2	Prime & Bond 2.1	Xeno III
LC 1	0.80	0	2.84	Nf
	0.72	7.75	2.76	Nf
	0.84	0	2.88	Nf
	0.81	0	2.92	Nf
	0.77	7.27	2.96	Nf
LC 2	0	nf	nf	Nf
	0	nf	nf	Nf
	6.31	nf	nf	Nf
	6.14	nf	nf	Nf
	0	nf	nf	Nf
LC 3	nf	0	1.97	0
	nf	0	3.44	0
	nf	3.33	3.49	2.51
	nf	2.64	3.44	2.54
	nf	0	3.05	0
LC 4	1.93	nf	1.25	Nf
	1.97	nf	0.97	Nf
	2.39	nf	1.28	Nf
	2.35	nf	1.25	Nf
	2.43	nf	1.31	Nf

Table 7: *Hybrid Layer Thickness in Group IV (Er:YAG Laser—400 mJ/6 Hz)*

Sample	Hybrid Layer Thickness (µm)			
	SBMP Plus II	Single Bond 2	Prime & Bond 2.1	Xeno III
LD 1	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
LD 2	nf	3.81	nf	0
	nf	1.02	nf	0
	nf	3.47	nf	3.01
	nf	3.06	nf	3.44
	nf	1.81	nf	2.51
LD 3	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
	nf	nf	nf	Nf
LD 4	nf	nf	nf	1.96
	nf	nf	nf	1.61
	nf	nf	nf	2.35
	nf	nf	nf	2.78
	nf	nf	nf	2.43

This study compared the thickness of the hybrid layer formed using four different adhesive systems on a dentin surface prepared with a diamond bur in a high-speed handpiece or with an Er:YAG laser used with two parameters of pulse energy and two parameters of frequency. In order to standardize the dentinal substrate where the dentinal adhesives had been used so that the comparisons could be done using the same dentin pattern, the authors decided to use tooth fragments, making up subgroups that always originate from the same tooth, according to the description in the Methods and Materials section. This contrasts with many other studies, where experiments are carried out on multiple teeth that could exhibit different dentin characteristics.

The mean results found in this study, which were statistically treated and listed in Table 8, and the data derived from the five groups, listed in Tables 3 to 7, must be evaluated together. The average result analysis obtained in Group I showed similarities to the existing literature.[24,28-30] In these studies, the mean results were similar to the results listed in Table 8, and the authors have also found high standard deviation values, which means that the methodology was in accordance with the literature.

When analyzing the results found in Group I, the thinnest hybrid layers were found in the Xeno III subgroup, the only subgroup where the hybrid layer was not constantly found. These results are in accordance with the existing literature.[18] Some authors[31-34] have already tried to improve the results found with self-etching adhesive

Table 8: *Mean Results in the Groups*

Surface Treatment	Adhesive System	N	Mean (µm)	SD
Laser 200 mJ/4 Hz	SBMP Plus II	15	1.92	2.966
	Single Bond 2	20	2.57	2.367
	Prime & Bond 2.1	15	2.08	1.981
	Xeno III	15	1.31	1.418
Laser 200 mJ/6 Hz	SBMP Plus II	20	1.73	2.162
	Single Bond 2	20	1.35	1.664
	Prime & Bond 2.1	20	2.05	3.052
	Xeno III	15	3.46	2.695
Laser 400 mJ/4 Hz	SBMP Plus II	20	1.37	1.887
	Single Bond 2	20	1.04	2.394
	Prime & Bond 2.1	20	1.79	1.329
	Xeno III	20	0.25	0.777
Laser 400 mJ/6 Hz	SBMP Plus II	20	0	0
	Single Bond 2	20	0.65	1.288
	Prime & Bond 2.1	20	0	0
	Xeno III	20	1.00	1.309
Diamond Bur	SBMP Plus II	20	3.91	1.381
	Single Bond 2	19	4.03	1.008
	Prime & Bond 2.1	20	3.13	0.811
	Xeno III	20	0.59	0.709
Total		379	1.68	2.079

