

# BACHELOR THESIS

---

DENTAL MEDICINE

VITH YEAR, 2019-2020

*Please read the materials  
carefully and solve the  
proposed themes*

---



## The use of sub-ablative Er:YAG laser irradiation in prevention of dental caries during orthodontic treatment

Carlo Fornaini<sup>1</sup>, Nathalie Brulat<sup>1</sup>, Giulia Milia<sup>1</sup>, Andrea Rockl<sup>1</sup>, Jean-Paul Rocca<sup>1</sup>

1: Faculty of Dentistry, University of Nice (F)

**Purpose:** This *in-vitro* study had two specific aims: the first, to test using a universal testing machine whether sub-ablative Er:YAG laser irradiation prior to acid etching is effective in orthodontic bracket bonding and secondly using micro-hardness measurements and Scanning Electron Microscopy (SEM) observations to investigate the effectiveness of de-mineralization reduction in enamel treated with sub-ablative Er:YAG laser irradiation followed by fluoride varnish application.

**Materials and Methods:** One hundred and eighty bovine permanent maxillary incisors were selected for shear bond strength testing and microhardness measurements. Sub-ablative Er:YAG laser irradiation was set at a power density of 2.5 J/cm<sup>2</sup>, a frequency of 7 Hz and air/water spray. Brackets were bonded with an auto-curing resin paste. The shear bond strength was measured comparing laser irradiated and non-irradiated enamel surface, followed by SEM observation of the bracket-resin-enamel interface.

Microhardness measurements were made on enamel samples before treatment, after samples preparation, and after demineralization.

**Results:** While the adhesion of orthodontic brackets to bovine enamel after sub-ablative Er:YAG laser irradiation and acid etching is comparable to that obtained after conventional acid etching, the effect of laser irradiation associated with topical application of fluoride varnish increases the microhardness of enamel.

**Conclusion:** Sub-ablative Er:YAG laser irradiation before the acid etching doesn't reduce the shear bond whereas when associated with fluoride application it may play a role in caries prevention. Further studies will be necessary to establish the mechanism by which the protective laser activated fluoride effect is achieved.

**Key words:** Er:YAG laser · Brackets · Bond strength · Micro-hardness · Orthodontics

### Introduction

The use of laser in dentistry began almost immediately after the development of the ruby laser by Maiman in 1960<sup>1)</sup>. Following the initial investigation by Stern and Sognnaes in 1964, this technology was used to remove carious enamel and dentin<sup>2)</sup>. Other applications such as cavity preparation, treatment of pits and fissures

etching teeth for resin bonding have also been investigated<sup>3)</sup>.

The laser irradiation of dental hard tissue with sufficient power leads to a variety of structural changes to the tissue surface. These changes depend on irradiation parameters such as wavelength, laser emission mode (pulsed or continuous), energy density, pulse duration, repetition rate, total irradiation and target tissue properties<sup>4)</sup>.

The main factors of enamel de-mineralization are as follows:

Etching performed with orthophosphoric acid during the direct bonding method before bracket setting increases the risk of dental caries and de-mineral-

Received Date: May 12, 2014  
Accepted Date: June 24, 2014

ization<sup>5)</sup>.

Placement of fixed orthodontic appliances is normally followed by an increase in oral colonization by "streptococcus mutans" concomitant with an elevated risk of dental caries development<sup>6)</sup>.

Increased risk in caries formation is also due to oral hygiene problems: it has been demonstrated that without a proper oral hygiene and plaque control, clinically visible white spot decalcifications can develop around fixed orthodontic appliances as early as 4 weeks after placement<sup>7)</sup>.

Nutrition facts may play a significant role: the erosive potential of different substances such as coke, sodas and red wine was demonstrated<sup>8)</sup>.

Systemic conditions and medical diseases like gastro-esophageal reflux and anatomic predisposition in enamel structure (hypoplasia for instance) are considered to be risk factors<sup>9)</sup>.

Sognnaes and Stern were the first to demonstrate an increased enamel resistance to de-mineralization as a result of laser irradiation. The treated enamel results in being more crystallized and acid-resistant<sup>10)</sup>.

