DOI: 10.7860/JCDR/2016/19739.7705

Original Article

Dentistry Section

Comparative Evaluation of the Etching Pattern of Er,Cr:YSGG & Acid Etching on Extracted Human Teeth-An ESEM Analysis

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ABSTRACT

Introduction: Etching of enamel and dentin surfaces increases the surface area of the substrate for better bonding of the tooth colored restorative materials. Acid etching is the most commonly used method. Recently, hard tissue lasers have been used for this purpose.

Aim: The aim of the present study was to evaluate and compare the etching pattern of Er,Cr:YSGG and conventional etching on extracted human enamel and dentin specimens.

Materials and Methods: Total 40 extracted non-diseased teeth were selected, 20 anterior and 20 posterior teeth each for enamel and dentin specimens respectively. The sectioned samples were polished by 400 grit Silicon Carbide (SiC) paper to a thickness of 1.0 ± 0.5 mm. The enamel and dentin specimens were grouped as: GrE1 & GrD1 as control specimens, GrE2 & GrD2 were acid etched and GrE3 & GrD3 were lased. Acid etching was done using Conditioner 36 (37 % phosphoric acid) according to manufacturer instructions. Laser etching was done using Er,Cr:YSGG (Erbium, Chromium : Ytrium Scandium Gallium Garnet) at power settings of 3W, air 70% and water 20%. After surface treatment with assigned agents the specimens were analyzed under ESEM (Environmental Scanning Electron Microscope) at X1000 and X5000 magnification.

Results: Chi Square and Student "t" statistical analysis was used to compare smear layer removal and etching patterns between GrE2-GrE3. GrD2 and GrD3 were compared for smear layer removal and diameter of dentinal tubule opening using the same statistical analysis. Chi-square test for removal of smear layer in any of the treated surfaces i.e., GrE2-E3 and GrD2-D3 did not differ significantly ($p>0.05$). While GrE2 showed predominantly type I etching pattern (Chi-square=2.78, $0.05<p>0.10$) and GrE3 showed type III etching (Chi-square=4.50, $p<0.05$). The tubule diameters were measured using GSA (Gesellschaft fur Softwareentwicklung und Analytik, Germany) image analyzer and the't' value of student 't' test was 18.10 which was a highly significant result ($p<.001$). GrD2 had a mean dentinal tubule diameter of 2.78μm and GrD3 of 1.09μm.

Conclusion: The present study revealed type I etching pattern after acid etching, while type III etching pattern in enamel after laser etching. The lased dentin showed preferential removal of intertubular dentin while acid etching had more effect on the peritubular dentin. No significant differences was observed in removal of smear layer between the acid etched and lased groups. Although diameter of the exposed dentinal tubules was lesser after lased treatment in comparison to acid etching, further long term in vivo studies are needed with different parameters to establish the usage of Er,Cr:YSGG as a sole etching agent.

Keywords: Power density, Smear layer, Target tissues, Wavelength

INTRODUCTION

Etching of enamel and dentin produces surface irregularities and helps forming microtags & macrotags which aids in micromechanical bonding to composite resins. Sazak et al., quoted many drawbacks of acid etching like damage to the tooth structure, multiple clinical steps making the procedure technique sensitive and time consuming and hybridization deficit [1].

As a possible alternative to acid conditioning the use of laser therapy has recently shown a promising front [2]. Er,Cr:YSGG(Erbium, Chromium : Ytrium Scandium Gallium Garnet) laser, a hydrokinetic laser system having a wavelength of 2780 nm was investigated by Usumez et al., and was found to have ablating effect on enamel and dentin [3].

The interest in the Erbium(Er) laser was based on the wavelength it can emit i.e., 1.54μm and 2.7μm-2.9μm. The former coincides with the absorption minimum of optical silica fibres, allowing long range optical communication incorporating optical amplifiers. The later wavelength coincides with the peak of water absorption. Water is present in every biological tissue so efficient interaction and dense optical energy deposition is guaranteed. In 1997 with the FDA clearance of Er,Cr:YSGG in US, came approval for caries removal, cavity preparation and conditioning of the tooth [4].

