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Original Article

Effect of bracket bonding with Er: YAG laser on nanomechanical properties of enamel

Shiva Alavi[1], **Reza Birang**[2], **Fatemeh Hajizadeh**[3], **Hamed Banimostafaee**[4]

[1]Dental Materials Research Center and Department of Orthodontics, [2]Department of Periodontics, Dental Implants Research Center, [3]Department of Orthodontics, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, [4]Department of Orthodontics, School of Dentistry, Arak University of Medical Sciences, Arak, Iran

ABSTRACT

Background: The aim of this study was to compare the effects of conventional acid etching and laser etching on the nano-mechanical properties of the dental enamel using nano-indentation test.
Materials and Methods: In this experimental *in vitro* study, buccal surfaces of 10 premolars were divided into three regions. One of the regions was etched with 37% phosphoric acid and another etched with Er:YAG laser, the third region was not etched. The brackets were bonded to both of etched regions. After thermocycling for 500 cycles, the brackets were removed and the teeth were decoronated from the bracket bonding area. Seven nano-indentations were applied at 1-31 μm depth from the enamel surface in each region. Mean values of the hardness and elastic modulus were analyzed with repeated measures analysis of variance and Tukey HSD tests, using the SPSS software (SPSS Inc., version16.0, Chicago, Il, USA). $P < 0.05$ was considered as significant.
Results: The hardness up to 21 μm in depth and elastic modulus up to 6 μm in depth from the enamel surface for laser-etched enamel had significantly higher values than control enamel and the hardness up to 11 μm in depth and elastic modulus up to 6 μm in depth for acid-etched enamel had significantly lower values than the control enamel.
Conclusion: The mechanical properties of the enamel were decreased after bracket bonding with conventional acid etching and increased after bonding with Er:YAG laser.

Key Words: Elastic modulus, enamel, Er:YAG laser, hardness, nano-indentation test, orthodontic bracket

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Address for correspondence:
Dr. Fatemeh Hajizadeh, Department of Orthodontics, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Golestan Street, Ahvaz, Postal Code: 6135715775, Iran.
E-mail: hajizadeh_fatemeh @yahoo.com

INTRODUCTION

The basis for the bonding of brackets to enamel has been enamel etching with phosphoric acid, which was first introduced by Buonocore in 1955.[1] Now orthodontists have successfully bonded the orthodontic appliances in offices around the world for about 40 years. However, a potential disadvantage of enamel acid etching is the demineralization of the superficial layer, which leaves the enamel more susceptible to the white-spot formation and caries, especially under orthodontic attachments.[2]

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Various preparation methods including maleic and polyacrilic acids, sandblasting and laser ablation have been suggested to etch enamel for orthodontic bonding.[3-5] Sandblasting and polyacrilic acid have been applied to control the enamel loss, but they have resulted in reduced bond strength on enamel.[6-8]

Thus, Er:YAG laser could be a suitable alternative for phosphoric acid because previous studies have cited that bonding strength was similar in groups etched either by acid or laser.[9-12]

The first application of laser in dentistry was reported in 1964, which was used to increase the resistance of enamel to demineralization and prevention of caries.[13] Some studies reported that Erbium lasers irradiation can decrease acid dissolution and increase fluoride uptake of enamel.[14,15] Furthermore, it has been found that Erbium lasers have an antimicrobial effect.[16]

Laser etching leads to thermally — induced changes such as melting and recrystallization of the enamel prisms.[17,18] It can produce non-specific and mixed-type pattern of the enamel prisms to a depth of 10-20 μm, depending upon the type of laser and its power setting.[19]

Apel *et al*.[20] in the microscopic examination following laser irradiation reported that the entire enamel surface was covered with fine cracks, extending up to 100 μm depth.

Uşümez *et al*.[4] found that the polished surface of the laser-etched samples had a more irregular pattern than that of the polished acid-etched samples with crack-like structures.