On the other hand, the effectiveness of topical fluoride as a carious-resistant re-mineralization agent is well established: topical fluoride incorporated into the enamel crystals forming a Fluor apatite-like mineral improves its resistance to the acid challenge. During the carious process, some of the mineral dissolves, and this apatitically bound fluoride can be released to act on the re-mineralization process<sup>11)</sup>.

Many authors have examined the process by which laser energy, in combination with topical fluoride therapies, increases the resistance of tooth structure to mineral loss from the organic acids involved in dental caries<sup>12)</sup>.

This "in vitro" study had two specific aims:

To test whether sub-ablative laser irradiation prior to acid etching (Er:YAG laser, output power: 80 mJ, frequency: 7Hz, fixed working distance = 30mm (de-focused), mirror handpiece, theoretical Fluence: 2.5 J/cm<sup>2</sup>) is effective in orthodontic bracket bonding as compared to the traditional bonding method (acid etching technique) using a universal testing machine.

To investigate the effectiveness of de-mineralization reduction in enamel treated with sub-ablative Er:YAG laser irradiation followed by fluoride varnish application using microhardness measurements (Vickers hardness test) and observations (SEM).

### Materials And Methods

One hundred and eighty freshly extracted bovine per-

manent incisors of the same age were obtained from a local slaughter house and stored in 0.5% chloramines solution before the experiment to inhibit microbial growth for 1h until ready for use. They were maintained for a maximum of 10 days before the tests in those conditions and then placed in distilled water according to ISO standards 11405 (Guidance on testing of adhesion to tooth structure)<sup>13)</sup>. The selected bovine incisor teeth were free of dental caries. Before the experiment the teeth were cleaned with ultrasonic scaling, and the labial surfaces of the crowns were polished using pumice and water slurry in a rubber cup. Then the teeth were rinsed with water for 15 seconds and blown dry with oil-free compressed air (Cattani Compressor). Samples were then assigned randomly to one of the four test groups (A B C D) and the witness group. (**Fig. 1**)

Upper central incisor BKT Tweed 0.022" slot stainless steel brackets (American Orthodontics, Sheboygan, USA) were used throughout the study. Standard edgewise specifications (0° torque and 0° tip) were chosen to minimize the implication of bracket face and design in the variation of load application. The average bracket base surface was 12.7 mm<sup>2</sup>.

Laser irradiation was performed on sixty samples prior to bracket bonding with an Er:YAG laser (Fidelis, Fotona, Slovenia, λ=2940 nm) using a non-contact mirror hand piece at a fixed distance of 30 mm.

The used parameters were approved by preliminary pilot experiments and SEM observation made at the University of Nice:

MSP mode (100 μsec), output power: 80mJ, frequency: 7Hz, de-focused spot of 2mm diameter, theoretical Fluence: 2.5 J/cm<sup>2</sup>. Treated areas (whatever technique used) was defined by a ceramic dime (sized 7mm per 7mm) which had been adapted to the bracket base and the surrounding enamel surfaces

The irradiated and non-irradiated teeth were etched with 37% orthophosphoric acid (AXJA-ETCH, Scientific Pharmaceutical Inc. USA) for 30 seconds, rinsed with de-ionized distilled water for 30 seconds and dried with oil-free compressed air. Brackets were bonded at room temperature strictly following the adhesive manufacturer's suggested procedure, first applying a primer on the enamel and on the bracket base and then a bonding resin (Rely-a-bond, Reliance Orthodontic Products Inc. USA) onto the base of the bracket.

The brackets were firmly pressed onto the teeth surface simulating clinical chair-side procedures. Excess composite was removed with a dental scaler. After bonding, teeth were stored in de-ionized water



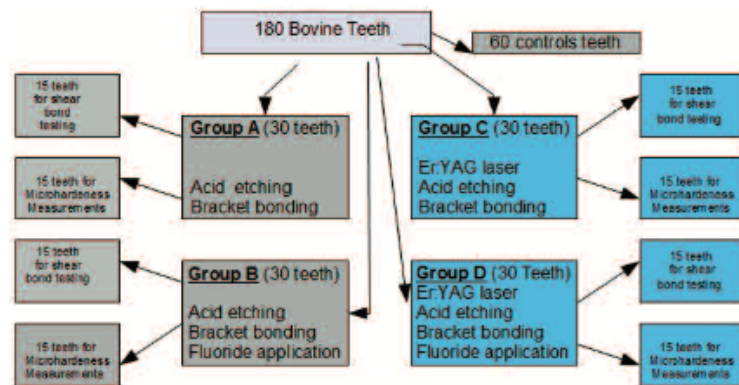


Fig. 1: Experimental design

for 48 hours at 37°C.

