



Evaluating the Effect of Diode Laser Irradiation on Microhardness of Bleached Enamel: An in Vitro Study

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Abstract

Aims: To investigate the effect of in-office dental bleaching techniques on the microhardness of enamel and the influence of post bleaching diode laser irradiation on the surface microhardness of bleached enamel. **Materials and methods:** Forty bovine teeth were prepared and then randomly allocated into two equal groups (n=20) according to bleaching technique as follows: CB: Conventional in-office bleaching technique and LB: Laser-assisted in-office bleaching technique. Each group was subdivided into two subgroups (n=10) according to laser irradiation setting as follows: C2: Conventionally bleached followed by diode laser irradiation at 2-Watt laser, C4: Conventionally bleached followed by diode laser irradiation at 4-Watt laser, L2: laser-assisted bleached followed by diode laser irradiation at 2-Watt laser and L4: Laser-assisted bleached followed by diode laser irradiation at 4-Watt laser. Vickers microhardness was assessed three times for all the specimens: before bleaching, 24 hours after bleaching and finally 24 hours after post bleaching diode laser irradiation. Paired samples t- test and independent samples t- test were utilized for statistical analysis. **Results:** Both bleached groups exhibited a significant decrease in micro harness after bleaching with no statistically significant difference between them. Post bleaching diode laser irradiation at 2Watt and 4Watt resulted in significant increase in microhardness with no significant difference was evident between them. **Conclusions:** Diode laser irradiation on bleached enamel had a positive influence on surface microhardness of enamel and it may represent a promising post bleaching treatment modality.

الخلاصة

الاهداف: تهدف هذه الدراسة الى تقييم تأثير تبييض الاسنان على صلابة سطح المينا وتأثير اشعاع ليزر الدايدود على صلابة سطح السن بعد التبييض. **المواد وطرائق العمل:** تم تحضير اربعين سن يقري ثم تم تقسيمها عشوائياً الى مجموعتين متساويتين (عشرون عينة) حسب طريقة تبييض الاسنان: CB: التبييض بالطريقة التقليدية، LB: التبييض بمساعدة الليزر. كل مجموعة قسمت الى مجموعتين فرعيتين حسب اعدادات اشعاع الليزر وكالاتي: C2: التبييض الاعتيادي متبوعاً بأشعاع الليزر عند قوة 2 واط، C4: التبييض الاعتيادي متبوعاً بأشعاع الليزر عند قوة 4 واط، L2: التبييض الليزري متبوعاً بأشعاع الليزر عند قوة 2 واط، L4: التبييض الليزري متبوعاً بأشعاع الليزر عند قوة 4 واط. تم تقييم صلابة السطح ثلاث مرات لكل العينات: قبل التبييض، 24 ساعة بعد التبييض و 24 ساعة بعد اشعاع الليزر باستخدام اختبار فيكرز للصلابة. تم استخدام اختبار T للعينتين المرتبطتين والعينتين المستقلتين لغرض التحليل الاحصائي. **النتائج:** اظهرت مجموعتي التبييض انخفاض ذات دلالة احصائية في صلابة سطح المينا بدون اي اختلاف معنوي بين المجموعتين. نتج عن اشعاع ليزر الدايدود بعد التبييض زيادة ذات دلالة احصائية في صلابة السطح عند قوة 2 واط و 4 واط بدون اي اختلاف معنوي بين المجموعتين. **الاستنتاجات:** تقنيات تبيض الاسنان كان لها تأثير سلبي على مينا الاسنان على مستوى صلابة السطح. كان لإشعاع ليزر الدايدود على المينا المبيضة تأثير إيجابي على الصلابة السطحية للمينا وقد يمثل طريقة معالجة واعدة بعد التبييض.