Márquez *et al*.[21] reported that Knoop hardness was increased after laser application on the enamel. Iijima *et al*.[22] showed that acid-etched enamel had significantly lower nano-hardness and elastic modulus values compared with the unetched enamel.

Therefore, recognizing mechanical changes in the microstructural features of enamel after removing orthodontic brackets is important to understand iatrogenic damages, such as enamel fracture or cracks during de-bonding. Recently, the nano-indentation test has become a common technique for measuring mechanical properties such as hardness and elastic modulus in biological hard tissues.[22-27]

The purpose of the present study was to evaluate the effects of conventional acid-etching and laser-etching on the nano-mechanical properties of the dental enamel, using nano-indentation test.

MATERIALS AND METHODS

In this experimental *in vitro* study, ten caries-free, intact human upper premolars, extracted for orthodontic purposes were used. After cleansing the root surface, the teeth were stored in Hank's balanced salt solution (HBSS, Sigma-Aldrich, St. Louis, MO, USA) in order to minimize enamel surface demineralization and subsequent alterations in the nano-mechanical properties of superficial enamel.[26,28] Antibiotics (penicillin 100×, metronidazole 100×, gentamicin 100×, amphotericin 100×) with 1% volume ratio were added to HBSS to prevent bacterial growth.

The teeth were embedded in self-cure acrylic resin (Dentsply Ltd., surrey, England) up to the cemento-enamel junction. For all teeth, buccal enamel surface was cleaned by non-fluoridated pumice and rubber cup, washed and dried before the etching procedure.

The buccal surface was divided into mesial and distal regions using masking tape (approximately 1.5 mm width). One of the regions was randomly etched with phosphoric acid and another with Er:YAG laser. Unetched enamel under the masking tape was assumed as the control region.

Laser-etched region

An Er:YAG laser (Fotona, fidelis plus, ljubljana, Slovenia) was used for etching one of the regions on the buccal enamel surface.

This device operates at a wavelength of 2940 nm with 2 watt power and 100 mj energy at 20 Hz, short pulse mode for 20 s. Energy density and power density were calculated 15.72 J/cm^2 and 31.45 W/cm^2 respectively. The laser beam was perpendicular to the enamel surface at a 1 mm distance (contact mode) and was moved in a sweeping fashion by hand. Laser application was performed with sufficient air and water cooling.

Acid-etched region

Another region was etched with 37% phosphoric acid gel (American orthodontics, Sheboygan, WI, USA) for 15 s, rinsed for 20 s and dried with an oil-free air spray until frosty white appearance was visible.

Bonding procedure

A thin coating of Transbond XT primer (3M Unitek, Monrovia, CA, USA) was applied on the both prepared regions with a microbrush. Twenty stainless-steel mandibular incisor brackets (American orthodontics, Sheboygan, WI, USA) were bonded with Transbond XT adhesive resin (3M Unitek, Monrovia, CA, USA) onto the mesial and distal etched regions. After removing the excessive resins, the specimens were cured with a light emitting diode (Starlight Pro, Carasco, Italy) for 20 s (10 s from each proximal side).

The masking tape was removed and specimens were immediately stored in distilled water bath at 37°C for 24 h and then were thermocycled for 500 cycles at 5-55°C to simulate the environment of the oral cavity.[4]

Afterwards, the metal brackets were deboned and the residual adhesives were removed by a low-speed tungsten carbide bur (REF 123-603-00, Dentaurum, Ispringen, Germany) and the buccal enamel was polished with non-fluoridated pumice.