Fluor-Opal Varnish, a 5% sodium fluoride in a resin carrier, was applied after bonding on the tooth enamel around the bracket.

Rectangular (0.018" x 0.025") stainless steel archwire segments with a length of 40mm were inserted and fixed to the brackets with stainless steel ligatures before embedding the samples in plastic cups filled with epoxy resin to avoid variability in the horizontal orientation of the crown.

After irradiation and treatment, the teeth were embedded in self-curing acrylic resin held in plastic rings in order to obtain a crown enamel surface as flat as possible.

Shear bond strength was tested for 60 samples (30 Er:YAG laser irradiated and acid etched samples and 30 acid etched only samples).

The shear bond strength test was carried out using a Gambaldini Sun 500 testing machine (University of Cagliari). The crosshead speed of the machine was set at 1 mm/min, which is commonly used in this type of testing.

Embedded crowns were fixed in the testing machine with their labial surfaces perpendicular to the horizontal plane to ensure consistency for the point of force application and direction of the de-bonding force for all samples.

A stainless steel wire loop (0.020 inch) was engaged under the occlusal bracket wings to produce a force parallel to the bracket base in a gingival-occlusal direction. Before the test, the wire loop was

detected for its physical characteristics and it showed a resistance of up to 150 Newton of traction load.

During testing, the slowly increasing force level was observed on the digital display of the machine and the force at de-bonding was automatically recorded. The machine was connected to a computer with specific software (Test Work Microsoft). The shear bond strength was measured in Newton and transformed into MPa by dividing the load by the bracket base area.

After bracket de-bonding, teeth were immersed into a 0.1 M lactic acid buffer solution at 37°C for 48 hours, then rinsed with de-ionized water and finally dried with oil-free compressed air.

Microhardness measurements were made for 60 samples before treatment (witness), after sample preparation (groups A B C and D: one measurement per tooth, total: 60 measurements) and after de-mineralization (groups A B C and D: four measurements per tooth, total: 240 measurements).

Microhardness measurements were made with a Vickers Diamond Microhardness Tester (Buehler Micromet 5101, obj. lens: 50x) in Vickers Hardness Units (VHN). Indentations were made using a 300g load perpendicularly orientated to the indentation surface for 15 sec.

After shear bond strength testing and microhardness measurements, two samples of each group were prepared and dried in an oven at 37°C for 48 hours. The surfaces were coated with a thin film of gold (Au) in a vacuum evaporator (Ion Sputter, JEOL) and were observed under SEM (JEOL JSL 5310-LV).

## Statistical Analysis

Mean shear bond strength values and mean microhardness values were calculated for each treatment and compared using Kruskal-Wallis test, Student t-test and Levene's test (MS Excel). Bonferroni corrections for multiple comparisons were applied to the data. Level of significance was set at  $p < 0.05$ .

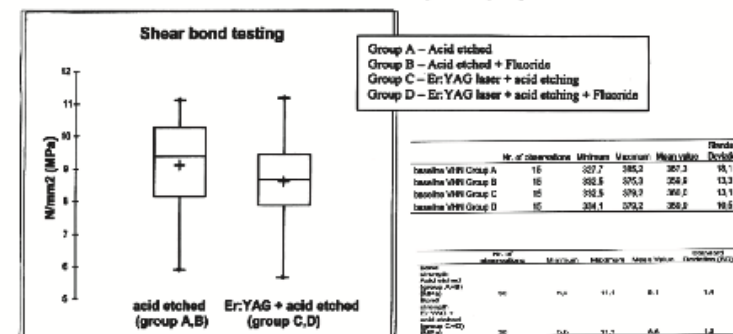
## Results

### Shear bond testing

To define the significance of the revealed shear bond strength values, a Student-t test was performed.

For groups A and B (30 samples) the mean value of shear bond strength measurements was 9.1 MPa as compared to groups C and D (30 samples) with a mean value of 8.6 MPa. Differences were not significant ( $p > 0.05$ ). Whatever the enamel surface treatment, the SD was quite identical and it may be considered that both surface treatments are highly reproducible.