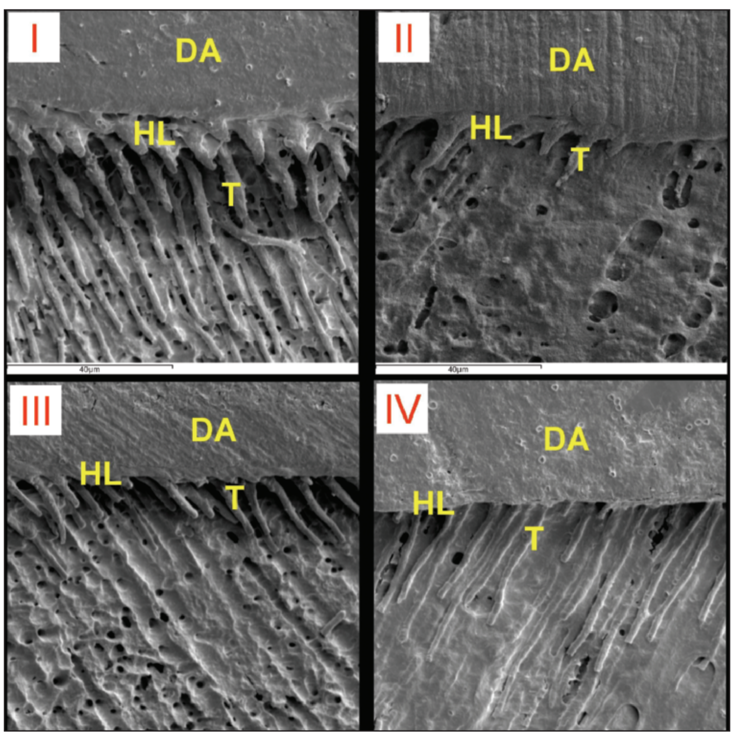

Figure 1. *Portion of the hybrid layers formed in the same tooth in Group 1 (Control). I—SBMP Subgroup; II—SB Subgroup; III—PB Subgroup; IV—XE Subgroup. DA=Dentin Adhesive; HL=Hybrid Layer; T=Tag.*

systems, but they failed. Theoretically, these self-etching adhesive systems are the better way to avoid nanoleakage[35] but, in fact, they still present many failures and their use should be avoided while new studies are conducted to improve their results.

The results found in groups where the dentin was treated with the Er:YAG laser were lower than the results in Group I in terms of all of the adhesive systems. Other authors[23,36] have already found thinner hybrid layers in dentin treated with Er:YAG laser, but the cause is still unknown. A simple comparison between the average results obtained in the different groups shows that the Er:YAG laser, with the parameters used in this experiment, is not a preparation method that allows for the formation of a thick hybrid layer when compared to the use of a diamond bur in a high speed turbine. Table 8 indicates that the groups were statistically heterogeneous—Group I > Group II = Group III > Group IV > Group V. However, more important than the comparison between the measurements obtained, is the fact that, in the samples belonging to Groups II through V, the hybrid layer was too irregular and its measurement was more difficult to obtain than in Group I. Unlike what happens in these laser groups, the hybrid layer could routinely be observed in Group I in a very even way, and the measurements could also be easily done. There were also a great number of samples where the hybrid layer was not found in the whole or part of the sample, indicating the instability of the dentin surface prepared with the Er:YAG laser. This fact was more constant where the

