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ORIGINAL ARTICLES

Effect Of Different Energy Levels And Exposure Times Of Ultra-Short Pulsed Laser On Dentin Surface Roughness And Shear Bond Strength Of Resin Based Composite.

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ABSTRACT

Objectives: to test surface roughness and evaluate the shear bond strength of dentin to resin based composite following different energy levels (200, 300 and 400 mJ) of ultra-short pulsed laser preparation for two exposure times (10 & 4o sec) using a total etch adhesive system. The microstructure of dentin surfaces after different energy levels of ultra-short pulsed laser ablation was investigated. **Materials and Methods**: A total of 120 of human dentin specimens were prepared by removal of occlusal enamel through sectioning till reaching the dentin just below the dentinoenamel junction. The total 120 samples were classified into two main groups group (I)**:** 60 samples for surface roughness testing (Ra). Group (II): 60 samples for shear bond strength measurements .Each group was subdivided into three subgroups according to Ultra short pulse laser energy used: *Subgroup (A1):* 200 mJ *Subgroup (A2):* 300 mJ *Subgroup (A3):* 400 mJ . Each subgroup was further divided into two divisions according to the laser exposure time: Division (B1): 10 second of USPL.Division (B2**)**: 40 second of USPL. The dentin surface was exposed to ultra short pulsed laser *at manufacturer's recommended settings. Dentin surface* ***roughness was*** **tested using profilmeter. Then dentin was etched with** 37% H3PO4 and thin layer of the tested adhesive material (Compobond 1, PROMEDICA, Germany) was carefully applied. Resin composite with (Composan LCM, PROMEDICA, Germany) was finally applied. **Results:** Surface roughness group results showed that the highest roughness was at an energy of 300 mJ and 40 seconds exposure time and the lowest roughness was at 400mJ/40 seconds. *With 200 mJ* there was no statistically significant difference between mean Ra after 10 sec. and after 40 sec while *With 300 & 400 mJ,* the mean Ra after 40 sec exposure time showed statistically significantly higher value of Surface roughness than after 10 sec exposure. The shear bond strength results showed that the highest results were at 300 mJ at 10 sec . While the lowest shear bond strength results was at 400mJ at both tested exposure times. *With 200 & 400 mJ,* there was no statistically significant difference between mean shear bond strength after 10 sec. and after 40- sec. While *With 300,* the mean shear bond strength after 10 sec. exposure showed statistically significant higher value than after 40 sec. exposure. **Conclusions:** 300 mJ of ultra-short pulsed laser for 10 seconds exposure time is considered the most appropriate of the tested energy levels for preparing dentin surface. At 400 mJ energy level there's massive destruction of dentin surface composition that decrease the shear bond strength. Moreover, Adhesion to lower energy levels ultra-short pulsed laser-ablated dentine was inferior to higher energy levels.

Key words:

Introduction

Since the 1990s, the laser in general is a well-established tool for hard tissue preparation in dentistry (Mehl A, *et al.,* 1997). Conventional laser ablated hard tissue preparation usually affects tissue removal by water-mediated ablation, and is commonly performed by erbium based laser systems. In spite of the high state of development of the current devices, some limiting factors still exist, such as the danger of inducing micro cracks and heat accumulation in case of insufficient external cooling (to maintain vitality, the maximum allowable temperature rise in the dental pulp is about 5°C (Zack L, Cohen G, 1965). These deficiencies are chiefly related to the ability of a given laser pulse to efficiently evaporate the water embedded in the tissue, and are governed by several factors, such as wavelength, pulse duration and pulse intensity (Strassl M, *et al.,* 2004; Meister J, *et al.,* 2003). To avoid these problems, a new approach was proposed already in the early 90s by several authors called ultra-short pulsed laser (USPL) having pulse durations in the picosecond and down to the femtosecond regime (Rubenchik AM, *et al.,* 1996; Kim BM, *et al.,* 2001). The dental application requires removal of a few mm of material, and clearly, speed is important. Marion and Kim 1999 stated that the lack of thermal and

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mechanical damage to surrounding tissue bodies is considered well for both patient discomfort and protection from tooth nerve damage (Marion II IE. Kim BM, 1999).