Table 1: Shear bond testing of the groups



### Microhardness measurements

Only well-shaped indentations were considered in this study.

The mean scores for baseline enamel hardness in the four groups were VHN 357 (group A) VHN 359 (group B) VHN 360 (group C) and VHN 359 (group D).

They did not differ significantly ( $p > 0.05$ ).

The mean value of microhardness of acid etched enamel prior to bracket bonding (group A) was  $216 \pm 21$  VHN. The mean value of microhardness after acid etching and fluoride application (group B) was  $237 \pm 21$ .

The mean value of microhardness in bovine enamel treated sub-ablative Er:YAG laser and acid etching prior to bracket bonding without fluoride treatment (group C) was  $2091 \pm 5$  VHN, while with fluoride treatment (group D) VHN was  $245 \pm 14$ .

The microhardness values after sample preparation showed significant differences between groups A and D, B and C, B and D (Tab. 2).

After de-mineralization, the mean value of microhardness of acid etched enamel prior to bracket bonding (group A) was  $129 \pm 22$  VHN. The mean value of microhardness after acid etching and fluoride application (group B) was  $163 \pm 21$ .

The mean value of microhardness in bovine enamel treated sub-ablative Er:YAG laser and acid etching prior to bracket bonding without fluoride treatment (group C) was  $124 \pm 12$  VHN, while with fluoride treatment (group D) VHN was  $175 \pm 19$ .

The microhardness values after sample de-mineralization showed significant differences between groups A and B, A and D, B and C, C and D (Tab. 3).

The data show that after sample preparation the decline in microhardness was nearly equal in groups A

Table 2: The microhardness values after sample preparation

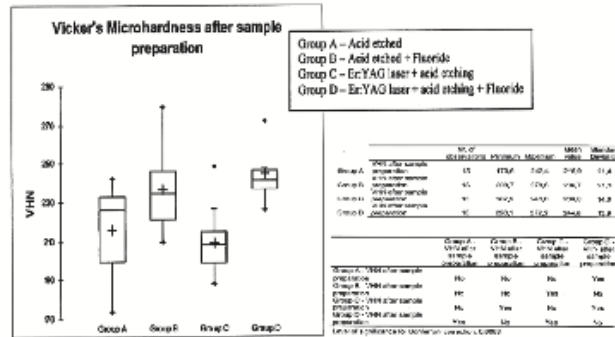
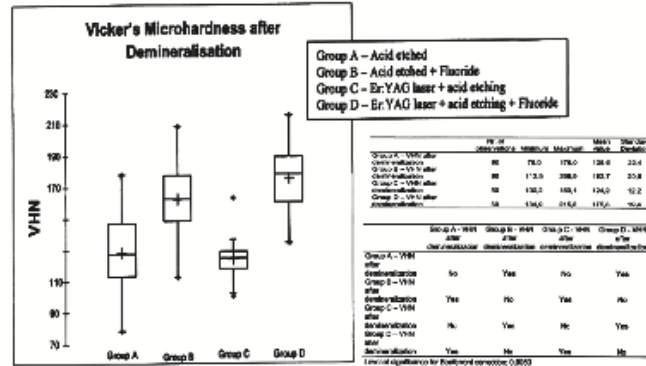


Table 3: The microhardness values after sample demineralization



and C and in groups B and D. In groups treated with fluoride, the microhardness values decreased less than in non-fluoride treated groups. The highest values were obtained for specimens after laser and fluoride treatment.

The specimens treated with laser and fluoride application (groups B and D) showed increased resistance to de-mineralization compared with the untreated specimens (group A and C).

There was no significant difference in decline of microhardness values between groups A and C (Fig. 2).

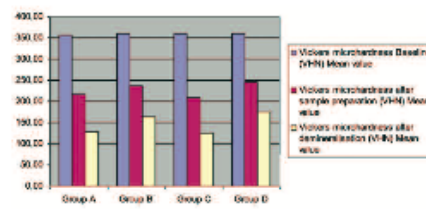


Fig. 2: Microhardness mean values at baseline, after sample preparation and after de-mineralization.