All the Er lasers have an affinity for the wavelengths to be highly absorbed by water, hydroxyapatite and collagen, thus are the target tissues. The highest peaks for the absorption of laser energy in water are 3μm and 10μm. The proposed mechanisms of ablation are cavitation bubbles, apatite crystal fragmentation and an acceleration of water droplets by laser light called the "hydrokinetic mechanism". Laser etching is through a process of continuous vaporization and micro explosions of water entrapped in the hydroxyapatite matrix. The energy level basically depends on the photon energy. The laser energy is absorbed by the water molecules which expand. Therefore they need more space and press and push the external microbulks of enamel towards the axis of light [5].

In comparison to acid etching, laser etching, reduces chair side time and can be used in moist conditions so is less technique sensitive. The use of fine laser tips gives more control over the area which needs to be precisely etched. Although cost has been a major factor for the limited usage of hard tissue lasers but its varied applications in other procedures, like tooth preparation, soft tissue excision makes it economical over the time [3].

The specific objectives of the present study was to compare the smear layer removal by Er,Cr:YSGG and acid etching on prepared specimens of enamel and dentin. This study also evaluated the type of etching pattern exhibited on enamel by both the methods of etching. Lastly, the difference in the etching zones on dentin (intertubular vs peritubular dentin) was also analyzed in this study by both laser and conventional etching methods. This study also measures and compares the diameter of dentinal tubules after the different surface treatments.

Our null hypothesis was that surface modification of enamel and dentin by Er,Cr:YSGG will result in better removal of smear layer as compared to that of 37% phosphoric acid. We also hypothesized that the diameter of the dentinal tubules was lesser after laser etching as compared to acid etching.

AIM

The aim of the present study was to compare and evaluate the etching pattern of Er,Cr:YSGG and conventional etching on prepared samples of human enamel and dentin by ESEM.

MATERIALS AND METHODS

This in vitro study was conducted in the Department of Conservative Dentistry & Endodontics, Dr, R. Ahmed Dental College & Hospital, Kolkata. Forty extracted non-diseased human teeth stored in normal saline after cleaning were taken as sample. Ethical Committee clearance was taken from West Bengal University of Health Sciences, Kolkata, India. Twenty anterior teeth for enamel specimens and 20 posterior teeth for dentin specimens were used [Table/Fig-1]. To obtain uniform thickness of samples anterior teeth were selected for enamel specimens. Vertical sections of enamel surfaces were prepared through middle third of the sample teeth by double sided diamond disk at slow speed. Horizontal sections of dentin surfaces were prepared through middle third of the sample teeth by double sided diamond disk at slow speed [Table/Fig-2]. Each specimen was polished by 400 grit SiC paper for 30sec to produce a smear layer. The thickness of each specimen was 1.0 mm ±0.5mm and was measured by a bur gauge of 0.1mm sensitivity. 20 enamel & 20 dentin specimens were subdivided into group: GrE1, GrD1 (two samples each) acting as control, GrE2, GrD2 (nine samples each) acid etched and GrE3, GrD3 (nine samples each) lased.

An acrylic resin platform was made and the specimens were placed in it stabilized by elastomeric impression material during the etching procedure. GrE2 and GrD2 were acid etched using 36% phosphoric acid gel (Conditioner 36, Dentsply) [Table/Fig-3]. The application time for enamel was 30sec and that for dentin was 15sec. The application time for enamel is longer due to the higher inorganic content of enamel (85% by volume) as compared to that of dentin (45% by volume). The specimens were then rinsed with water for 30sec and dried by oil free compressed air for 15sec [6].

Er,Cr:YSGG laser of 2.78 µm wavelength with the following settings was used: power -3W, air- 70%, water- 20%. The beam for enamel and dentin specimens was aligned perpendicular at 1mm distance and moved in a sweeping fashion over the specimen for 15sec [Table/Fig-4]. The specimens were then dried with an oil free air source for 15sec.

[Table/Fig-4]: Samples lased.