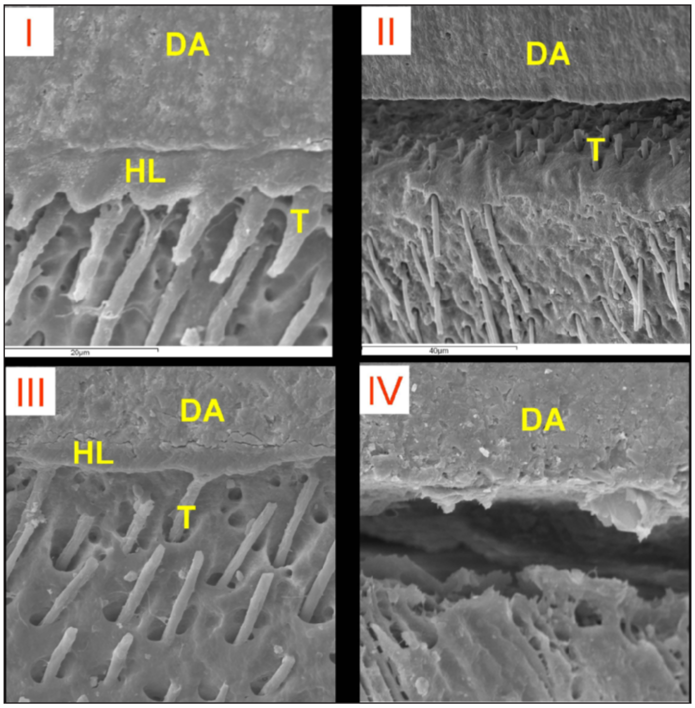

Figure 2. *Portion of the hybrid layers formed in Group 2. Note the differences in hybrid layer morphology with the same adhesive. I—Adper Single Bond 2; II—Adper Single Bond; III—Xeno III; IV—Xeno III. DA=Dentin Adhesive; HL=Hybrid Layer; T=Tag.*

authors used higher parameters. Figure 2 shows a great hybrid layer formation failure, which is a good example of this problem.

Mello[37] described the Er:YAG laser, when used on healthy dentin according to certain parameters, as promoting fusion between the dentinal components and a later solidification, leading to a solid structure formation with a mineral composition that is still unknown. According to Mello,[37] the structure is known to be extremely acid resistant and presents a completely altered collagen fiber composition mixed in the formed structure. It is believed that the structure is more mineralized and could be a poor substrate for the formation of a hybrid layer, which would agree with the statement by Perdigão and others[7] that a more mineralized structure is a poor substratum for adhesion.

It was not possible, with the applied methodology, to discover the cause of the obtained results in Groups II through V regarding both the hybrid layer measurement results and the relation to the inconsistency of the hybrid layer formation. The authors of the current study believe that the results obtained may be related to the following explanation: In the place where there is a perpendicular laser beam incidence, an acid resistant structure will form, as has already been described. Therefore, the hybrid layer formation would suffer damage in this location. In the region around the laser beam incidence, a lower temperature increase would occur, promoting a less stressing alteration in the structure of the collagen fibers,[21] and the formed structure would be less acid resistant, leading to an irregular and not so thick hybrid layer formation. This explains the inconsistency of the hybrid layer formation; in fact, in the places where it was possible to observe a hybrid layer, a hybrid layer over the peripheral region of the laser beam incidence might be what is actually observed.

When looking for a better quality hybrid layer over dentin prepared with the Er:YAG laser, the current authors believe that some changes in the parameters used could generate different results. Additional studies are in order, including alterations in parameters used by the laser device, which would promote less alterations in the dentinal structure; the use of acid etching for a longer period or the use of more concentrated acids; trying to promote a greater demineralization of the dentinal structure altered by laser or use of adhesive systems that are different from the adhesive systems used in the experiment. Another suggestion for future research would be to develop experiments that, using all the parameters used in this experiment, would try to explain how a hybrid layer that is so different from what is commonly found can obtain microleakage results or adhesion force results similar to that found in tests that use substrates where adhesion occurs, as described by Nakabayahi and others.[38] That is, how is it possible that works using the same parameters used in previous studies[13-14,27,39] have had microleakage results statistically similar between groups prepared with a high-speed diamond bur and groups prepared with the Er:YAG laser.

Even knowing the efficiency of the Er:YAG laser for dentistry, the remaining dentin surface is not favorable for the bonding mechanism, based on hybrid layer formation. Further studies should be conducted in order to develop new bonding mechanisms, specifically for irradiated surfaces, thus forming a new pattern of interaction for the bonding of resin-based materials.

CONCLUSIONS

Through analysis of the results obtained in this *in vitro* study, it was possible to conclude that:

1. On a dentin surface prepared with a high speed diamond bur, the four tested adhesive systems produced hybrid layers with an average thickness of 2.90 ± 1.71 μm, with a consistent, regular format in a continuous way;
2. On a dentin surface prepared with the Er:YAG laser, the four tested adhesive systems produced hybrid layers with an average thickness rang-

ing from 0.41 ± 1.00 µm to 2.06 ± 2.49 µm, with an inconsistent and irregular format in an intermittent way;

3. A preparation with high speed diamond point allows for a thicker hybrid layer formation than a preparation done with the Er:YAG laser.

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