In Vitro Comparative Assessment of Composite Nanoleakage Using Various Dentine Surface Treatments

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ABSTRACT

Background: The treatment of dental tissues proceeding to adhesive procedures is a crucial step in the bonding protocol and decides the clinical success ofrestorations. This study was conducted *in vitro,* with the aim of evaluating thenanoleakage on the interface between the adhesive system and the dentine treated by five surface modalities using scanning electron microscopy and energydispersiveX-ray spectrometry.

Materials and methods: Twenty five extracted premolars teeth were selected in the study. Standardized class V cavities were prepared on the buccal and lingual surfaces then the teeth divided into five main groups of (5 teeth in each group n=10) according to the type of dentine surface treatment that was used: Group (A): dentine was conditioning with Er, Cr: YSGG laser. Group (B): dentine was conditioning with (Er, Cr: YSGG laser + acid). Group (C): dentine was conditioning with (acid + Er, Cr: YSGG laser). Group (D): dentine was conditioning with acid etch. Group (E): dentine was conditioning with acid + 10% sodium hypochlorite. For All the teeth SBMP adhesive were used and restored with Z250 composite restoration then all specimens were subjected to thermocycling 500 cycles, at 5° to 55 °C. The teeth were soaked in silver nitrate suspension. Then the teeth sectioned bucco-lingually across the centre of the restorations. The specimens were characterized using scanning electron microscopy and the amount of nanoleakage was measured by EDX spectro-analysis to identify the existence of metallic silver particles.

Results: Data were analysed statistically by one way ANOVA test and (LSD) Tests. The results showed that there were statistically highly significant differences among all groups of the present study.The resultshowed that the acid and laser (group **III**) exhibited the lowest mean value of nanoleakage at around (6.14 %), whereas the highest mean value of nanoleakage (12.83 %) was determined by the only acid (group **IV**).

Conclusions: Treating the acid etched dentine with Er: Cr: YSGG laser showed promising results as it exhibits lowest amount of nanoleakage of the adhesive bonding system.

Keywords: Er, Cr: YSGG laser, silver nitrate, Nanoleakage, SEM/EDX. **(J Bagh Coll Dentistry 2016; 28(4):49-55)**

INTRODUCTION

The seal of a restorative material against the tooth structure, and the quality and durability of the seal, are major considerations for the longevity of restorations. Since the introduction of the acid-etching technique for the pre-treatment ofdental hard tissues **[(1)]**, new techniques and adhesive materials thatcan fulfil their function of bonding to enamel and dentine have been developed andmodified **[(2)]**. Yet, a disadvantage distinguished to acid etchingis the demineralization of tooth tissues, which create them more permeable and liableto acid attacks, particularly if the demineralized substrates are not entirely filled byadhesive resins **[(3)]**.

The clinical significance beyond studying thenanoleakage measurement using different surface treatments is to improve the qualityof bonding of composite restoration. Thus, improving the durability of restoration canbe achieved. Despite the significant improvement in bondingcapacity, up-to-date, no method and/or restorative material being capable ofeliminating nanoleakage. For this reason, new methods have been developed toimprove the quality and longevity of restorations **[(4)]**.

In order to overcome this limitation, recent investigations Suchas dental lasershave been introduced to be used as Alternative tools that could better prepare theenamel and dentine surfaces for future bonding procedures. High power lasers havebeen produced in dentistry to carry out cavity preparations and to promote chemical/morphological changes on the tooth surface. Erbium lasers (Er:YAG andEr,Cr:YSGG) have been considered the most promising lasers to be used onmineralized tissues because both wavelengths show high absorption by water andhydroxyapatite **[(5)]**.

Previous studies have evaluated the effect of erbium lasers in surfacemorphology of dentine, wherein they have found an irregular appearance, withoutsmear layer, with open dentinal tubules and prominent peritubular dentine, with a microretentive morphological pattern possibly favourable to bonding procedures **[(6)]**.

Nanoleakage is originally defined as the phenomenon of tracer penetration *via* 20- to100-nm spaces into hybrid layers, even in the absence of a marginal gap **[(1)]**. Nanoleakage is considered an important indicator of the sealing ability ofrestorative materials and hybrid-layer quality, which subsequently affect thedurability of the restoration **[(1,7)]**.