Again, the specimens were mounted in acrylic resin blocks so that both control and bonded regions were covered with acrylic resin. The crown of the teeth was sectioned with a slow-speed water-cooled diamond saw (TC3000, Vafaei industrial, Tehran, Iran), at the previously bonded regions and parallel to the occlusal surface. The sliced surface of the cervical half of the crown was polished with a series of silicon carbide abrasive papers up to 4000 grit size to obtain a flat and smooth polished surface for the nano-indentation test. The final polishing was performed with diamond paste (3 and 1 μm particle size). The sliced surfaces were gently cleansed of debris using distilled water after each polishing step. Ultrasonic cleaning was avoided because it could alter the plastic-elastic response of the enamel and loosen their mounting.[25]

Each specimen was stored for 24 h before the test in the same ambient environment, which the nano-indentation test was conducted, to reach a stable dry condition so that there were no significant changes during testing.[25,29]

Nano-indentation test

Nano-indentation testing was performed by the CSM Indentation Tester (Nano-Hardness Tester, CSM, Switzerland) with maximum load of 10 millinewton (10 mN) by a diamond Berkovich indenter tip. Each test included three parts: 10 s for loading to the maximum value, 1 s of holding at the maximum load and 10 s for unloading. The indentations were applied at 1-31 μm depth (7 locations spaced 5 μm apart) from the external enamel surface for the three regions (control, acid-etched and laser-etched regions) on each specimen [Figure 1]. The performer was blind regarding the type of preparation of each region when recording the amounts of hardness and elastic modulus.

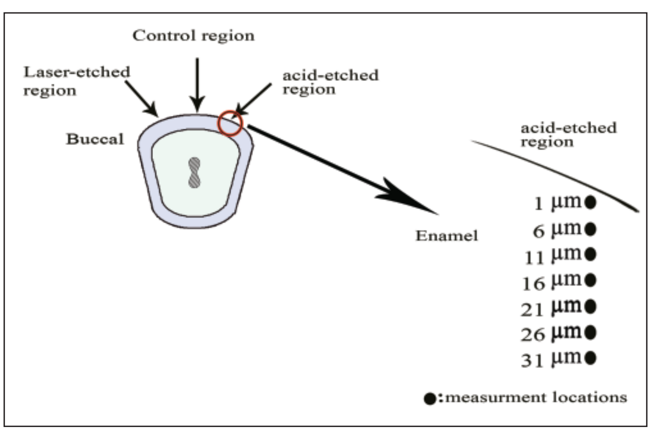

Figure 1: The cross-sectional view of the premolar tooth after removing the brackets and residual adhesives, the nano-indentation test was applied at seven locations in each region

Hardness and elastic modulus were calculated using the software available with the CSM Indentation Tester by Oliver-Pharr method.[30] As illustrated in Figure 2, hardness was calculated using maximum force divided by the contact area at maximum load. Elastic modulus was calculated from the slop of the linear portion of the unloading curve at maximum load.[31] According to the literature, the Poisson's ratio of 0.28 was assumed for calculating elastic modulus.[32,33]

Statistical analysis

Both hardness and elastic modulus data were tested for normal distribution and were found to be normally distribution (Kolmogorov-Smirnov test). The mean values of hardness and elastic modulus for the three groups were compared with repeated measure analysis of variance (ANOVA) test followed with the Tukey HSD test and $P < 0.05$ was considered as significant. The one-way ANOVA was used to compare the hardness and elastic modulus values of the three groups at each of the enamel's depth (1, 6, 11, 16, 21, 26 and 31 μm from the enamel surface). The Tukey HSD test was performed afterward. The results were analyzed using the SPSS software (SPSS Inc., version16.0, Chicago, Il, USA).

RESULTS

As illustrated in Figures 3 and 4, mean values for hardness and elastic modulus of the unetched enamel (control group) were 3.22-4.4 GPa and 78.82-92.65 GPa respectively. The enamel etched with laser, showed increased hardness (3.47-6.58 GPa) and elastic modulus values (81.17-116.14 GPa) and the acid-etched enamel showed decreased