The specimens were then observed under ESEM at X1000 & X5000 magnification. Parameters studied for enamel specimens were: 1) removal of smear layer; 2) type of etching pattern. Parameters studied for dentin specimens were: 1) removal of smear layer; 2) diameter of dentinal tubule opening measured by GSA (Gesellschaft fur Softwareentwicklung und Analytik, Germany) image analyser. The collected data values were subjected to the Chi square & Student‘t’ test.

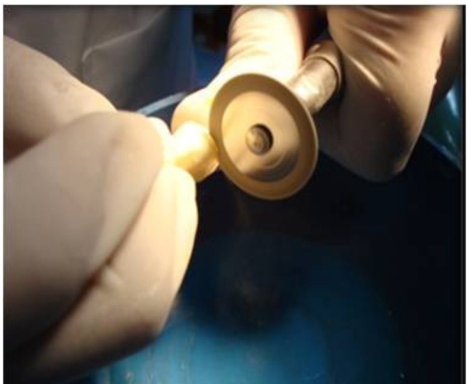
RESULTS

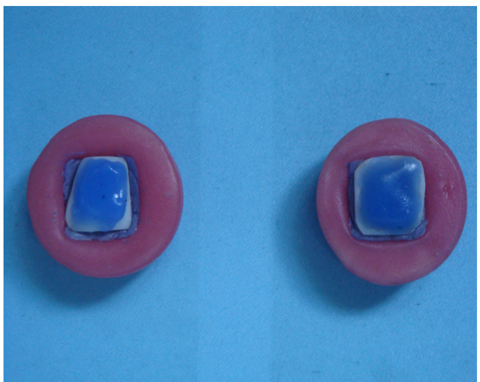
In the present study Chi- square & Student‘t’ statistical comparison between GrE2 & E3 for smear layer removal and predominant type of etching pattern. While GrD2 & D3 were analyzed for smear layer removal and diameter of dentinal tubules after surface treatment.

GrE2 showed absence of smear layer in seven (77.8%) specimens where as two specimens showed presence of smear layer. Eight (88.9%) specimen of GrE3 specimens showed absence of smear layer where as one specimen showed presence of smear layer [Table/Fig-5].

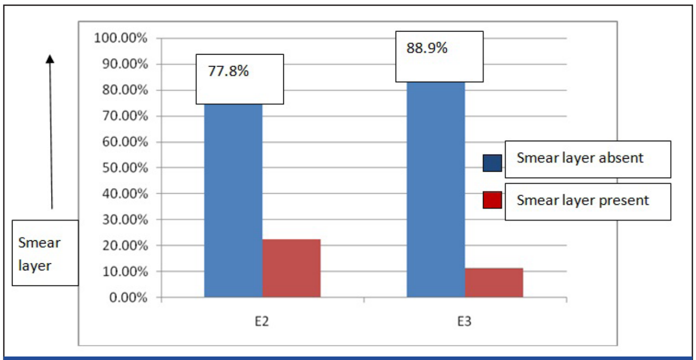
Chi-square test revealed that comparison of removal of smear layer in the two groups viz E2 & E3, do not differ significantly ($p>0.05$). However in GrE3, absence of smear layer is 88.9% which is significant ($\chi^2= 5.44$, $p<0.05$) at 5% level & in GrE2 ($\chi^2= 2.78$) the removal of smear layer is significant at 10% level ($p<0.10$). For the two groups the absence of smear layer is 83.33% (15 out of 18) and it is significant ($z=2.80$, $p<0.01$).

GrE2 showed type I etching pattern in seven (77.8%) specimens, type II & type III etching patterns in one specimen each. GrE3