The present study aimed at evaluating the nanoleakage on the interface between adhesive

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system and the dentine with five different dentine surfacetreatmentsby scanning electron microscopy and energy-dispersive X-ray spectrometry.

MATERIALS AND METHODS

Teeth selection

Non-carious, non-restored twenty five sound maxillary first premolar teeth extracted for orthodontic purposes were conducted in the present study. Teeth were cleaned from adhering soft tissues and calculus deposits with a hand scaler.

The teeth were cleaned and polished using water and pumice with a prophylaxis rubber cup, and rinsed with water. All teeth were visually examined under a magnifying lens and by transillumination fibre optic from a light curing unit for the presence of cracks and the ones that showed any defects were excluded from the study[(8)].

Teeth mounting

The teeth were mounted individually in a specifically designed locally- manufactured rubber mold with cold cure acrylic (Major, Italy) with the long axis of the tooth parallel to centre of the mold. Each tooth was suspended in the middle of the mold using a Ney Surveyor (Bego, Germany) to ensure vertical positioning of the tooth inside the mold, as follows; the centre of the occlusal surface of each tooth was attached to the vertically moving arm of the surveyor along its long axis with sticky wax.

When the axis of the tooth was positioned correctly, acrylic resin was poured into the mold and before it reached the dough stage the tooth was inserted. All specimens were embedded up to 3mm apical to the CEJ. After initial polymerization, the samples were placed in water to avoid overheating due to resin polymerization [(4)].

Cavity Preparation

On the buccal and lingual surfaces standardized class V cavities were prepared (2mm height, 2mm width, 2mm depth)**.** A high speed hand piece was fitted to the surveyor (horizontal arm) in a manner that the long axis of the bur being vertical to the tooth, by a medium grain diamond bur No. 848, underneath water coolant.

On the tooth surface the outline of the cavity was drawn by a 0.5 mechanical pencil by a matrix band with a previously made cut hole of 2 x 2 mm which was secure on the tooth using a retainer in which the gingival floor of the cavity was set at (1 mm) below the cemento-enamel junction of the tooth. The cavity form was finished by round bur in a low speed hand piece by water coolant. The teeth were arbitrarily divided into five groups each one consists of five teeth (n=10).

Samples grouping

The teeth were randomly divided into five main groups (5 teeth in each group n=10) according to the type of dentine conditioning: Group one: the class V conditioning only with laser. Group 2: the class V conditioning with laser and acid etching, Group 3: the class V conditioning with acid etching and laser, Group 4: the class V conditioning with only acid etching, Group 5: the class V conditioning with acid etching and 10% NaOCl.

Laser irradiation

The Er, Cr:YSGG laser used in this study was Waterlasei-plus (Biolase Technology Inc., San Clemente, CA, USA), which emits at a 2.78-μm wavelength. It has fixed pulse duration for hard tissues of 140 μs. The irradiation was accomplished in both vertical and horizontal directions, using 30 % of water spray and 60 % of air spray.

Laser tip was positioned upright to the target area of the dentine surface, with working distance of 1 mm [(9)] was kept from the dentine surface throughout the procedures. To ensure this distance between dentine and radiation tip, an endodontic file was fixed at the handpiece [(10)].

Scanning the surface in both orders (vertical and horizontal). Each fibre tip was discarded after five times of use [(11)]. Laser hand-piece moved in a sweeping fashion by surveyor to obtain a homogeneous surface appearance over the entire area [(12)]. The laser etching was performed for 15 s for each surface [(13)]. Using laser tip MZ8 in the gold MD handpiece (manufacture instruction).

Conditioning of dentine with acid

The teeth were etched by the total Etch technique (a 35% phosphoric acid etchant (3M) which was spread over the enamel and dentine start with the enamel borders of the restoration for 15 seconds.

The cavities were carefully rinsed from phosphoric acid gel by water for 15 sec. The dentine surface was dry by an air syringe for two seconds using triple syringe at a distant of 1.5 cm to achieve a little moist surface (the surface is considerably glossy); however, no noticeable excess water should persist on the tooth surface.