INTRODUCTION

Dental bleaching is becoming a very popular non-invasive dental procedure because of increasing demands for whiter teeth. It can be categorized generally into two main types: In-office dental bleaching, which is performed by the dental professional at the dental clinic, and at-home dental bleaching in which the patient applies the bleaching material into fabricated tray that fits the patient's teeth at home and under the supervision of the dentist. The in-office dental bleaching procedure incorporates a higher concentration of bleaching agent (35–38 % carbamide peroxide or 25–40 % hydrogen peroxide) for a shorter duration of bleaching time, while lower concentrations of bleaching agents (10–16 % carbamide peroxide or 3–6 % hydrogen peroxide) are typically utilized in at-home bleaching technique for a longer duration of time ⁽¹⁾.

The exact mechanism of a tooth bleaching is not fully clear, but it has been attributed to the changes in the chemical structures of tooth by free radicals that are released from hydrogen peroxide by either oxidation or reduction reactions that take place during bleaching ⁽²⁾. These free radicals are considered highly unstable agents because they have one or more unpaired electrons in their outer atomic orbit. To stabilize their molecular structure, they tend to acquire electrons from adjacent compounds by acting as oxidative agents. As a result, the pigment

molecules within the tooth structure become oxidized leading to their breaking down with subsequent decrease or elimination of tooth discoloration ⁽³⁾.

A relatively new advancement in the field of in-office dental bleaching technique is the activation of bleaching material by laser light and to accomplish this technique, the laser energy must be absorbed by the bleaching gel by incorporating specific chromophores for each specific wavelength ⁽⁴⁾. Although there is no doubt regarding the efficacy of dental bleaching in terms of tooth color enhancement (5-8), the experimental data are rather controversial with no general agreement about the safety of this procedure on the microhardness of enamel. Some authors reported that dental bleaching did not affect the microhardness of enamel ⁽⁵⁾, while others reported that the microhardness was decreased after dental bleaching ⁽⁶⁾. Many posts bleaching protocols were conducted to enhance the microhardness of bleached enamel including the use of topical fluoride with different concentrations and regimes, nano hydroxyapatite and laser ^(9,10). A wide range of lasers have been utilized in an attempt to increase the microhardness of dental enamel. The phenomenon responsible for that effect is related to the chemical and physical alterations in enamel caused by photothermal or photochemical interactions induced by laser ⁽¹¹⁾. Different effects were obtained

depending on the temperature reached during laser irradiation ⁽¹²⁾. The evidence in literature is scarce regarding the use of diode laser on bleached enamel and to the best of our knowledge there are very limited studies investigated its effect on of enamel ⁽¹³⁾. Therefore, the aim beyond the current research was to investigate the effect of in-office dental bleaching techniques on the microhardness of enamel and the possible influence of post bleaching diode laser irradiation at two power parameters on microhardness of bleached enamel with two different in-office bleaching techniques. The first null hypothesis to be tested was that in-office dental bleaching techniques did not affect the microhardness of bleached enamel. While the second null hypothesis was that post bleaching diode laser irradiation at two power parameters did not affect the microhardness of bleached enamel.

MATERIALS AND METHODS

Specimen's collection and preparation

Forty permanent bovine incisors ⁽¹⁴⁾ were utilized in the present study. They were extracted by one of the investigators on the same day of cattle slaughtering to prevent the need for refrigeration and to avoid dehydration of teeth at the same time. The teeth were stored in 0.1% thymol solution for disinfection at room temperature before starting the study. The remnant of soft tissues from the teeth was gently removed by using dental hand scaler. They were examined under stereo

microscope (X40) to ensure that the specimens were free from caries, surface cracks or any enamel defect. The samples were polished by non-fluoridated pumice (Bilkim LTD/ Turkey). Then the roots were cut by utilizing a diamond disc with copious water cooling at the level of cement-enamel junction then the roots were discarded. The crowns were embedded in cold-cure acrylic resin in custom made Poly Venile Chloride (PVC) rings with their labial surface facing upward. The middle of the middle third of the labial surface was made flat by wet sanding by using ascending grit waterproof silicon carbide papers (King spor/ Germany) started from #400 up to #1,200 under running water for a total of 25 seconds (5second/ grit). The teeth were examined again under stereo microscope to exclude any sample with exposed dentin. The teeth were placed in ultrasonic bath (Biosonic UC50DB, Coltene whaledent/ USA) to remove all the impurities for 15 minutes ⁽⁷⁾. The target area (middle of the middle third of the labial surface) was defined by using masking tape with 5 mm diameter hole to standardize the area of measurements. The samples were stored in deionized water during conducting the study.