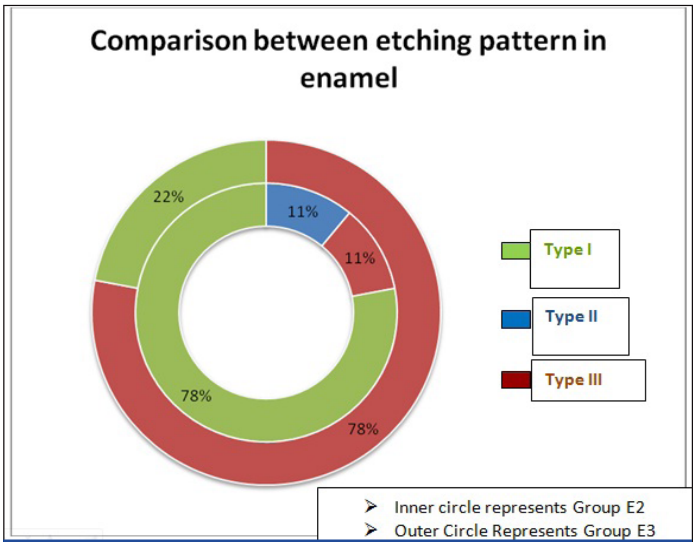




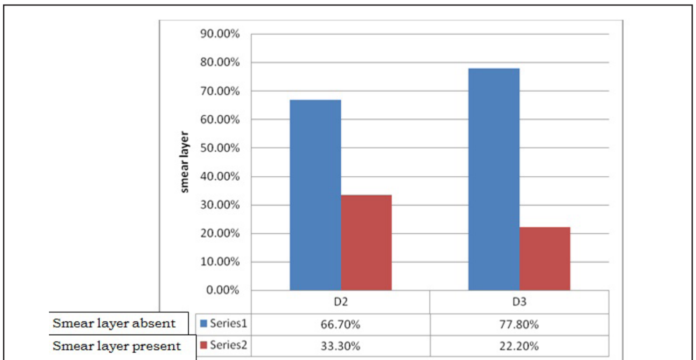
[Table/Fig-1]: Tooth samples & armamentarium for sectioning. **[Table/Fig-2]:** Tooth sample sectioned by diamond disc. **[Table/Fig-3]:** Samples acid etched.



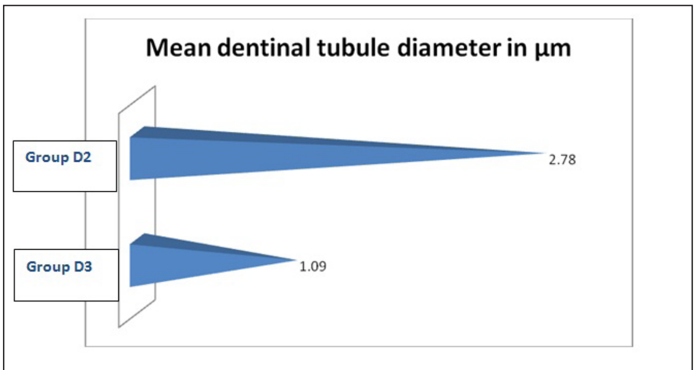
[Table/Fig-5]: Comparison of absence & presence of smear layer removal between GrE2 & E3.



[Table/Fig-6]: Comparison between GrE2 & E3 for type of etching pattern.



[Table/Fig-7]: Comparison of absence & presence of smear layer between group D2 & D3.



[Table/Fig-8]: Comparison of tubule diameter between Group D2 & D3.

showed distinguishable type I etching pattern in two specimens & rest seven (77.8%) showed type III etching pattern [Table/Fig-6].

In Type I etching pattern, the proportion in GrE2 is higher and significant at 10% level (χ^2=2.78, 0.05<p>0.10) as compared to GrE3. In Type III etching pattern, the proportion in GrE3 is higher & significant (χ^2= 4.50, p<0.05) as compared to GrE2.

GrD2 showed six (66.7%) specimen free of smear layer and three specimens revealed presence of smear layer. Seven (77.8%) of Group D3 specimens showed absence of smear layer where as two specimens showed presence of smear layer [Table/Fig-7].

Chi-square test reveals that comparison of removal of smear layer in the two groups viz D2 & D3, do not differ significantly (p>0.05). Comparison between the groups reveals non- significant results (chi-square=0.28, p>0.05). Absence of smear layer in two groups combined is 13 out of 18(72.2%). SND test shows that z=1.89, p=0.061; indicating significance at 10% level.