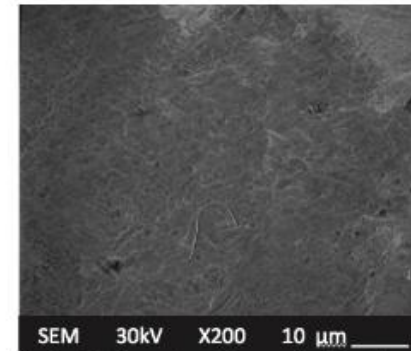


Fig. 3: SEM observation of the enamel surface after Er:YAG laser irradiation with 2.5 J/cm² (original magnification: x200)

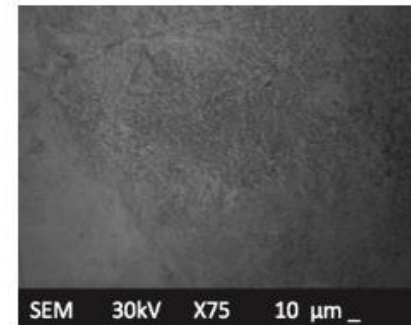


Fig. 4: SEM observation of the enamel surface after acid treatment (original magnification: x75)

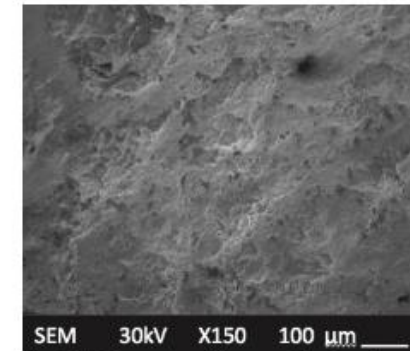


Fig. 5: SEM observation of the enamel surface after Er:YAG laser irradiation with 2.5 J/cm² and acid etching (original magnification: x150)

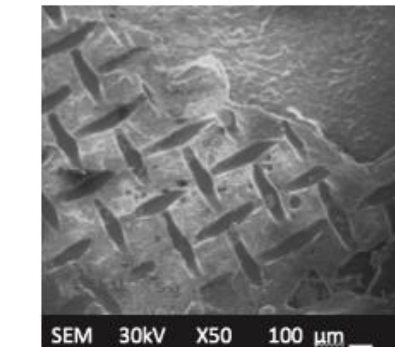


Fig. 6: SEM observation of the enamel surface after bracket de-bonding (original magnification x50)

## Discussion

Many researchers have studied adhesion to enamel and at present, acid etching is probably the best method of bonding resins to enamel.

Unfortunately, de-mineralization and initiation of caries around brackets are typical complications of orthodontic treatment. Laser-induced resistance to caries would be of interest in orthodontics.

Many researchers have affirmed that adhesion to hard dental tissues after Er:YAG laser etching is lower than that obtained after conventional acid etching: enamel and dentin surfaces prepared by Er:YAG laser etching only show extensive subsurface fissuring that is

adverse to adhesion<sup>14)</sup>.

Numerous studies on shear bond strength using laser irradiation at different energy levels (80 mJ to 350 mJ output power) for enamel conditioning show that shear bond strength increases proportionally to the increase of energy density<sup>6)</sup>. Gonçalves et al.<sup>15)</sup> did not find significant differences in the tensile bond strength of enamel as compared to acid conditioning only when treating enamel surface by Er:YAG laser (at 80 mJ output power) followed by acid etching.

Different studies have been investigated also the possible thermal damages caused by laser irradiation. Hoke et al.<sup>16)</sup> showed that water/air spray associated to laser irradiation has no negative effect on ablation



depth in dental enamel or dentin, but decreases the rise in temperature ( $< 5^{\circ}\text{C}$ ).

In our research we studied the effects of Er:YAG laser irradiation on enamel structure using a sub-ablative power density of  $2.5 \text{ J/cm}^2$ . With a pulse duration of  $100 \text{ } \mu\text{s}$  coupled with air/water spray cooling it is possible to prevent the formation of micro-fissures and cracks in the enamel as well as an adverse temperature rise in the pulp chamber <sup>17</sup>.