Neev *et al.,* 1996 reported the major advantages of the ultra-short pulsed lasers:

- 1) Efficient ablation due to small input of laser energy per ablated volume of tissue and the resulting decrease of energy intensity needed to ablate material.
- 2) Minimal collateral mechanical damage due to the efficient ablation and the short duration of the stress impulse.
- 3) Minimal collateral thermal damage due to the extremely short deposition time and the fact that a large fraction of the deposited thermal and kinetic energy is carried away with the ablated tissue.
- 4) Extreme precision in ablation depth is achievable because only a small amount of tissue is ablated per pulse and the number of pulses can be controlled by feedback mechanisms;
- 5) Low, operating, noise level and minimized pain due to localization of energy deposition and damage (Neev J. *et al.,* 1996).
- Most commonly, the dentin surface morphology, chemical composition and smear layer will be different according to the way of dentin preparation (Tokonabe H, *et al.,* 1999; Hossain M, *et al.,* 1999). Also the surface roughness and adhesive bond strength will be variable according to either the preparation technique or the pretreatment protocols before resin based composite application (Harris, D.M and Pick, R.M *et al.,* 1995; Tokonabe H, *et al.,* 1999). However , unanswered questions remain of this technique: what will be the results of different energy levels of ultra-short pulsed laser on dentin surface roughness and shear bond strength of lased dentin to resin based composite. Therefore, this study was designed to study Effect of different energies of USPL on dentin surface roughness and shear bond strength of resin based composite to lased dentin.

Materials and Method

I- Preparation of the samples:

A total of 120 of human molar teeth were extracted, from 50 to 60 years old patients, for periodontal diseases. The teeth were free from visible caries and other surface defects. Teeth were cleaned with a rotary brush and pumice and stored in saline and used within 6 months from time of extraction to inclusion in the study. The roots were sectioned 2 mm beyond the cemento-enamel junction .Teeth were individually embedded in polyvinyl chloride cylinder (2 cm diameter and 1.5 cm high) filled with an auto-polymerized acrylic resin (JET, Cla´ssico, Sa˜o Paulo, SP 05458-001).

The occlusal enamel was removed by horizontal sectioning till reaching the dentin just below the dentino-enamel junction using the Isomet slow-speed diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). The dentin surface was abraded with decreasing grits of silicon carbide (SiC) paper (from #800 to # 1200) under water-cooling for 30 s / paper. A standard superficial dentin surface of about 0.5 mm from dentinoenamel with standard smear layer was produced. Dentin surface area for testing was determined with the aid of an adhesive tape punched by a modified Ainsworth rubber-dam punch to provide 3 mm diameter holes. This was necessary to ensure that the restorative material was applied to the tested areas (Hossain M, *et al.,* 1999; De Munck J, *et al.,* 2002; De saouza A , *et al.,* 2004; Souza-Gabriel A.E, *et al.,* 2006).

II- Sample classification:

The total 120 samples were classified into two main groups:
Group I: 60 samples for surface roughness testing.
Group II: 60 samples for shear bond strength measurements
Each group was divided into three subgroups according to Ultra short pulse laser energy used:
Subgroup 1 (A1): 200 mJ
Subgroup 2 (A2): 300 mJ
Subgroup 3 (A3): 400 mJ
Each subgroup was further divided into two divisions according to the exposure time:
Division 1(B1): 10 second of USPL.
Division 2 (B2): 40 second of USPL.

III- Laser ablation condition:

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Dentin surfaces were ablated by Ultra short pulsed laser (USPL). Its wavelength was 2940 nm in infra red region. Spot size was 3 mm. The beam was applied perpendicularly to the specimens, with the tested different energies of (200, 300, 400 mJ).

IV- Measurement of surface roughness:

Five points were determined on all samples of each division of group I samples. These points were one on the upper part of the tested area, one in the lower part, one on the right part, one on the left part and one on the center. Dentin roughness was tested using a profilmeter. The profilmeter, which is based on a diamond tracer of 0.5 μ in diameter, was adjusted to traveling distance of 0.8. It is based on measuring the (Ra) value which is the arithmetic mean of the movement of the profile above and below the central line of the surface. The mean of five tracing for every specimen was calculated and taken as the surface roughness value of the specimen. Dentin roughness was measured for all samples before and after laser ablation.