Application of Scotchbond multipurpose bond

The Scotchbond multipurpose bond was used onto the conditioned tooth structure by a bonding applicator. In a gentle movement apply the primer with disposable brush tip then gently dried with air stream for 5 seconds. Apply the adhesive bond and light cure for 10 seconds (manufacture instructions).

Placing the restoration

The resin based composite was spread over in one incremental layer. Before curing a transparent matrix was positioned to contour the restoration and cure for 20 second. The borders of the restoration were finished and polished by using sof-lex (3M) discs.

Application of sodium hypochlorite

A sodium hypochlorite 10%solution was gently spread over for 1 min, rinsed by using runningwater for 20 seconds [14]. The dentine surface was dry by an air syringe for two seconds using triple syringe at a distant of 1.5 cm.

Thermocycling procedure

In an attempt to simulate the temperature changes that take place in the oral cavity, all specimens were subjected to thermocycling according to the International Organization for Standardization (ISO) TR11405 standard of 500 cycles, at 5° to 55 °C, with a 15 second dwell time [15].

Ability to resist Nanoleakage

All the teeth were characterized using a stereo microscope to confirm that no flash was left along borders of the restoration. And then the entire tooth, excluding for the bonded interface and also the 1 mm of the tooth surface adjacent to the interface coated by two layers of nail varnish. The teeth were put in a 50% (weight/volume) silver nitrate liquid in total darkness for 24 hours, rinsed in running water for 5 minutes, immersed in photo-developing liquid, and subjected to a fluorescent light for 8 hours (which reduce the silver ions to metallic silver). Then remove the teeth from the developing solution, the teeth were put in running water for 5 minutes and sectioned bucco-lingually through the centre of the restorations by a low speed diamond disk [1, 16].

The cut surfaces were fixed in epoxy resin and polished by using increasingly fine diamond pastes (6, 3, 1 µm; Buehler Ltd, Lake Bluf, IL, USA). Then the specimens were placed in ultrasonic cleaner contain distilled water for 5 minutes, and They were dehydrated in increasing concentrations of ethanol (50%, 60%, 70%, 80%, and 90%) for 2 h each and in 100% ethanol for 24 h. Final chemical using hexamamethyldisilazane [17], stable on aluminium stubs. The nanoleakage patterns were detected by scanning electron microscopy/ energy dispersive X-ray spectroscopy (SEM-EDX) (Elemental chemical analysis by means of Energy-dispersive X-ray spectroscopy (EDX) using the backscattered electron image mode.

The use of SEM in combination with EDX had the ability to present both distinct images and sensitive quantification of silver ion penetration accurate. As it permits analysis for the element composition of the scanned square area. Thus, provides accurate identification for the presence or absence of metallic silver particles along the adhesive tooth / restoration interface. By this, both false negative and false positive results were excluded.For each specimen makes 3 readings by EDS in the center of the gingival surface and 0.3 in the left and the right to the center to determine nanoleakage value by silver ions uptake [18].

RESULTS

The descriptive statistics of nanoleakage qualification detected by silver ion percentage for each group are shown in Table 1.

Table 1: Means and Standard deviations of the experimental groups tested in this study, n=10

Groups	Mean	±SD	Min.	Max.
Only laser	12.01	3.29	3.39	16.23
Laser + acid	8.66	2.66	3.02	13.74
Acid + laser	6.14	2.55	0.84	9.99
Only acid	12.83	4.96	4.02	18.22
NaOCL	7.65	2.87	1.06	13.91

One-way ANOVA test revealed that there were statisticallyhighly significant differences among all the groups tested in the present study ($P \leq 0.01$) as reported in table 2.

Table 2: ANOVA test for the nanoleakage of the experimental groups tested in this study, n=10

ANOVA test	Sum of Squares	df	Mean Square	F-test	p-value
Between Groups	365.899	4	91.475	8.26	0.000 HS
Within Groups	498.349	45	11.074		
Total	864.248	49			

Further comparisons among groups were done using the Least Significant Difference test (LSD test) to see where the significant difference occurred. The results showed that there was no significant difference ($p > 0.01$) when comparing group (**I**) and groups (**II** and **IV**), whereas a statistically significant difference ($p < 0.01$) was observed when comparing group (**I**) and groups (**III** and **V**).

