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Research Article

MICROTENSILE BOND STRENGTH OF UNIVERSAL ADHESIVE TO DENTIN AFTER ER: YAG LASER PRETREATMENT WITH DIFFERENT CONDITIONING MODES - AN *IN VITRO* STUDY

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ABSTRACT

Adhesion to dentin remains challenging due to its complex nature that prevents complete infiltration of hydrophobic monomer to hydrophilic dentinal substrate. Recently, Er:YAG laser has been used in dentin pretreatment to enhance the bond strength. A deficient hybrid layer is formed following laser etching as it causes thermal damage and collagen denaturation of dentinal substrate. As the results are controversial, attempts are made to modify laser etching technique with respect to energy density, pulse duration and water spray. Thus, the present study aimed to evaluate the influence of Er:YAG laser conditioning of dentin by activation of Chlorhexidine collagen stabilising agent on the bond strength.

Samples included 60 freshly extracted premolar which were divided into three groups (n=20) based on the protocol of dentin pretreatment done: Group 1 (Control) - Phosphoric acid etching followed by universal adhesive; Group 2 (Experimental) - Conventional Er:YAG laser dentioning; Group3 (Experimental) - Er:YAG laser dentin conditioning with coronal reservoir of 0.12% Chlorhexidine. Occlusal class 1 cavity was prepared on the samples followed by which dentin conditioning protocol was carried out for the respective groups. Universal adhesive was applied followed by Composite resin restoration. 1000 cycles of Thermocycling was done and the samples were sectioned to obtain microsections. Microtensile bond strength values were obtained by subjecting the specimens to Universal testing machine.

Highest microtensile bond strength value was obtained for group 1 followed by group 3 with least value obtained for Group2 with statistically significant values between the groups. ($p<0.0001$). Within the limitations of the present invitro study, it can be concluded that preetching with phosphoric acid increases the bond strength of universal adhesive to dentin. Laser conditioning of dentin through activation of chlorhexidine provided better bond strength value of universal adhesive when compared to conventional laser dentin conditioning.

Keywords:

1. INTRODUCTION

Clinical success of composite restoration depends on the durability of bond between the dental substrate and the restorative material. Most efficacious mechanism for adhesion is micromechanical retention [1, 2]. Adequate bond strength for enamel is achieved by phosphoric acid etching whereas for dentin which has higher water and organic content, its efficiency is questionable [3]. Dentin adhesive interface is marked by the formation of hybrid layer which is an in situ tissue engineering in which resin enveloped collagen scaffold is formed [4].

Van Meerbeek et al., classified adhesives as etch and rinse adhesives and self-etch adhesives based on their mode of application [5]. Later, universal adhesive was introduced to be used in either Self etch, Total etch or Dual etch mode based on the substrate [6].

Drawbacks of phosphoric acid etching include its technical difficulty, postoperative sensitivity and demineralisation and incomplete infiltration of resin that makes the hybrid layer more prone to nano-leakage [3]. To obtain equally effective bond to different dental substrates, additional pre-treatment is required that makes the substrate more receptive and ultimately improve their bond strength to tooth structure.

Currently emphasis has been placed on laser dentin conditioning. Er:YAG laser is an attractive tool in operative dentistry which has been used successfully with various applications. There is ongoing research in regard to the effect of Er:YAG laser conditioning on bond strength. However its effect remains questionable due to controversial results obtained which is influenced by factors like laser wavelength, the emission mode, energy density, frequency, tissue water content, air/water spray cooling. This leads to changes in morphological characteristics of dentin along with changes in chemical composition following laser irradiation [7-9].

Upon irradiation with Er:YAG laser, thermal energy is absorbed by water containing collagen which in turn gets denatured hindering the formation of hybrid layer. Though, adequate water spray is used to avoid thermal damages of the surrounding tissue, water spray is primarily utilised for the ablation process than serving as a cooling system [10]. Thus, It was hypothesized that laser conditioning with additional supply of solution like Chlorhexidine (CHX) might prevent denaturation of collagen thereby providing a more receptible surface for bonding.

Thus, the aim of this study was to investigate the microtensile bond strength of composite resin bonded with universal adhesive to dentinal surface treated with Phosphoric acid etching/two different modes of laser dentin conditioning. The null hypothesis to be tested was that there was no difference in microtensile bond strength of composite to dentin treated by phosphoric acid or laser conditioning.

2. MATERIAL AND METHODS

Sixty freshly extracted human premolar teeth without any caries, cracks, restorations or structural deformities was used in the study. The teeth were stored in 0.5% chloramine-T, that was replaced once in 15 days to avoid contamination.

On each tooth, occlusal class I cavities were prepared using a high speed handpiece and diamond burs (EX-41,Dia burs-MANI, Tochigi, Japan) with air and water coolant. The final dimensions of the prepared cavity was approximately 3mm wide, 2.5 mm deep. Tooth samples were randomly divided into three groups (n=20), according to the etching protocol used for bonding and restoration of class I cavities.