V- Pre-treatment of Dentin surface:

Each sample of group II was etched, according to manufacturer instructions, for 10 sec with etching agent (35 % of phosphoric acid, Cica, PROMEDICA, Germany) and rinsed for 20 sec. Then oil free air dried. A thin layer of the tested adhesive material (Compobond 1, PROMEDICA, Germany) was carefully applied to etched specimens on the isolated dentin surface with disposable brush tips to avoid excess and pooling of adhesive along the edges of the insulating tape that could compromise the distribution of fracture during the test. The specimen was gently air-dried for 5 seconds and photopolymerized for 20 seconds using a light-curing unit [(XL 3000, 3M Dental Products, USA)] with an output of 600 mW/cm2. The intensity of light was verified with a handheld radiometer [Demetron/Kerr, Danbury, CT, USA] every five samples.

A circular Teflon mould was positioned over the tested areas, resulting in a cylindrical cavity with the diameter coincide with the lased area and determined bonding area (φ = 3mm) and 3 mm in height.

Resin composite with shade A1 (Composan LCM, PROMEDICA, Germany) was applied, in two successive layers and cured for 40 sec, to the treated surface to give a disc of 3 mm in width and 3 mm in depth. The teflon mould was removed and samples were stored in water at 37◦C for 24 hours.

Measurement of shear bond strength:

The samples of group II were subjected to shear bond strength testing. The samples with their restorative material were clamped to a universal testing machine. Each specimen in its resin block was hold in the lower jaw of the testing machine. In the upper jaw, a knife edge chisel was attached and allowed force application on interface between the test material and the tooth surface, the test machine was run at a constant speed of 0.5 mm/min and until fracture. The software (windap version 3.2 on IBM compatible computer) connected to the machine was used to collect data and draw a load-displacement curve for each specimen.

Shear bond strength values were registered in Newton and transformed into MPa by dividing the maximum load by the surface area .The surface area (A) was calculated according the equation:

$A = \pi r^2$, where

$\Pi = 22/7 = 3.14$

$r = 1.5$ mm so

$A = 22/7 \text{ X } (1.5)^2 = 7.065 \text{ mm}^2$.

VI- Topographic evaluation:

The Scanning Electron Microscopic (SEM) examination was carried out using QUANTA 200 scanning electron microscope attached with EDX unit, with accelerating voltage 30 K.V., magnification 10x up to 400.00x. Selected samples were rinsed in distilled water and cleaned with a gauze wetted with 98% ethanol alcohol and then dried in an oven at 75 ° C for 15 min. The specimens were mounted on a metallic holder of one cm² in diameter & two mm in height , using a carbon adhesive paste. Then the samples were examined with SEM and photographed

VII- Statistical analysis:

Numerical data were presented as mean and standard deviation values. *Paired t-test* was used to compare between mean surface roughness and mean shear bond strength. *One-way ANOVA (Analysis of Variance)* was used to compare between means surface roughness and shear bond strength after application of different energy levels. *Duncan's post-hoc test* was used for pair-wise comparison between the means when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with SPSS 14.0 (Statistical Package for Scientific Studies) for Windows.

Results:

I- surface roughness results:

Comparison between energy levels:

Table 1: The means, standard deviation (SD) values of surface roughness, results of ANOVA and Tukey's test for comparison between the three energy levels after 10 seconds exposure

A1		A2		A3		*P*-value
Mean	SD	Mean	SD	Mean	SD	
2.59 [b]	0.2	2.45 [b]	0.5	2.73 [b]	0.17	0.010*

**: Significant at P ≤ 0.05, Means with different letters are statistically significantly different according to Tukey's test*

There was no statistically significant difference between A1, A2 and A3 .
A2 showed the lowest means Ra.