DISCUSSION

The clinical relevance of the current study was to improve the marginal adaptation of composite restoration by conditioning the dentine surface with a variety of treatments. Among the most promising systems of laser is the family of erbium lasers because their wavelength coincides with the main absorption peak of water and hydroxyapatite **(19)**. Thus Er: YAG and Er, Cr: YSGG lasers interact well with all biological tissues, including enamel and dentine surface **(9)**. It has been reported that there is often a discrepancy between the depth of acid-etching and the degree of resin infiltration and exposed collagen network **(20)**. This region may be another site for silver uptake. Although the amount of nanoleakage may be very small (nanometre-size) in the bonded assembly, it has the potential to serve as a pathway for water movement within the adhesive‐dentine interface over time **(21)**. Evaluation of silver uptake (i.e. nanoleakage evaluation) provides good spatial resolution of submicron defects in resin infiltration or inadequate polymerization **(22)**.

The findings of the present study are discussed in details and justified as shown in the following sections below:

Group one (only laser conditioning figure 1)

There are non-significant differences with (only acid conditioning group) and (laser + acid conditioning group) While there are a highly significant differences between this group and (acid + laser conditioning group) and (10% NaOCL conditioning group)this result could be attributed to the effect of laser on the dentine surface that will effect on the monomer diffusion, the characteristics of erbium-irradiated dentine, such as the absence of smear layer, opened dentinal tubules and the presence of an irregular surface after irradiation, could be favourable for adhesive procedures **(23)**. Hossain et al **(24)** showed that in addition to the lack of smear layer and presence of intact enamel rods as well as exposed dentinal tubular openings in their SEM observations, laser prepared surfaces provided better bond abilities with restorative materials and acid etching could be easily replaced by laser use. Similarly, Türkmen et al **(25)** found that the Er,Cr:YSGG laser "etches" the enamel surface more effectively than 37% phosphoric acid for subsequent attachment of composite material.

The type III acid etching pattern with a regular rough surface and spaces could be seen with acid etching **(26)**. Dissolution of hydroxyapatite by acid produced tags and rough surfaces that afforded the mechanical lock for resin. The preferred type III pattern was seen by the Er:YAG laser at a distance 1 and 2 mm and with the Er,Cr:YSGG laser at 1 mm. A uniform honeycomb appearance was evident. Enamel irradiated with the Er:YAG laser at 4 ad 6 mm and with the Er,Cr:YSGG laser at a distance 2, 4 and 6 mm had the type IV etching pattern as described by Silverstone et al **(26)**. The surface characteristics and shear bond strength are suitable for enamel etching. The dentine surface after Er,Cr:YSGG laser irradiation shows no smear layer, dentine tubules are open and the subsurface is not demineralized. Irradiation of dentine with an Er,Cr:YSGG laser creates a rough surface with chimney-like formations due to the preferential removal of intertubular dentine.

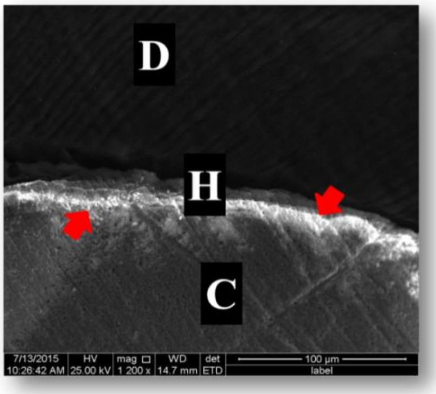

Figure 1: Representative backscattered SEM images of the resin–dentine interfaces of group (I) only laser surface conditioning. (C composite, H hybrid layer, D dentine, red arrow refer to nanoleakage).