Energies not promoting thermal damages in pulp and periodontal tissues must be considered to choose an appropriate irradiation condition for a clinical application. Zach and Cohen demonstrated that increments of  $5.5^{\circ}\text{C}$  are tolerable by the dental pulp even if above this threshold the temperature rises are potentially threatening and can result in pulpitis and pulpal necrosis, while below  $2.5^{\circ}\text{C}$  no histological changes to the pulp tissue could be seen.

It was reported that Er:YAG laser without air/water spray is more effective in caries prevention when compared with Er:YAG laser with water mist <sup>18</sup>. Several theories have been described to explain the mechanism of an increase of enamel acid resistance induced by laser irradiation:

1. The decrease of enamel permeability due to melting and re-crystallization of enamel surface <sup>19</sup>
2. The decrease in the enamel's solubility due to the formation of less soluble substances such as tetra calcium di-phosphate monoxide <sup>20</sup>
3. The decrease in the enamel's solubility due to changes in its ultra-structure, as the reduction of water and carbonate contents, the increase in hydroxyl ion contents, formation of pyrophosphates and the decomposition of proteins <sup>21</sup>
4. Development of micro pores causing a slight contraction of the 4-axis in the apatite crystal because of a reduction in the water and carbonate content. It is assumed in this context that dissolved ions are caught in the micro pores and thus prevent demineralization <sup>22</sup>

The crystallographic changes on enamel are promoted by heating the surface with laser irradiation. Temperature rises of  $100 - 650^{\circ}\text{C}$  are reported to be necessary to promote these changes <sup>23</sup>.

The sub-ablative energies applied in the present study were considered safe concerning the pulp vitality. Nonetheless the temperature rise is assumed to be high enough to induce a loss of carbonate in dental enamel, which is reported to begin at a temperature of  $100^{\circ}\text{C}$  <sup>24</sup>.

Topical fluoridation can be achieved with tooth-pastes, varnishes or gels. Acidulated phosphate fluo-

ride gels containing amine fluoride or sodium fluoride reduced caries formation successfully in both children and adults <sup>25</sup>. Topical fluoridation with highly concentrated fluoride regimes results in an increase of both KOH-soluble fluoride and structurally bound fluoride in enamel <sup>26</sup>.

Several physical-chemical changes are described in literature to explain the mechanisms that occur during LAF treatment: deposition of calcium fluoride <sup>27</sup>, formation of micro-spaces in the dental hard tissue <sup>22</sup>, formation of tri-calcium phosphate <sup>28</sup> and phase transformation of hydroxyapatite to fluorapatite. <sup>29</sup>

Amaechi et al. showed that de-mineralization of bovine enamel leads to superficial mineral loss with formation of a lesion body beneath, thereby closely resembling an initial carious lesion. However, acid exposure for only 48 hours might produce caries-like lesions but also only a softened or de-mineralized enamel surface layer <sup>30</sup>.

Buffer solutions with a pH value of less than 5 are usually used for this purpose <sup>31</sup>. In the present study a  $0.1 \text{ M}$  lactic-acid buffer solution was used with a pH value of  $4.75$  ( $0.75 \text{ mM CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $0.45 \text{ mM K}_2\text{H}_2\text{P}_4\text{O}_{14}$ ) <sup>32</sup>.

Microhardness alterations are directly associated with mineral changes in superficial enamel layers <sup>33</sup>. Kielbassa et al. <sup>34</sup> found a clear relationship between microhardness and the mineral content of in-situ-induced enamel lesions. Consequently, in the present study microhardness testing was performed to evaluate the capacity of sub-ablative Er:YAG laser irradiation and fluoride varnish application during orthodontic treatment; this to re-harden de-mineralized enamel and prevent further demineralization, as suggested by recent studies.

Seppa observed that the efficacy of fluoride varnish was not proportional to the fluoride concentration but rather to the number of applications on pre-softened enamel <sup>35</sup> while Wiegand et al. showed that protection from enamel microhardness loss due to a demineralization depends on fluoride concentration of the gel applied.

In this study, only well-shaped indentations were considered and special attention was given to sample preparation. The indentation can be easily observed by microscope and immediately accepted or rejected according to the shape they showed.

Microhardness values achieved by the use of sub-ablative Er:YAG laser at a Fluence of  $2.5 \text{ J/cm}^2$  showed no notable difference in de-mineralization reduction compared to acid-etched enamel, which agrees with the findings in comparable studies <sup>36</sup>.