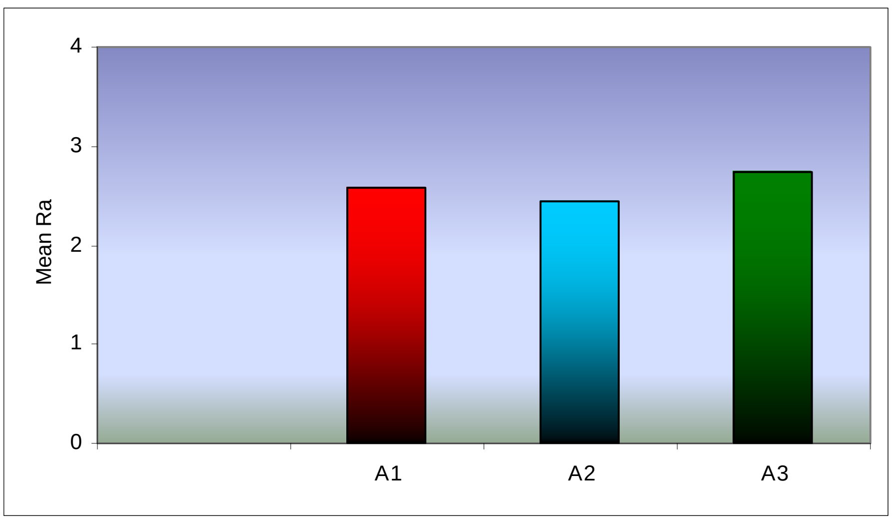

Fig. 1: The mean values of surface roughness, results of ANOVA and Tukey's test for comparison between the three energy levels after 10 seconds exposure.

Table 2: The means, standard deviation (SD) values of surface roughness, results of ANOVA and Tukey's test for comparison between the three energy levels after 40 seconds exposure

A1		A2		A3		*P*-value
Mean	SD	Mean	SD	Mean	SD	
2.70 [b]	0.2	3.30 [a]	0.3	2.10 [c]	0.2	<0.001*

**: Significant at P ≤ 0.05, Means with different letters are statistically significantly different according to Tukey's test*

A2 showed the statistically significantly highest mean Ra. This was followed by A1 which showed lower value. A3 showed the statistically significantly lowest mean Ra.

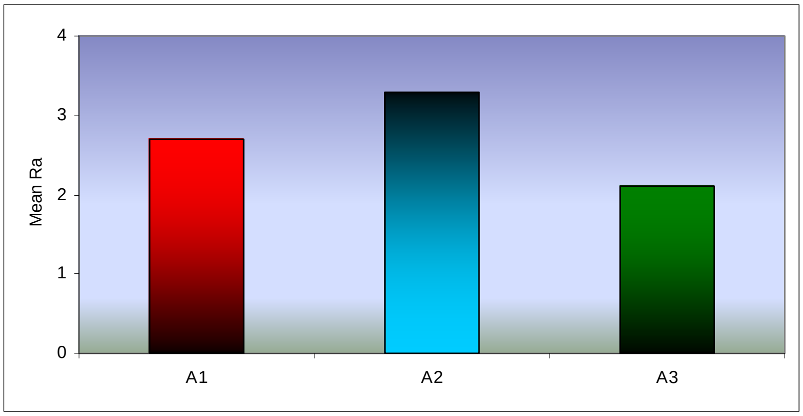

Fig. 2: The mea values of surface roughness, results of ANOVA and Tukey's test for comparison between the three energy levels after 40 seconds exposure

Comparison between exposure time :

Table 3: The means, standard deviation (SD) values and results of Student's t-test for comparison between exposure times

Time / Energy level	10 Sec.		40 Sec.		*P*-value
	Mean	SD	Mean	SD	
A1	2.59	0.2	2.70	0.2	0.412
A2	2.45	0.5	3.30	0.3	0.010*
A3	2.73	0.17	2.10	0.2	0.002*

**: Significant at P ≤ 0.05*

With A1, there was no statistically significant difference between mean Ra after 10 sec. and after 40 sec.

With A2, the mean Ra after 40 sec. exposure showed statistically significantly higher value than after 10 sec. exposure.

With A3, the mean Ra after 10 sec. exposure showed statistically significantly higher value than after 40 sec. exposure.