Group 1: Conventional Dentin conditioning using Phosphoric Acid

A 37% phosphoric acid gel was used to etch dentine surfaces for 15 s. After this, etched dentine surfaces were rinsed for 10 s to remove etching gel completely. The bonding procedures was performed according to the manufacturer's instructions. The primer was applied for 15 s, and then gently air-dried for 5 s. The adhesive (Single Bond Universal, ESPE, St. Paul, Minnesota, USA) was applied and light cured for 20 s with a light curing unit.

Group 2: Conventional Er:YAG laser conditioning of dentine

Er:YAG laser with contact hand piece with a sapphire tip (8 mm long, 1.3 mm diameter) was used to treat dentin surfaces with laser irradiation. The parameters considered for irradiation will be 80 mJ/pulse, a frequency of 10Hz for duration of 30 seconds. Continuous air water spray (40% of air/60% of water = 21 ml/min) was used throughout the study. Laser beam was perpendicular to the dentin surface at a focal distance of 7 mm from the target point (focused mode). The adhesive (Single Bond Universal, ESPE, St. Paul, Minnesota, USA) was applied and light cured for 20 s with a light curing unit.

Group 3: Er:YAG Laser Dentin conditioning with CHX

0.5 mL of 2% chlorhexidine liquid was inserted into the cavity and activated by laser for 1 min. When the irrigating solution in the coronal reservoir decreased, the 2% chlorhexidine liquid was refreshed, resulting in a total of 3 mL. Laser activation was performed with an Er/YAG laser at a wavelength of 2,940 nm. A 14-mm-long and 300-μm-diameter quartz tip was applied with 0.9 W, 30 Hz, and 30mJ/pulse. The water and air on the laser system was turned off, and the optical fiber was placed into the cavity and irradiated for a period of 30 secs. The adhesive (Single Bond Universal, ESPE, St. Paul, Minnesota, USA) was applied and light cured for 20 s with a light curing unit.

Once the bonding agent was applied, samples in each group were restored with composite. The restored teeth were stored in an incubator with 100% humidity at 37 degree C for 1 week and will be subjected to thermocycling for 1000 cycles. The teeth were then mounted in acrylic resin blocks and a low speed diamond was used to section the teeth under copious water coolant. Two cuts were made in a mesio-distal direction along the long axis of the teeth with a 1 mm thick diamond disc and then the centre restorative part of the tooth was sectioned bucco-lingually by giving four cuts. Thus, three bonded stick shaped specimens of 0.9 mm ± 0.1 mm2 cross -sectional area was obtained from each tooth.

Samples from all the groups were subjected to bond strength evaluation using universal testing machine. Each beam was attached to a custom-made jig using cyanoacrylate glue and a tensile load was applied at a cross head speed of 1 mm/min until the beam fractured.

The amount of load required for fracture recorded in newtons was converted to megapascals (Mpa) by using the formula,

S = L / A

Where S is the bond strength in mega pascals (MPa)

L= Test load (N)

A= Adhesive area (mm^2)

2.1. Statistical Analysis

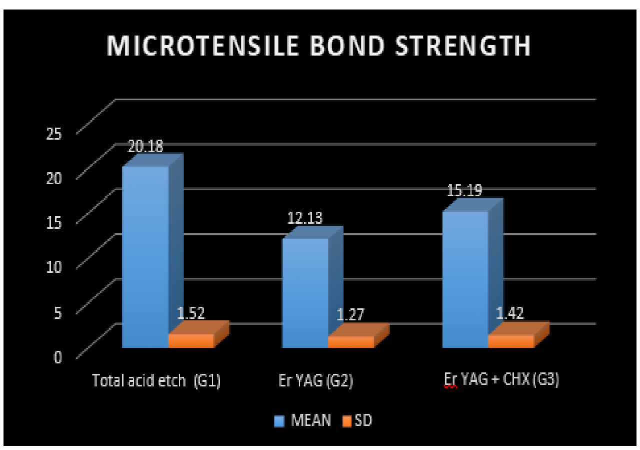
All statistical analysis was performed using the statistical package SPSS 22.0 (SPSS Inc., Chicago, IL) and level of significance will be set at $p<0.05$. Inferential statistics was done to find out the difference between and within the groups using Student T test, one way ANOVA and Tukeys post hoc test.

3. RESULTS

The average microtensile bond strength were G1: 20.18, G2: 12.13, G3: 15.19 ($p<0.001$) [Table1]. Statistical analysis revealed statistically significant difference between Group1, Group2 & Group 3. Group 1 revealed significantly higher bond strength values whereas samples in Group 2 had least values for bond strength. Intergroup comparison was done using Post Hoc HSD test. The results showed significant difference between all the groups.