Study design and specimens grouping

Forty bovine teeth were randomly allocated into two groups (n=20) according to the bleaching technique being used as follows:

CB group: Conventional in-office bleaching technique was performed by utilizing 35% hydrogen peroxide (Quick White in surgery bleaching kit/ UK). According to manufacturer instructions, the hydrogen peroxide liquid was mixed with powder until thick homogenous mixture was obtained. Uniform layer of 2mm thickness was applied to the target area and left for 10 minutes and considered as one treatment cycle then rinsed, dried and the treatment cycle was repeated two more times. Total bleaching time was 30 minutes.

LB group: Laser-assisted in-office technique was accomplished by using Laser white 20 whitening gel kit (Biolase, Irvine/ California, USA). According to manufacturer instructions, the base gel syringe and activator gel syringe were connected together and mixed for 25 times. The concentration of hydrogen peroxide of the resulted gel was 35%. The teeth were arranged so that each 4 teeth were bleached at the same time to simulate the 4 quadrants of the patient's mouth in order to follow the manufacturer instructions strictly. Uniform layer of resulted bleaching gel (about 1 mm thickness) was applied on the four teeth. After the disposable protective shield was placed on the whitening hand piece of the diode laser device with 940 nanometer (Epic 10, Biolase, Irvine/ California, USA), the handpiece was placed perpendicular and in close proximity to the

bleaching gel of one tooth without being in contact with it. Each of the four teeth were subjected individually to a preset whitening mode (continuous wave mode, 7 Watt) for 30 seconds resulting in a total resting time of 1.5 minutes for each tooth (which is the time required to lase the remaining three teeth). This procedure was repeated twice which produced a total time of 1 minute laser exposure and 3 minutes of rest (without laser irradiation) for each tooth. According to manufacturer instructions, the bleaching gel was allowed to remain on the teeth for 5 minutes after the second laser exposure. After that the bleaching gel was suctioned and the teeth were rinsed with deionized water. All of the above-mentioned procedure was considered as one bleaching cycle that was repeated again. The total bleaching time after two bleaching cycles was 18 minutes with 2 minutes of laser activation for each tooth.

Post bleaching diode laser irradiation

Both conventionally and laser-assisted in-office bleaching groups were sub divided into two sub groups according to diode laser irradiation setting that it was received after bleaching as follows:

C2: Conventionally bleached followed by diode laser irradiation at 2Watt power parameter.

C4: Conventionally bleached followed by diode laser irradiation at 4Watt power parameter.

L2: Laser bleached followed by diode laser irradiation at 2Watt power parameter.
L4: Laser bleached followed by diode laser irradiation at 4Watt power parameter.
Forty-eight hours after dental bleaching, all the specimens were irradiated by a diode laser device (Epic 10, Biolase, Irvine/ California, USA), the same diode laser device that was previously used for the activation of the aforementioned bleaching gel. The energy of laser was transmitted via an optical fiber delivery system (EZ tips, Biolase, Irvine/ California, USA) with 400 μm in diameter and 4 mm length. The tip of the laser was positioned in non-contact mode with standard distance of 1 mm from the enamel surface. For standardization of the distance and perpendicularity of the laser

tip, milling machine (Bio art 1000 Ma/ Brazil) was used to stabilize the laser hand piece with the aid of condensation silicone impression material (DUROSIL L/ Germany). The milling machine permitted only the horizontal movement of the laser hand piece during the irradiation to ensure that all the specimens were lased in a similar manner (Figure 1). The laser irradiation was performed at 2Watt power parameter for (C2 and L2 groups) and 4Watt for (C4 and L4 groups) with continuous-wave mode. The irradiation time was 15 seconds⁽¹³⁾ during which the treatment area was irradiated for three times during the 15 seconds period with uniform scanning motion over the entire target area in inciso- cervical direction.