GrD2 & D3 showed dentinal tubule opening in all the samples. For comparison of SEM impression between GrD2 & D3, the mean diameter of tubules was tested for significance of their difference. Student't' test was applied and the't' value with 16 df came out to be 18.10 which is highly significant (p<0.001). The mean tubule diameter in GrD2 was 2.78µm & in GrD3 was 1.09µm & the comparison between the groups showed a highly significant result [Table/Fig-8].

DISCUSSION

Er,CR: YSGG laser etching has the advantage of no heat production due to the use of water coolant and absence of vibration. The laser can be easily handled making this treatment highly attractive for routine clinical use [7]. Er,Cr:YSGG laser irradiated surfaces exhibited microirregulaties and removal of smear layer [8].

In this study 3W energy density was used so as to avoid any cavity formation [1]. According to T. Dostalova et al., at 3 W power settings it is possible to etch the tooth surface without removing the enamel & dentin, the border is well defined & roughness is clearly visible. Sun X et al., in their study showed that samples irradiated with Er,Cr:YSGG laser, in the lower range of output power (0–4 W), showed increased surface roughness of the substrate [9]. The higher air to water percentage 70% air & 20% water was used to reduce the thermal effect of laser and increase the cleaning & cooling of the substrate [10].

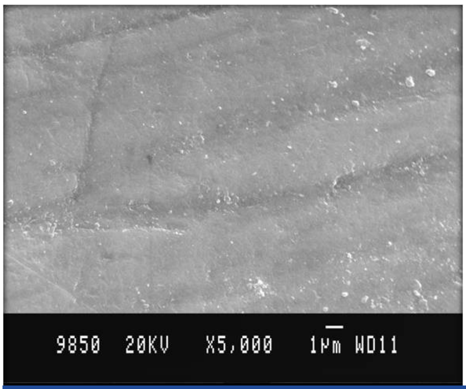
The laser beam was aligned perpendicular to all the specimens for maximum cutting effectiveness of the laser beam. According to Usumez et al., the advantage of aligning laser beam perpendicular were clean surfaces of the substrate, absence of debris and open dentinal tubules [3].

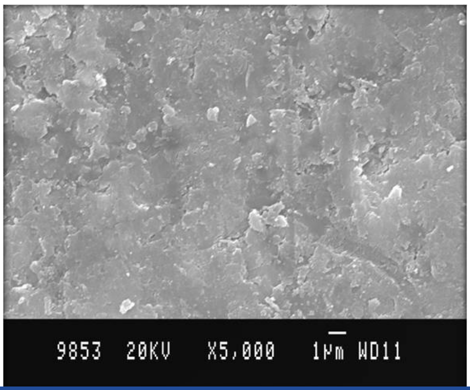
The control specimens of both enamel and dentin did not show any obvious morphology and were covered by smear layer [Table/Fig-9,10]. Almost all the specimens of treated enamel & dentin surfaces showed the absence of smear layer in the present study & no statistically significant difference was found between acid etched & laser etched surface. Thus the hypothesis that smear layer removal was better for Er,Cr:YSGG as compared to acid etching was rejected. This observation concurs well with previous studies by M. Hossain et al., Piyamart et al., G. Olivi et al., [11-13].

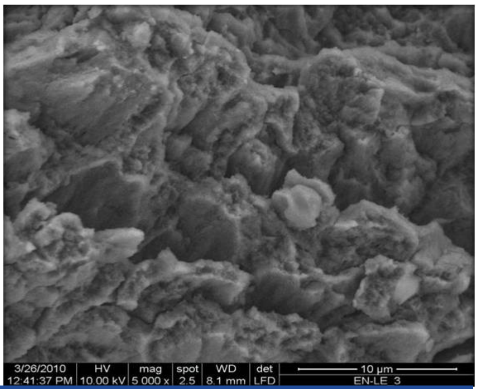
ESEM observation showed that laser irradiation produces recrystallized enamel & dentin surface [Table/Fig-11,12] which was also reported in studies by Secilmis et al., Tachibana et al., [10,14].

On observation of SEM photos of lased enamel in the present study the classic features of laser-treated enamel was seen: grooves, flakes, shelves and sharp edges [Table/Fig-11]; all these aspects were more indicative of microexplosion than of melting as was seen by Olivi G et al., [10].