	GROUPS	MEAN	SD
Microtensile bond strength (Mpa)	Total acid etch (G1)	20.18	1.52
	Er:YAG (G2)	12.13	1.27
	Er:YAG with Chlorhexidine(G3)	15.19	1.42
***P* value (<0.0001) (ONE WAY ANOVA TEST)**			
Tukeys hsd test	G1 vs G2	0.0001*	
	G1 vs G3	0.0001*	
	G2 vs G3	0.0001*	

(p <0.0001)



4. DISCUSSION

Latest generation of bonding agent is universal adhesive. Xiaofang et al., in his meta analysis reports superior performance of etch and rinse technique when compared to self etch approach in terms of retention and adaptation. This necessitates additional pretreatment of dentinal substrate to enhance bond strength in self etch mode [11].

Er:YAG hard tissue laser with wavelength of 2940nm has several applications in dentistry as its wavelength coincides with the absorption peak of water and hydroxyapatite crystals. Er:YAG laser has been used successfully in minimally invasive cavity preparation with remarkable patient comfort, selective caries removal with antibacterial effect, treatment of dentin hypersensitivity, prevention of secondary caries by decreasing enamel solubility, as cavity disinfectant and in bleaching [8,12].

In terms of bonding, its mechanism of action produces dentin surface that is free of smear layer with open dentinal tubules theoretically favoring adhesion of restoration to the dentin. However, various studies reveal that, upon Er:YAG laser dentin conditioning, there is deficient hybridization. This occurs due to the thermal damage upon laser irradiation that results in fusion of collagen fibrils without interfibrillar space impeding the formation of hybrid layer. This occurs even in presence of water spray, as it majorly acts as source for ablation rather than serving as coolant to avoid thermal damage [8, 12, 13].

So far, there are no studies evaluating the bond strength following laser dentin conditioning by flooding the cavity with collagen stabilizing agent. In present study, chlorhexidine was used for laser activation as it serves the purpose of cavity disinfection and acts as collagen stabiliser by inhibiting the activity of matrix metalloproteinase, thereby preserving the hybrid layer and enhancing the longevity of restoration [14, 15].

The results of the present study revealed significant difference between three groups where group conditioned with phosphoric acid presented highest bond strength followed by group conditioned with Er:YAG activation of chlorhexidine with least bond strength values for conventional laser conditioning group.

Nesrine *et al.,* compared the bond strength of composite resin to dentin treated with phosphoric acid etching and laser in low energy mode followed by universal adhesive application. He concluded that, when laser was used in high energy mode, bond strength was inferior to that obtained by phosphoric acid etching whereas when laser conditioning was done in low energy mode followed by application of Universal adhesive, bond strength was equivalent to phosphoric acid etching. This is similar to the results obtained in the present study, where lowest bond strength values are obtained for convention laser etching. The high energy causes morphologically altered zone with carbonization of top layer and melting of dentin with fused interfibrillar space in subsurface layer hindering the penetration of resin monomer [16, 17].

However, there are studies reporting the bond strength following Er:YAG laser conditioning with low fluence. When used in low energy mode, there is less thermally altered layer favoring resin infiltration [17, 18].

In Endodontics, Laser tips are used to activate irrigating solution which results in formation of bubbles and consequent evaporation of water molecules leading to efficient removal of smear layer leading to increased bond strength of fibre post to root dentin [19, 20]. Influence of laser modification of coronal dentin by activation of chlorhexidine solution has not been previously addressed in the literature.

It was found that, when chlorhexidine was activated with Er:YAG laser, there was significantly higher bond strength values than that obtained by conventional laser conditioning. Similarly, when laser conditioning was done with chlorhexidine, it might have prevented thermal damage by causing expansion of solution without causing damage to the dentin by ablative process.

Edson et al., in his study states that 0.12% chlorhexidine did not interfere with the performance of self etch adhesive systems [15]. Thus, laser activation of chlorhexidine might have reduced the thermal damage preventing cracks and denaturation of collagen and additionally rehydrating dried mineralised dentin preserving the humidity required to keep collagen network intact [21].

Further studies are required to confirm the efficacy of Er:YAG Laser dentin conditioning through activation of solution to overcome the drawbacks of direct activation of laser tip on dentin. Fewer samples with small number of groups were used in the study as it was a preliminary study which is the limitations of the present study.

5. CONCLUSION

Within the limitations of the present invitro study, it can be concluded that preetching with phosphoric acid increases the bond strength of universal adhesive to dentin. Laser conditioning of dentin through activation of chlorhexidine provided better bond strength value of universal adhesive when compared to conventional laser dentin conditioning. Thus, laser dentin conditioning with modifications it technique could enhance the bond strength which is otherwise difficult to achieve.

Conflict of interest

None declared

Source of funding

None declared

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