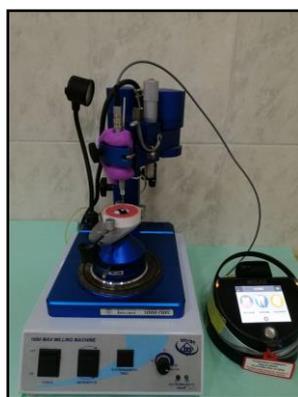


Figure (1): Diode laser handpiece stabilized by milling machine

Surface microhardness assessment

Surface microhardness test was performed by using Vickers microhardness testing machine (Otto wolpert, werke GMBH/ Germany) using X70 magnification. A load of 500 gram and dwell time of 10 seconds were used

during testing⁽¹⁴⁾. Three equally placed indentations were made for each specimen⁽¹³⁾. The Vickers microhardness values were calculated by using the following equation and expressed in Kg/mm^2 ⁽¹⁵⁾:

$HV = 1.854 P/d^2$ in which, P is the applied load in Kilogram (Kg) and d is the arithmetic mean of the two diagonals in mm. The mean of three microhardness values was calculated and reported as Vickers hardness number (VHN) for each specimen. The microhardness was assessed three times for all the specimens: after polishing procedure and considered as the base line data then 24 hours after the bleaching procedure and finally 24 hours after the post bleaching diode laser irradiation procedure.

Statistical analysis

The normal distribution of data was verified by performing normality test (Shapiro- Wilk). All the data showed normal distribution. A paired samples t -test and independent samples t -test were utilized to compare between before and after treatment and between groups respectively. The confidence level was set at 95% for all tests.

RESULTS

The data in Table (1) illustrates the findings of t -tests for microhardness

assessment of conventional and laser-assisted bleached groups. Conventional bleaching and laser-assisted bleaching techniques resulted in a significant reduction in microhardness of enamel from $(309.074 \pm 18.55$ and $305.791 \pm 10.95)$ to $(244.416 \pm 13.18$ and $241.282 \pm 6.76)$ respectively. No significant difference was evident between bleached groups ($P=0.472$). Based on Table (2) and Figure (2), the post bleaching diode laser irradiation significantly increased the microhardness of conventional bleached enamel at both 2Watt and 4Watt power parameters. However, there was no statically significant difference between them ($P= 0.966$). Similarly, diode laser irradiation at 2Watt and 4Watt power parameters after laser-assisted in-office dental bleaching resulted in a significant increase in microhardness of bleached enamel without significant difference between the two power parameters ($P=0.785$) as shown in Table (3) and Figure (3).

Table (1): Paired samples t -test and independent samples t - test for VHN of conventionally and laser-assisted bleached groups.

Groups	Descriptive statistics	Baseline	Bleached	P^a
CB.	Mean \pm SD	309.074 ± 18.55	244.416 ± 13.18	0.000*
LB.	Mean \pm SD	305.791 ± 10.95	241.282 ± 6.76	0.000*
P^b		0.603 *	0.472 *	

CB: Conventional bleaching; LB: Laser-assisted bleaching; SD: Standard deviation; P^a : Based on paired samples t -test; P^b : Based on independent samples t -test.

*Indicates a significant difference at $P \leq 0.05$. * indicates non-significant difference at $P > 0.05$.

Table (2): Paired samples *t*-test and independent samples *t*- test for VHN of conventional bleached specimens irradiated after bleaching with laser at two different power parameters.