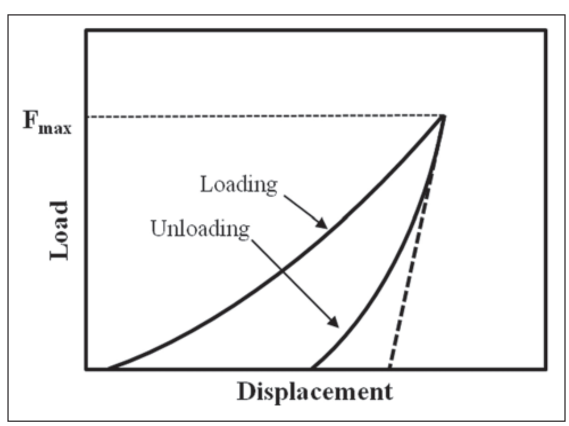

Figure 2: Load-displacement curve for a nano-indentation test

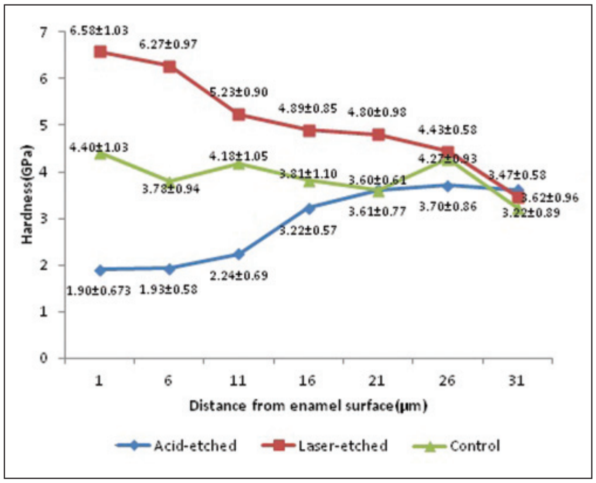

Figure 3: Mean values and standard deviations of the hardness of the enamel at different distances from the external surface after bracket de-bonding. Square, laser-etched region; triangle, control region; rhomboid, acid-etched region

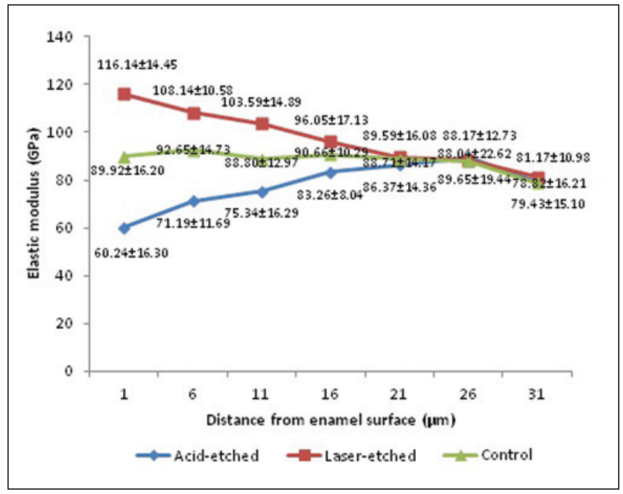

Figure 4: Mean values and standard deviations of the elastic modulus of the enamel at different distances from the external surface after bracket de-bonding. Square, laser-etched region; triangle, control region; rhomboid, acid-etched region

hardness (1.90-3.70 GPa) and elastic modulus values (60.24-89.65 GPa).

Repeated measures ANOVA test indicated that three groups (acid-etched, laser-etched and control) were significantly different, regarding hardness and elastic modulus values ($P < 0.001$).

Indentation hardness

The hardness values using 1-way ANOVA and *post hoc* Tukey HSD were compared in different enamel's depth (1, 6, 11, 16, 21, 26 and 31 µm from the enamel surface) between three groups and the following results were obtained:

Three groups at locations 1 µm and 6 µm from the enamel surface had significant differences in hardness values ($P < 0.001$). Laser-etched group had higher values than control enamel ($P < 0.001$) and acid-etched group had lower values than control enamel ($P < 0.001$).

At the location 11 µm from the enamel surface, there was significant differences between three groups ($P < 0.001$) and the laser-etched group was significantly harder than the control group ($P = 0.036$). On the contrary, the acid-etched group had significantly lower hardness than the control group ($P < 0.001$).