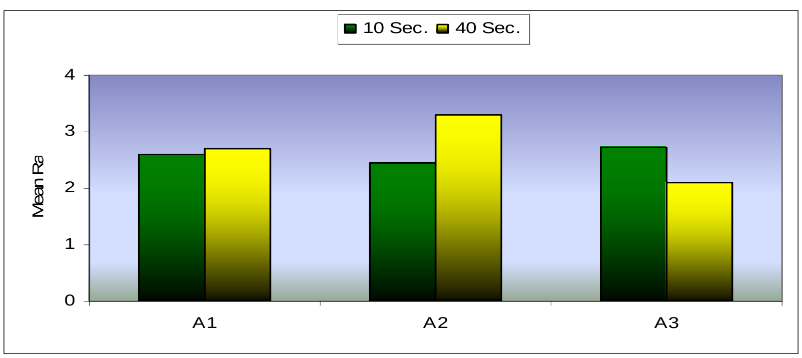

Fig. 3: The mean values and results of Student's t-test for comparison between exposure times

Overall comparison between the groups:

Table 4: The means, standard deviation (SD) values, results of ANOVA and Tukey's tests for comparison between all the groups

Group	Mean	SD	*P*-value
A1/10 sec.	2.59 [b]	0.2	<0.001*
A1/40 sec.	2.70 [b]	0.2	
A2/10 sec.	2.45 [c]	0.5	
A2/40 sec.	3.30 [a]	0.3	
A3/10 sec.	2.73 [b]	0.17	
A3/40 sec.	2.10 [d]	0.2	

**: Significant at P ≤ 0.05, Means with different letters are statistically significantly different according to Tukey's test*

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A2/40 sec. which showed the statistically significantly highest means Ra. There was no statistically significant difference between A1/10 sec., A1/40 sec. and A3/10 sec. which showed lower values. This was followed by A2/10 sec. A3/40 sec. showed the statistically significantly lowest mean Ra.

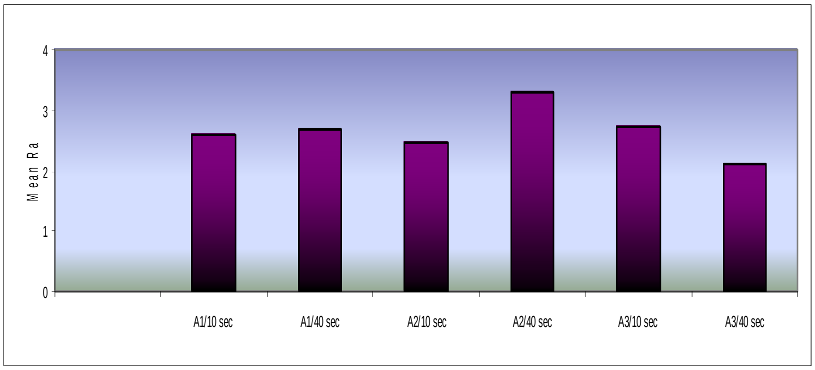

Fig. 4: The mean values, results of ANOVA and Tukey's tests for comparison between all the groups

II- Shear bond results:

Comparison between energy levels:

Table 5: The means, standard deviation (SD) values, results of ANOVA and Tukey's test for comparison between the three energy levels after 10 seconds exposure

A1		A2		A3		*P*-value
Mean	SD	Mean	SD	Mean	SD	
203.5 ^{b}	2.5	277.6 ^{a}	11.6	155.9 ^{c}	7.9	<0.001*

**: Significant at $P \leq 0.05$, Means with different letters are statistically significantly different according to Tukey's test*

A2 showed the statistically significantly highest mean shear bond strength. A1 showed lower values. A3 showed the statistically significantly lowest mean shear bond strength.