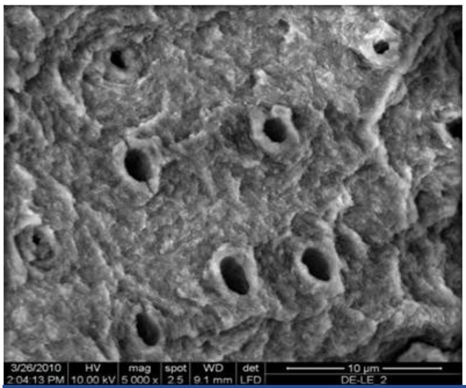
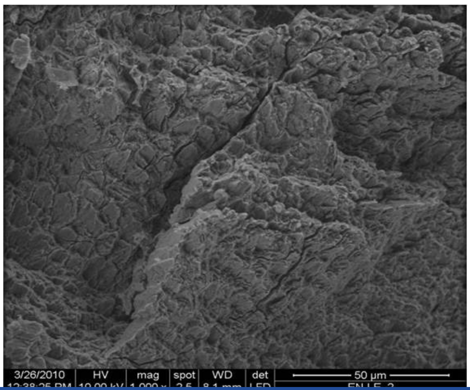
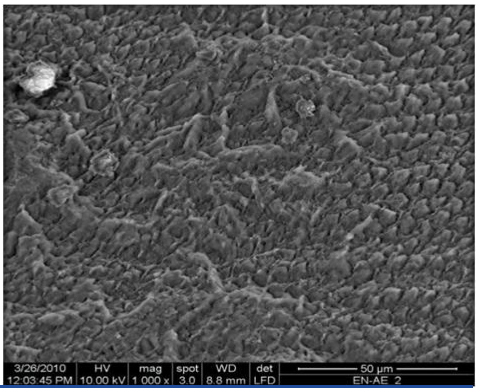
The predominant enamel etching pattern after laser etching seen was Type III [Table/Fig-11,13] as compared to the Type I in case of acid etching [Table/Fig-14] Lin et al., using Er:YAG observed the same etching pattern [15]. However in a study by Patricia et al., typical honey comb pattern of etching (type I) was observed after irradiation by Er laser [16].



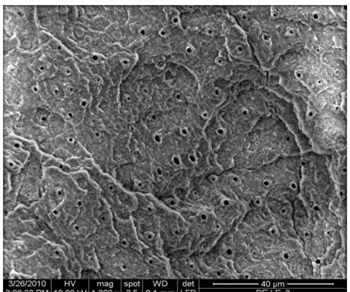
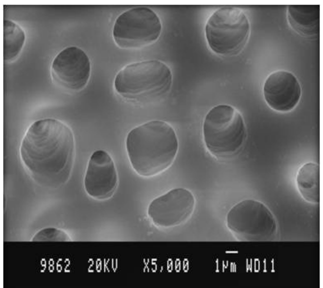




[Table/Fig-9]: Enamel control. **[Table/Fig-10]:** Dentin control. **[Table/Fig-11]:** Enamel after laser etching at (X5000)-uneven ablation of prism cores & peripheries seen.

[Table/Fig-12]: Collared up appearance surface observed of dentinal tubules- depicting more removal of intertubular dentin (X5000) by laser etching. **[Table/Fig-13]:** Enamel after laser etching (X1000)-Type III etching pattern seen & microcracks observed. **[Table/Fig-14]:** Enamel after acid etching- Type I pattern with signs of erosion seen (X1000).

[Table/Fig-15]: Dentin after laser etching –open dentinal tubules with ablated surface(X1300). **[Table/Fig-16]:** Typical funneled appearance of dentinal tubules after acid etching (X5000).

There was evidence of minor surface cracking on the laser etched enamel & dentin surfaces [Table/Fig-13,15] which may be attributed to the 3 W power setting. These cracks were also evident in a study by Sun X et al., were higher power settings of 5 W and 6 W was used [9]. A probable explanation for this according to Keller Hibst et al., may be that the cracking occurs as a result of local thermal stresses induced during the irradiation process.