At the location 16 and 21 µm, the laser-etched enamel had significantly higher hardness than the acid-etched ($P = 0.001$, $P = 0.007$, respectively) and unetched enamel ($P = 0.027$, $P = 0.007$, respectively). Contrarily, hardness values were not significantly different between acid-etched and control group ($P = 0.293$, $P = 0.99$, respectively).

Hardness values between three groups were not significantly different at the locations 26 and 31 µm from the enamel surface ($P = 0.125$, $P = 0.565$, respectively).

Indentation elastic modulus

The elastic modulus values between three groups (laser-etched, acid-etched and control) were evaluated in different locations (1-31 µm from the enamel surface) using one-way ANOVA and Tukey HSD test.

At the location 1 µm and 6 µm from the enamel surface, elastic modulus values had significant differences between the three groups ($P < 0.001$), which elastic modulus values in the laser-etched group were significantly higher than the control enamel ($P = 0.002$, $P = 0.026$, respectively) and the acid-etched group had significantly lower elastic modulus than the control group ($P = 0.001$, $P = 0.002$, respectively).

In the depth 11 µm from the enamel surface, the laser etched group had significantly higher elastic modulus value than the acid-etched group ($P = 0.001$). Otherwise, elastic modulus values in the acid-etched and laser-etched groups did not have significant differences with the control group ($P = 0.123$, $P = 0.083$, respectively).

No statistically significant differences in the elastic modulus were found for the three groups at the locations 16, 21, 26 and 31 µm ($P = 0.088$, $P = 0.883$, $P = 0.978$, $P = 0.930$, respectively).

Pearson correlation test indicated that hardness and elastic modulus values were correlated ($r = 0.58$, $P < 0.001$).

DISCUSSION

In the present study, the hardness values up to 21 µm in depth and elastic modulus values up to 6 µm in depth from the enamel surface for laser-etched enamel were significantly higher than those for unetched enamel. In contrast, the hardness up to 11 µm in depth and elastic modulus up to 6 µm in depth from the enamel surface for phosphoric acid-etched enamel were significantly lower than unetched control enamel.

Iijima *et al*.[22] evaluated the effect of bracket bonding on nano-mechanical properties of enamel and reported that the enamel surface at 1 and 5 µm had significantly lower hardness and elastic modulus values in the acid phosphoric etched region compared with the control region, which was similar to our results. Our study also showed the same results for hardness at the 11 µm location, which could be due to higher peak load (10 mN) and higher sample numbers (10 teeth).

Márquez *et al*.[21] evaluated effects of Nd:YAG laser using Knoop hardness test on the mechanical properties of the sound enamel and stated that laser application leads to increase the Knoop hardness in all samples. Therefore, their results are similar to ours, but they used the Knoop test for evaluating the hardness, which cannot measure the modulus of elasticity.

In this study, the mean hardness and elastic modulus values for unetched control enamel ranged from 3.22-4.4 GPa and 78.82-92.65 GPa respectively. These values were similar to recent studies using the nano-indentation test.[23,24,28,32,34]

Dental enamel contains by volume 85-95% hydroxyapatite crystallites, 8-12% water and 2-3% organic components.[35] The hardness and elastic modulus values for the enamel is different for each tooth because the mechanical behaviors vary based on the variation in enamel mineralization, age and health condition of the donors.[23,36,37] Moreover, these values may be affected by the location (depth from the enamel surface), applied load, type of indenter, organic components[34,38] and orientation of the enamel rods.[24-26,32] Thus, these cited factors may explain some variations in the studies.