## Conclusion

Microhardness values found in the present study showed a significant correlation between lased and unlased groups as well as between sample groups with and without fluoride application. The laser irradiated and fluoride treated samples (Group D) showed the least diminution in enamel surface microhardness after bracket bonding and after de-mineralization. Our results did not show a statistically significant difference in microhardness diminution between enamel samples

## References

1. Maiman T (1960) Stimulated optical radiation in ruby. *Nature*, 187: p. 493-494.
2. Stern R.H, Sognnaes RF (1964) Laser beam effect on dental hard tissues. *J Dent Res*, 43: p. 873.
3. Bahar A, Tagomori S (1994) The effect of normal pulsed Nd:YAG laser irradiation on pits and fissures in human teeth. *Caries Res*, 28: p. 460-467.
4. Kwon YH et al. (2003) Nd:YAG laser ablation of enamel for orthodontic use: tensile band strength and surface modification. *Dent Mater J*, 22(3): p.397-403.
5. Harazaki M et al. (2001) The Nd:YAG laser is useful in prevention of dental caries during orthodontic treatment. *Bull Tokyo Dent Coll*, 42(2): p. 79-86.
6. Corbett JA et al. (1981) Comparison of *Streptococcus mutans* concentrations in non-banded and banded orthodontic patients. *J Dent Res*, 60(12): p.1936-42.
7. Ogaard B, Rolla G and Arends J (1988) Orthodontic appliances and enamel de-mineralization. Part I. Lesion development. *Am J Orthod Dentofacial Orthop*, 94(1): p. 68-73.
8. Lupi-Pegurier L et al. (2003) In vitro action of Bordeaux red wine on the microhardness of human dental enamel. *Arch Oral Biol*, 48(2): p.141-5.
9. Moazzaz R, Bartlett D and Anggiansah A (2004) Dental erosion, gastroesophageal reflux disease and saliva: how are they related? *J Dent*, 32(6): p. 489-94.
10. Sognnaes RF and Stern RH (1965) Laser Effect on Resistance of Human Dental Enamel to De-mineralization in Vitro. *J South Calif Dent Assoc*, 33: p. 328-9.
11. Wiegand A and Attin T (2003) Influence of fluoride on the prevention of erosive lesions: a review. *Oral Health Prev Dent*, 1 (4): p. 245-53.

that did not undergo laser irradiation but were treated with fluoride varnish and laser and fluoride-treated samples.

We may conclude that, whereas sub-ablative Er:YAG laser irradiation before the acid etching doesn't reduce the shear bond, it may play a role in caries prevention when associated with fluoride application. Further studies will be necessary to establish the mechanism by which the protective laser activated fluoride effect is achieved.

12. Vlacic J, Meyers LA and Walsh LJ (2007) Laser-activated fluoride treatment of enamel as prevention against erosion. *Aust Dent J*, 52(3): p. 175-80.
13. Oilo G (1993) Bond strength testing: what does it mean? *Int Dent J*, 43(5): p. 492-8.
14. Dunn WJ, Davis JT and Bush AC (2005) Shear bond strength and SEM evaluation of composite bonded to Er:YAG laser-prepared dentin and enamel. *Dent Mater*, 21(7): p. 616-24.
15. Goncalves M et al. (2003) Influence of the frequency of Er:YAG laser on the bond strength of dental enamel. *J Clin Laser Med Surg*, 21(2): p.105-8.
16. Hoke JA et al. (1990) Erbium:YAG (2.94 nm) laser effects on dental tissues. *J Laser Appl*, 2(3-4): p. 61-5.
17. Dostalova, T et al (1996) Evaluation of the surface changes in enamel and dentin due to possibility of thermal overheating induced by Er:YAG laser radiation. *Scanning Microsc*, 10(1): p. 285-90
18. Zach L and Cohen G (1965) Pulp Response to Externally Applied Heat. *Oral Surg Oral Med Oral Pathol*, 19: p. 515-30.
19. Apel C, Schafer C and Gutknecht N (2003) De-mineralization of Er:YAG and Er,Cr:YSGG laser-prepared enamel cavities in vitro. *Caries Res*, 37(1): p. 34-7.
20. Morioka T, Tagomori S, Oho T (1991) Acid resistance of lased human enamel with Er:YAG laser. *J Clin Laser Med Surg*, 9: p. 215-217.
21. Hossain M et al. (2000) Caries-preventive effect of Er:YAG laser irradiation with or without water mist. *J Clin Laser Med Surg*, 18(2): p. 61-5.
22. Oho T and Morioka T (1990) A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. *Caries Res*, 24(2): p. 86-92.
23. Nelson DG et al. (1987) Morphology, histology and crystallography of human dental enamel treated