Groups	Descriptive statistics	Bleached	Lased	<i>P</i> ^a
C2	Mean ± SD	243.941 ± 8.26	345.087 ± 5.54	0.000*
C4	Mean ± SD	249.390 ± 10.15	345.341 ± 12.98	0.000*
<i>P</i> ^b		0.332 ×	0.966 ×	

C2: Laser irradiated group at 2 W; C4: Laser irradiated group at 4W; SD: Standard deviation; *P*^a: Based on paired samples *t*-test; *P*^b: Based on independent samples *t*-test.

*Indicates a significant difference at $P \leq 0.05$. × indicates non-significant difference at $P > 0.05$.

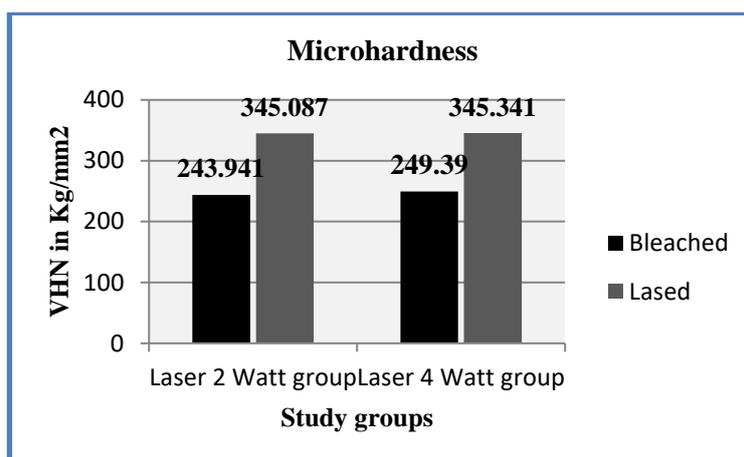


Figure (2): Bar chart represents the VHN of conventionally bleached groups irradiated after bleaching with laser at two different power parameters.

Table (3): Paired samples *t*-test and independent samples *t*- test for VHN of laser–assisted bleached specimens irradiated after bleaching with laser at two different power parameters.

Groups	Descriptive statistics	Bleached	Lased	<i>P</i> ^a
L2	Mean ± SD	238.623 ± 3.92	319.027 ± 24.44	0.001*
L4	Mean ± SD	243.941 ± 8.26	323.097 ± 25.79	0.001*
<i>P</i> ^b		0.185 ×	0.785 ×	

L2: Laser irradiated group at 2 W; L4: Laser irradiated group at 4W; SD: Standard deviation; *P*^a: Based on paired samples *t*-test; *P*^b: Based on independent samples *t*-test.

*indicates a significant difference at $P \leq 0.05$. × indicates non-significant difference at $P > 0.05$.

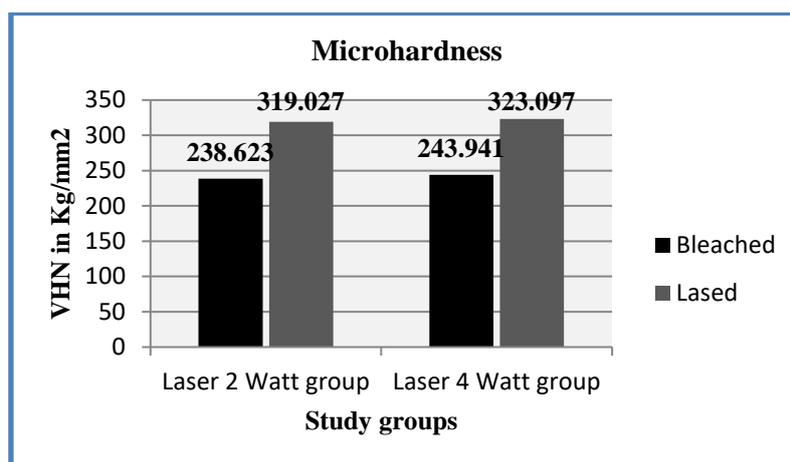


Figure (3): Bar chart represents the VHN for laser–assisted bleached specimens irradiated after bleaching with laser at two power parameters