Group two (laser + acid conditioning figure 2)

There are non-significant differences between this group and (only laser conditioning group), (acid + laser conditioning group) and (10%NaOCL conditioning group), While there are a significant differences between this group and (only acid conditioning group). This is possibly related to the creation a more homogeneous dentine surface by the acid etched that follow the laser etched widened dentine tubule orifices were seen when acid was applied after Er,Cr:YSGG laser irradiation, because of removing the mineral content of the dentine. Flat surfaces were seen after acid etching **(27)**.

Acid etching following Er,Cr:YSGG laser irradiation could demineralize the inorganic portion of surface dentine and reproduce a suitable environment for molecular entanglement of polymer chains with collagen fibrils. Furthermore, the widened dentinal tubule orifices also facilitated the deep infiltration of bonding agent. The extensive branching of the resin tags into lateral branches of dentinal tubules could be found acid etching of lased dentine could reinforce the hybrid layer; and formation of resin tags.

Acid etching following Er,Cr:YSGG laser conditioning can help reduce microleakage in class V restorations [13].

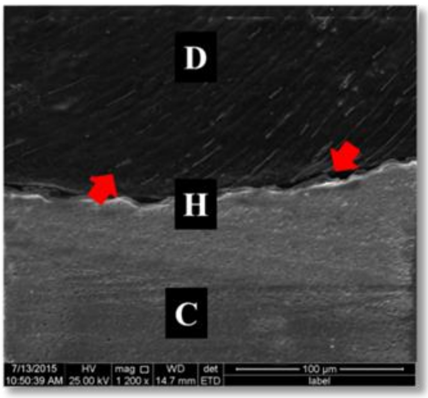

Figure 2: Representative backscattered SEM images of the resin–dentine interfaces of group (II) laser + acid surface conditioning. (C composite, H hybrid layer, D dentine, red arrow refer to nanoleakage).

Group three (acid + laser conditioning. Figure 3)

There are non-significant differences between this group and (10% NaOCL conditioning group) and (laser + acid conditioning group), while there are a highly significant differences between this group and (only acid conditioning group) and (only laser conditioning group). This is due to the ability of Er,Cr:YSGG laser to remove the collagen network away from acid etched dentine and this will lead to enhance the infiltration potential of the monomer to the intact dentine and minimizing the nanoleakage. Also removing of the collagen network away from acid etched dentine substrate will make the chemical composition of dentine more similar to that of enamel by decreasing the organic component of dentine substrate and this will cause a changing in the hydrophilic properties of the dentine. The depletion of collagen network from the surface of acid etched dentine results in: The permeability of dentine substrate will be enhancing due to the enlargement of dentinal tubules nearby the outer dentine surface; this will increase the distribution and spreading of adhesive monomers through dentine [28].

The surface energy of dentine will be improved, because the high surface energy of the hydroxyapatite while the collagen has a low surface energy and this would produce improving in the diffusion of adhesive monomers through dentine. This description complies with that of Bedran et al [29].

The dentine is very rough and porous with many lateral branches of tubules are noticeable in main tubules which may contribute to enhance the diffusion of adhesive monomers through dentine, this complies with Ferrari et al [30] wherein they justified the findings with the same explanation.

Er,Cr:YSGG radiation is highly absorbed not only by the hydroxyapatite of dentine but also by the protein and lipid in collagen fibre [31].

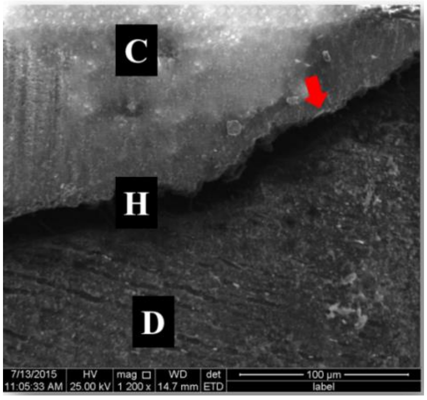

Figure 3: Representative backscattered SEM images of the resin–dentine interfaces of group (III) acid +laser surface conditioning. (C composite, H hybrid layer, D dentine, red arrow refer to nanoleakage).