Surface cracking was also evident in studies conducted by Usumez et al., Shuinn Lee et al., Tachibana et al., [3,14,17]. As lower power setting was used in a study by Torun Ozer et al., no cracks were reported in their study [8]. A study by Lin et al., showed that occasional cracks enhances retention and is ideal for resin penetration [15].

In the present study SEM observation of the lased dentin specimens revealed absence of smear layer, open dentinal tubules, microroughness, and crater-like appearance [Table/Fig-12,15]. Oznurhan et al., in their study confirmed this appearance of lased dentin surfaces with opening of dentinal tubules [18].

Cuff-like appearance of peritubular dentin was evident in the SEM images from the current study [Table/Fig-12] Goes et al., attributed this cuff-like appearance to the preferential removal of water from the organic tissue in the intertubular dentin region, leading to the raised appearance of the peritubular region [19].

When an acid etchant is applied due to the higher inorganic content of peritubular dentin it is preferentially etched, resulting in funnel-shaped openings to the tubules [Table/Fig-16]. According to Ozer et al. this shape of dentinal tubule leads to polymerization shrinkage, pulling the tags away from the walls and thus leading to microleakage [8].

On the other hand, laser irradiation produces lesser demineralization of peritubular dentin, and the dentinal tubules remain open, parallel, with no widening. This might probably contribute to the reduced microleakge after laser etching [8]. The dentinal tubules after laser etching were, approximately 1μm in diameter (GSA image analyser) [Table/Fig-12,15]. While the acid etched dentin surface displayed patent dentine tubules of around 2.8 μm [Table/Fig-16]. Thus our second hypothesis of lesser diameter of dentinal tubules after laser etching was confirmed.

According to Dunn et al. the tubule diameter in the middle third of coronal dentin normally ranges from 0.8 -1.2 μm. In the present study the diameter of dentinal tubules after laser etching was found to be an average of 1 μm which implies that Er, Cr:YSGG laser had no effect on tubule diameter [20,21].

Widely open dentinal tubules as seen after acid etching permits access of bacteria, it's by- products & toxic chemicals like acids to the pulp. This is a major cause for postoperative pain, sensitivity & pulpal damage after bonding of tooth coloured restorations [22].

As Er,Cr:YSGG might have no effect on dentinal tubule diameter & thus it may lead to reduction in hypersensitivity clinically.

The present study is a preliminary step towards incorporating laser in routine dental practice for esthetic restorations and needs to be further validated by other in vitro & long term clinical studies at different power settings. Further studies including variables like microleakage and bond strength is needed to prove the efficacy of laser clinically.

Within the limitations of the present study it can be concluded that laser in the near future Er,Cr:YSGG laser can be used as a reliable etching agent by providing a substrate which is free of smear layer with microporosities for better long term bonding.

CONCLUSION

Within the limits of this in vitro study it can be concluded that both conventional & laser etching showed smear layer removal & no statistical difference was found for smear layer removal between the two groups (p>0.05). After laser etching the dentinal tubule diameter was 1µm and after acid etching it was 2.8µm, which was a highly statistically significant result (p<0.001). Predominant type III etching pattern observed after laser etching, while type I etching pattern after acid etching.