- with pulsed low-energy infrared laser radiation. *Caries Res*, 21(5): p. 411-26.
- 24: Holcomb DW and Young RA (1980) Thermal decomposition of human tooth enamel. *Calcif Tissue Int*, 31(3): p. 189-201.
- 25: Marinho VC et al. (2003) Systematic review of controlled trials on the effectiveness of fluoride gels for the prevention of dental caries in children. *J Dent Educ*, 67(4): p. 448-58.
- 26: Attin T et al. (1995) Fluoride retention of incipient enamel lesions after treatment with a calcium fluoride varnish in vivo. *Arch Oral Biol*, 40(3): p. 169-74.
- 27: Westernnan GH et al. (1999) Combined effects of acidulated phosphate fluoride and argon laser on sound root surface morphology: an in vitro SEM study. *J Clin Laser Med Surg*, 17(2): p. 63-8.
- 28: Aminzadeh A, Shahabi S and Walsh LJ (1999) Raman spectroscopic studies of CO<sub>2</sub> laser-irradiated human dental enamel. *Spectrochim Acta A Mol Biomol Spectrosc*, 55A(6): p. 1303-8.
- 29: Meurman JH et al. (1997) Transformation of hydroxyapatite to fluorapatite by irradiation with high-energy CO<sub>2</sub> laser. *Caries Res*, 31(5): p. 397-400.
- 30: Amaechi BT, Higham SM and Edgar WM, (1998) Factors affecting the development of carious lesions in bovine teeth in vitro. *Arch Oral Biol*, 43(8): p. 619-28.
- 31: Theuns HM et al. (1985) Effect of time, degree of saturation, pH and acid concentration of buffer solutions on the rate of in-vitro demineralization of human enamel. *Arch Oral Biol*, 30(1): p. 37-42.
- 32: Fuhrmann R et al. (2001) Conditioning of enamel with Nd:YAG and CO<sub>2</sub> dental laser systems and with phosphoric acid. An in-vitro comparison of the tensile bond strength and the morphology of the enamel surface. *J Orofac Orthop*, 62(5): p. 375-86.
- 33: Featherstone JD, Duncan JF and. Cutress TW, (1978) Surface layer phenomena in in-vitro early caries-like lesions of human tooth enamel. *Arch Oral Biol*, 23(5):p. 397-404.
- 34: Kielbassa AM et al. (1999) Correlation of transversal micro-radiography and microhardness on in situ-induced de-mineralization in irradiated and non irradiated human dental enamel. *Arch Oral Biol*, 44(3): p. 243-51..
- 35: Seppa L, (1988) Effects of a sodium fluoride solution and a varnish with different fluoride concentrations on enamel re-mineralization in vitro. *Scand J Dent Res*, 96(4): p. 304-9.
- 36: Apel C et al. (2002) Calcium solubility of dental enamel following sub-ablative Er:YAG and Er:YSGG laser irradiation in vitro. *Lasers Surg Med*, 30(5): p. 337-41.

**[Acknowledgements]**

The Authors want to thank the Dental School of the Faculty of medicine of the University of Cagliari, and particularly its Dean Prof. Vincenzo Piras for contributing to the bond strength analysis.

# ANSWER THE FOLLOWING REQUIREMENTS BASED ON THE ARTICLE PRESENTED BELOW:

---

- 1. Identify the purpose of the study.
- 2. Identify the type of study (review, experimental - in vitro, experimental - in vivo, etc.)
- 3. Identify the inclusion criteria.
- 4. Identify the materials used in the study (samples, devices, etc.)
- 5. Identify which treatment method was performed, respectively with which device(s).
- 6. Identify the time period of the entire experiment.
- 7. Identify the method / methods for analyzing / interpreting the results obtained after performing the procedure.