Group four (only acid conditioning. Figure 4)

There are non-significant differences between this group and (only laser conditioning group), and there are a significant differences between this group and (laser + acid conditioning group), and highly significant differences between this group and (acid + laser conditioning group) and (10%NaOCL conditioning group). These differences were possibly due to the possibility of the adhesive system monomers not being able to penetrate completely the demineralized dentine after the acid etching, leaving a porosity zone that could lead to leakage. This porosity may permit the hydrolysis of collagen fibres which is believed to degrade the adhesive resin.

Strong acids such as phosphoric acid create demineralization deeper than the diffusion capacity of the resin monomers thus leaving collagen fibres in the deep tissue layers unprotected [32].

The deposition of silver ions occurred in most of the specimens along the base on the hybrid layer, and, according to the author, the difficulty of the penetration of the resin monomers all over the demineralized dentine extension is, in part, due to the limited size, length and sinuosity of the canals created around the collagen fibres that collagen fibrils within the hybrid layer are not fully embedded by dentine adhesives revealing different degrees of infiltration from the top to the bottom of the hybrid layer [33].

For total-etch systems, these areas of sparse, imperfect resin infiltration exist because it is physically difficult for the adhesive monomers to penetrate the highly hydrophilic matrix of demineralized collagen fibrils, as previously indicated by other studies [34].

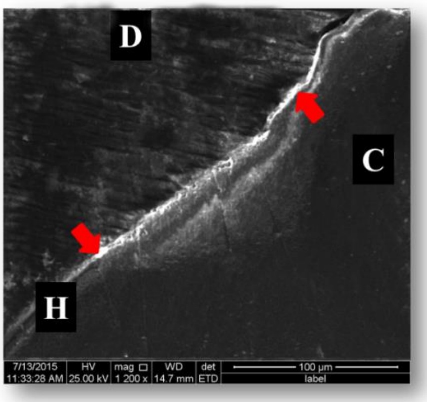

Figure 4: Representative backscattered SEM images of the resin–dentine interfaces of group (IV) only acid surface conditioning. (C composite, H hybrid layer, D dentine, red arrow refer to nanoleakage).

Group five (10% NaOCL conditioning. Figure 5)

There are non-significant differences between this group and (acid + laser conditioning group) and (laser + acid conditioning group), While there are a highly significant differences between this group and (only acid conditioning group) and (only laser conditioning group). This differences in the nanoleakage could related to the deprotinization procedure, this procedure first removes the exposed collagen and then dissolves the fibrils into the underlying mineralized matrix to create submicron porosities within the mineral phase. Channels created are available for resin infiltration within the mineralized matrix.

They show a complete difference in the morphology of deproteinized dentine as compared with the acid etched dentine. The diameter of tubule orifices increased after the NaOCL treatment of acid etched and demineralized dentine due to loss of demineralized peritubular dentine. This substrate has more hydroxyapatite crystals and may result in a more durable interface over time as it is basically made of minerals [14].

Our results are in accordance with the study done by Goes and Montes [35], wherein they concluded that collagen depletion prior to bonding application may prevent nanoleakage occurrence in dentinal walls.

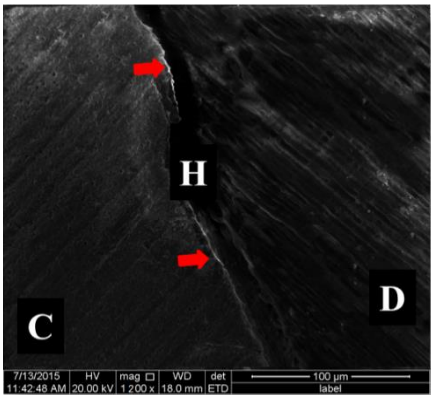

Figure 5: Representative backscattered SEM images of the resin–dentine interfaces of group (V) 10% NaOCL surface conditioning. (C composite, H hybrid layer, D dentine, red arrow refer to nanoleakage).

The following conclusions are drawn in this study:

1. None of the surface conditioning techniques used in the present study can prevent nanoleakage in class V cavity.
2. Treating the acid etched dentine with Er: Cr: YSGG laser has led to a significant decrease the nanoleakage value at the adhesive bonding system.
3. Using acid etch is important with the laser to reduce nanoleakage.

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