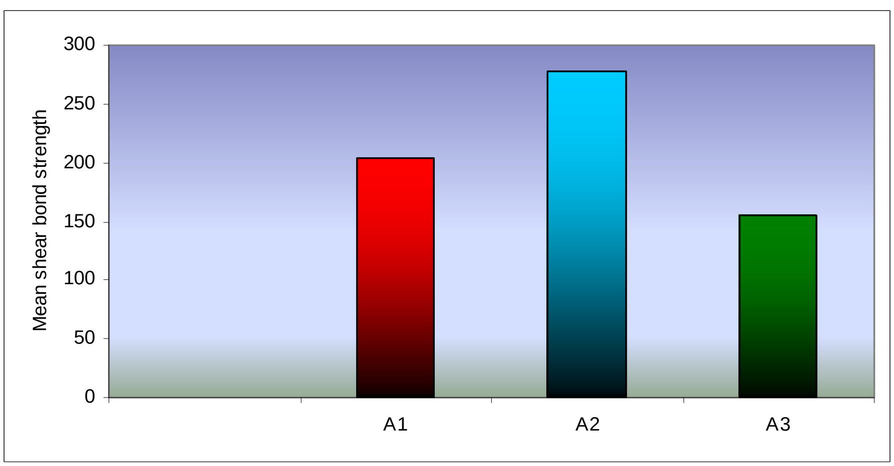

Fig. 5: The mean values, results of ANOVA and Tukey's test for comparison between the three energy levels after 10 seconds exposure

Table. 6: The means, standard deviation (SD) values, results of ANOVA and Tukey's test for comparison between the three energy levels after 40 seconds exposure

A1		A2		A3		*P*-value
Mean	SD	Mean	SD	Mean	SD	
199 ^{a}	7.5	179.6 ^{b}	5.3	165.4 ^{c}	10.4	0.006*

**: Significant at $P \leq 0.05$, Means with different letters are statistically significantly different according to Tukey's test*

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A1 showed the statistically significantly highest mean shear bond strength. This was followed by A2 which showed lower value. A3 showed the statistically significantly lowest mean shear bond strength.

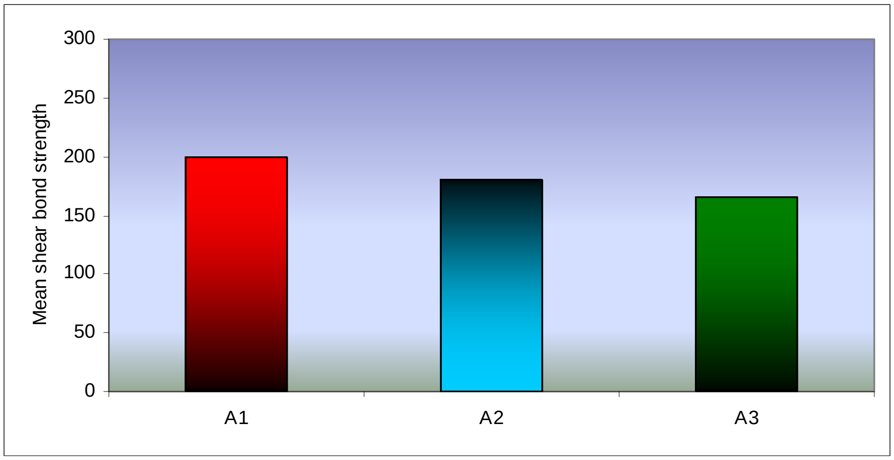

Fig. 6: The mean values, results of ANOVA and Tukey's test for comparison between the three energy levels after 40 seconds exposure

Comparison between exposure times:

Table 7: The means, standard deviation (SD) values and results of Student's t-test for comparison between exposure times

Time / Energy level	10 Sec.		40 Sec.		*P*-value
	Mean	SD	Mean	SD	
A1	203.5	2.5	199	7.5	0.377
A2	277.6	11.6	179.6	5.3	<0.001*
A3	155.9	7.9	165.4	10.4	0.277

**: Significant at $P \leq 0.05$*

With A1, there was no statistically significant difference between mean shear bond strength after 10 sec. and after 40 sec.

With A2, the mean shear bond strength after 10 sec. exposure showed statistically significantly higher value than after 40 sec. exposure.

With A3, there was no statistically significant difference between mean shear bond strength after 10 sec. and after 40- sec.