REFERENCES

[1] Sazak H, Turkmen C, Gunday M. Effects of Nd:YAG laser, air-abrasion and acid-etching on human enamel and dentin. *Operative Dentistry.* 2001; 26: 476-81.
[2] Parker S Introduction, history of lasers and laser light production. *BDJ*. 2007; 1:21-31.
[3] Usumez S, Orhan M, Usumez A. Laser etching of enamel for direct bonding with an Er,Cr:YSGG hydrokinetic laser system. *Am J Dentofacial Orthop*. 2002;122:649-56.
[4] Beer F, Goharkhay K, Schoop U, Strassl M, Verheyen P, Walsh LJ, et al. Oral Laser Application. 1st Ed. Berlin:Quintessence Publishing;2006.
[5] Maziar M, Norbert G, Reinhart P, Leon V, Friedrich L. Visualizing the procedures in the influence of water on the ablation of dental hard tissue with Er,Cr:YSGG laser pulses. *Laser in Med. Sci.* 2008:451-57.
[6] Sasaki H, Lobo DC, Moriyama Y, Watanabe S, Villaverde A, Tanaka S, et al. Tensile bond strength and SEM analysis of enamel etched with Er:YAG laser and phohsphoric acid: a comparative study In vitro. *Braz Dent J*. 2008;19: 1.
[7] Brannstrom.M, Nordenvall KJ. The effects of acid etching on the pulpodentin complex. *Operative Dentistry*. 1977; 17: 229-42.
[8] Ozer T, Basara G, Berk N. Laser etching of enamel for orthodontic bonding. *Am J Dentofacial Orthop*. 2008;134:193-97.
[9] Sun X, Ban J, Sha X, Wang W, Jiao Y, Wang W, et al. Effect of Er,Cr:YSGG laser at different output powers on the, micromorphology and the bond property of non-carious sclerotic dentin to resin composites. *PLoS ONE*. 2015; 10:11.
[10] Secilmis A, Altintas S, Usumez A, Berk G. Evaluation of mineral content of dentin prepared by Er,Cr:YSGG. *Laser in Med Sci.* 2007;23:421-25.
[11] Olivi G, Angiero F, Benedicent S. Use of the Er,Cr:YSGG on human enamel tissues. Influence of the air water spray on the laser tissue interaction: SEM evaluations. *Lasers Med Sci*. 2010; 25:793-97.
[12] Hossain M, Nakumura Y, Yamada Y, Suzuki N, Murakami Y, Matsumoto K. Analysis of surface roughness of enamel and dentin after Er,Cr:YSGG laser irradiation. *Journal of Clinical Laser Medicine & Surgery*. 2001;19: 297-303.
[13] Piyanart E, Sharanbir KS, John FM. Effect of different power parameters of Er,Cr:YSGG laser on human dentin. *Lasers in Med*. Sci. 2007;22:175-82.
[14] Tachibana A, Marques M, Soler J, Matos A. Er,Cr:YSGG laser for caries removal : influence on bonding of a self etching adhesive system. *Lasers Med Sci.* 2008;23:435-41.
[15] Lin S, Caputo A, Eversole L, Rizoiu I. Topographical characteristics and shear bond strength of tooth surfaces cut with a laser-powered hydrokinetic system. *Journal of Prosthetic Dentistry.* 1999; 82:451-45.
[16] Patricia T, Joao CF, Sofia AO, Alvaro FA, Walter RD, Paulo RM. Shear bond strength and SEM morphology evaluation of different dental adhesives to enamel prepared with Er:YAG laser. *Contemp Cl Dent*. 2013;4:20-26.
[17] Lee B, Hsieh T, Lee Y. Bond strengths of orthodontic bracket after acid etched, Er:YAG laser irradiated & combined treatment on enamel surface. *The Angle Orthodontist*. 2002;73:565-70.
[18] Oznurhan F, Buldur B, Ozturk C. Influence of erbium: yttrium-aluminum-garnet laser, potassium titanyl phosphate laser irradiation, and acid etch on microtensile bond strength of adhesives. *J Pediatr Dent*. 2015;3:46-51.
[19] Goes M, Coelho M, Simnoides. Morphological effect of type, concentration & etching time of acid solutions on enamel & dentin surfaces. *Braz Dent J*. 1998;9(1):3-10.
[20] Dunn W, Davis J, Bush A.Shear bond strength and SEM evaluation of composite bonded to Er:YAG laser-prepared dentin and enamel. *Dental Materials*. 2005;21:616-24.
[21] Ramos R, Chimello D, Chinelatti M. Effect of Er:YAG laser on bond strength to dentin of a self etching primer & two single bottle adhesive systems. *Lasers Med Sci.* 2002;31:164-70.
[22] Hossain M, Yamada Y, Nakamura Y. A study on surface roughness & microleakage test in cavities prepared by Er:YAG laser irradiation & etched bur cavities. *Lasers Med Sci*. 2003;18:25-31.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 23, 2016**
Date of Peer Review: **Mar 03, 2016**
Date of Acceptance: **Mar 16, 2016**
Date of Publishing: **May 01, 2016**