An established operative technique involves acid etching the enamel surface with a 35-50% solution of phosphoric acid. This etching produces an irregular and pitted surface with numerous microscopic undercuts by an uneven dissolution of enamel rod heads and tails and leaves the organic rich inter-rod space intact. The composite resin is bonded to the enamel surface by resin tags formed in the acid-etched enamel rod structures. Thus, the mechanical features of the etched enamel surface are affected by penetration of resin tags, high amounts of elastic and soft organic tissue and demineralization.[22]

Dickinson *et al*.[27] also evaluated nano-mechanical characterization of incipient caries lesions and found that there is a close relation between the low mineral content of lesions and the weak mechanical properties. Moreover, they reported that the lesions with a significant organic content have lower mechanical properties.

The question is what the mechanism of the changes induced by laser is and what is the kind of relation between these changes and increase in the resistance of enamel to decalcification.

Márquez *et al*.[21] suggested that laser irradiation could increase the mineralization of the enamel while decreasing the water and organic to inorganic content ratio without affecting the amount of the mineral content.

Also, in previous studies regarding the enamel structural changes induced by Er:YAG laser, these changes were ascribed to the alteration in the mineral composition of the enamel, such as calcium (Ca), phosphorus (P), chlorine and Ca/P ratio,[39,40] reduction of carbonate and modification of the organic content.[41] On the other hand, Secilmis *et al*.[42] found that the mean percentage weights of Ca, P, sodium, potassium and Ca/P ratio of the enamel were not changed by the Er, Cr:YSGG laser irradiation.

There are three theories about the mechanism of acid resistance of enamel treated with laser that could be used for explaining increased hardness and elastic modulus in laser-etched enamel:

1. formation of low soluble compositions, such as tetracalcium diphosphate monoxide,[17]
2 melting and recrystallization of enamel surface,[43] and
3. alterations in enamel's ultra-structure, such as the decrease of the amount of water and carbonate, increase in the hydroxyl ion contents, formation of pyrophosphates and the breakdown of proteins. These changes occur due to heating the enamel surface up to approximately 650-1100°C during ablation.[44]

Although, some investigator have reported that laser creates changes in the morphology of the enamel as cracks and micro-crater like appearance of irradiated surface,[15,18,39] which, these cracks in the enamel surface acts as a starting location for fracture during debonding, acid attack and enamel demineralization. Therefore, the positive effect of Er:YAG laser in increasing enamel acid resistance and prevention of caries formation around the orthodontic attachments is decreased or eliminated.[20]

We chose nano-indentation test to measure hardness and elastic modulus at multiple locations in the buccal surface of the enamel, because this is an accurate and excellent method to evaluate the mechanical properties of small sub-micrometer volumes of materials.[34] The nano-indentation method continuously recorded the load and displacement of the nano-indenter throughout the indentation process, during this process contact area and subsequent mechanical properties are determined automatically, which eliminates the need to image the area of the indentation.[27]

The nano-indentation test on the enamel was performed using a 10 mN peak load, which created indentation with about 2 μm width and length thus the 5 μm space eliminated any overlapping between the adjacent indentations.[24]

Since a greater peak load produces more stable and accurate values, we used 10 mN maximum loading. In addition, this load allowed that the indentation depth become considerably larger than surface roughness, which minimizes the effect of surface polishing on the values of the hardness and elastic modulus.[23,24]

Because the nano-indentation test is performed in an ambient environment over a period of many hours, the samples were kept in a similar dry environment. Although, previous studies reported that the hardness and elastic modulus values obtained from dry enamel were slightly higher than those from wet samples,[25,29,45] this enhancement was expected for all specimens and therefore would not influence the data.

The results show that both etching procedures have statistically significant effects on the hardness and elastic modulus values of the enamel surface, but this was an *in vitro* study and the results may be different when the procedures are performed clinically. Further investigations are suggested to clear the mechanism of the effect Er:YAG laser on mechanical properties of the tooth.

CONCLUSION

Based on the results of present study, the mechanical properties (hardness and elastic modulus) of the enamel surface increased after bracket bonding with Er:YAG laser etching, in contrast, conventional acid etching decreased the hardness and elastic modulus values of the enamel surface as a result of bracket bonding.

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