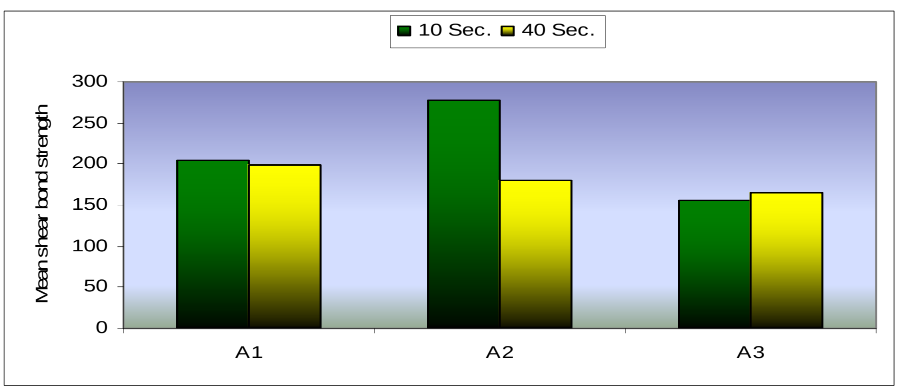

Fig. 7: The means values and results of Student's t-test for comparison between exposure times

Overall comparison between the groups:

Table 8: The means, standard deviation (SD) values, results of ANOVA and Tukey's tests for comparison between all the groups

Group	Mean	SD	*P*-value
A1/10 sec.	203.5 [b]	2.5	<0.001*
A1/40 sec.	199 [b]	7.5	
A2/10 sec.	277.6 [a]	11.6	
A2/40 sec.	179.6 [c]	5.3	
A3/10 sec.	155.9 [d]	7.9	
A3/40 sec.	165.4 [d]	10.4	

**: Significant at P ≤ 0.05, Means with different letters are statistically significantly different according to Tukey's test*

A2/10 sec. showed the statistically significantly highest mean shear bond strength. A1/40 sec. which showed lower values. This was followed by A2/40 sec. There was no statistically significant difference between A3/10 sec. and A3/40 sec. which showed the statistically significantly lowest means shear bond strength.

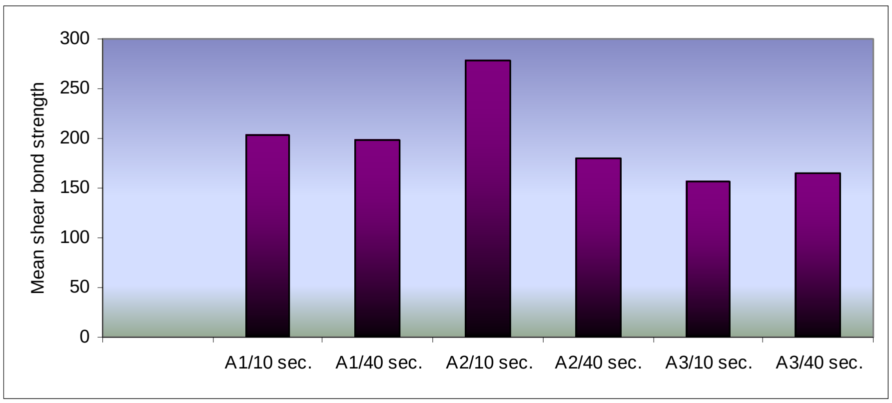

Fig. 8: The means values, results of ANOVA and Tukey's tests for comparison between all the groups

Fig. 9: SEM magnification 800 showing dentin surface ablated with ultra short pulse laser at 200 mJ for 10 sec showing surface roughness with long roughness folds

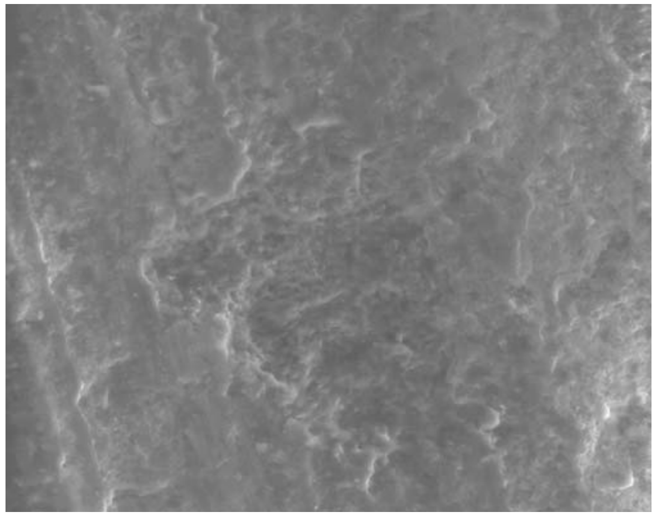

Fig. 10: SEM at magnification 800 showing dentin surface ablated with ultra short pulsed laser at 300 mJ for 10 sec showing increasing numbers in surface roughness and shorter roughness folds

Discussion:

The ultra-short pulsed laser was chosen for this study as it was proposed by many investigators to be a promising type of laser for cutting hard dental tissues with a major advancement in cutting technology. None of cutting hard tooth tissue, tools can achieve the precision of the femtosecond laser machine tool (0.1 millimeters).

It was found (tables 1-4) that there was increase in the dentin surface roughness when energy levels were increased. This may be explained as the increase the energy densities of USPL causing increase the ablasive process at fixed time of exposure. Also for A1 and A2 the increase of ablation time lead to increase of surface roughness. It was coinciding with Rosane de Fátifna *et al.,* (2002) who found that the surface configuration of the dentin was having pits and grooves. In addition, it lacks of smear layer and the orifices of many of the tubules were exposed. Also Uchizono *et al.,* (2007) mentioned that in irradiated dentin samples, the surface showed opened dentinal tubules and no smear layer. Ablation depth by USPL depends on the average power density. USPL has the possibility that can control the precision and non-thermal ablation with depth direction by adjusting the irradiated average power density. And this may explain that in our study at 300mJ the mean Ra after 10 sec. exposure showed statistically significantly lower mean value than after 40 sec. exposure as the roughness depths increases when exposure time increase

It was found that the highest shear bond results was at A2/10 sec and the lowest shear bond at A3/10 sec (table 8) which may show that adjusting the irradiated average power density lead to proper roughness results and highest shear bond results. These results was in agreement with Uchizono *et al.,* (2007) and Wieger *et al.,* (2007) who mentioned that the novelty of performance of USPLs with various pulse durations regime were applied to dental hard tissue.

In this study scanning electron microscope results showed that the surface of the irradiated dentine samples showed open dentinal tubules and no smear layer. This was also in agreement with Uchizono *et al.,* (2007). who studied the morphology of the tissue surfaces remaining after laser preparation. They stated that Smooth cavity rims, no microcracks, melting or carbonization and precise geometry are the advantages of scanned USLP ablation.

It was found that a statistically significant negative correlation between shear bond strength and energy levels and so surface roughness. We may consider that the lack of resin penetration in laser-ablated dentin is the most likely explanation for lower bond strengths through SEM images. The bonding mechanism of resin to acid-etched dentin is well known and understood to be micromechanical (De Munck J, *et al.,* 2002; Perdigao J, *et al.,* 1994). Little is known about the adhesion of resin to laser ablated dentin, but it appears that the formation of an interdiffusion zone, which is the basis for dentin hybridization in acid-etched dentin, is unlikely (William J, *et al.,* 2005). Instead, laser ablated dentin probably acquires its bond strength solely from the penetration of resin tags into dentin tubules. In this topic, Ceballos *et al.,* (2002) mentioned that laser ablation of dentin produced a modified superficial layer in which collagen fibers are poorly attached to the underlying substrate, because they lost part of their cross-banding, and the thermal effects due to higher densities could extend into the dentin subsurface, thus impairing interdiffusion zone formation.

Conclusions:

This study compared different energy levels dentin ablation rates at two different exposure time. Evaluation the surface roughness changes and measured the shear bond strength results to resin based composite.

1- regarding surface roughness, a moderate energy level (300 mJ/10 s) of ultra-short pulsed laser is considered the most appropriate.

2- Increasing of exposure time with higher energy levels leads to decrease in surface roughness and shear bond strength.

3- USPL is a novel method for hard dental tissue preparation because it is considered a painless method without anesthesia

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