DISCUSSION

Dental bleaching is considered as effective and relatively safe procedure ⁽¹⁶⁾. However, dental bleaching may cause harmful effect on tooth structure ⁽¹⁷⁾. The bovine teeth were utilized in the current research as they have been suggested as possible substitute for human teeth for dental research by showing no significant difference in microhardness value and chemical composition between them ⁽¹⁴⁾. In the current research, the conventional in-office dental bleaching caused significant decrease in microhardness of enamel, which is in agreement with Vieira *et al.* ⁽⁷⁾ and Scribante *et al.* ⁽¹⁵⁾. This effect may be attributed to the reaction of free radicals, which are highly unstable oxidative agents released during oxidation reduction reactions of bleaching agent, with organic matrix of enamel ⁽¹⁸⁾. Although organic matrix represents only minor percentage of enamel structure (1%), but it has crucial role for its integrity since it glues the

mineral crystals together, avoiding the loss of microhardness ⁽¹⁹⁾. However, other researchers reported loss of calcium (Ca^{2+}) from enamel after in-office bleaching and this loss may be attributed to the extended reaction of these free radicals to involve the inorganic component of enamel ⁽²⁰⁾. The hydroxyapatite crystals of enamel are surrounded by a layer of tightly bound water; this hydration shell makes the crystals electrically charged and thus can attract ions and participate in demineralization reaction ⁽²¹⁾. Nevertheless, this finding is in disparity with Mushashe *et al.* ⁽⁵⁾ who found that 35% H_2O_2 had no significant effect on enamel microhardness. This discrepancy in findings may be attributed to the methodological variations of opposing study such as the application of fluoride gel immediately after bleaching for 4 minutes following the manufacturer's instructions and then the microhardness measurement was performed.

Laser-assisted in-office bleaching, based on the present study, also resulted in significant decrease in microhardness, which is in concordance with Nematianaraki *et al.* ⁽²²⁾. No significant difference was evident between microhardness of enamel after conventional and laser-assisted bleaching despite the fact that conventional bleaching time was 30 minutes as compared to 18 minutes of laser-assisted bleaching. Laser light may decrease the operation time by maximizing the concentration of perhydroxyl free radicals that were formed as a result of the acceleration of hydrogen peroxide decomposition ⁽²³⁾. This finding contradicts with Mondelli *et al.* ⁽²⁴⁾, who reported that conventional in-office bleaching caused a significantly lower level of microhardness reduction compared to laser-assisted in-office bleaching. This contradictory may be due to methodological variation of opposing study such as different bleaching protocol, different laser type and setting in addition to using Knoop hardness test instead of Vickers hardness test. The small size of diode laser device, portability, affordability in addition to exceptional ease of use in oral cavity as it is equipped with optic fiber delivery system favor its usage at dental clinic and encourage conducting the current research. In this research, post bleaching diode laser irradiation at both power parameters produced the same significant increase in VHN. To the best of

our knowledge and according to the literature there is no previous study has utilized diode laser irradiation to enhance the microhardness of bleached enamel and resulted in significant increase. Other researchers conveyed a study on the effect of diode laser irradiation on simulated white lesion of enamel and their findings were in line with our findings ⁽¹³⁾.

The diode laser wavelength of 940 nm is located within the near infrared region of thermal invisible part of electromagnetic spectrum. This wavelength is poorly absorbed by dental enamel ⁽¹¹⁾ in addition to low thermal conductivity of enamel ⁽²⁵⁾, which all could result in rapid rise in surface temperature during laser radiation and rapid decay once the radiation has been stopped. However, such elevated temperature may lead to serious structural changes in enamel which may lead to microhardness increase. Different effects were obtained depending on the temperature reached during laser irradiation ⁽¹²⁾. Loss of water occurred between 100 -200 °C ⁽¹²⁾, organic matrix swelling and denaturation was observed at temperature range 300-350 °C ⁽¹¹⁾, carbonate group loss was reported around 400 °C ⁽²⁶⁾ and pyrophosphate ions formation at temperature range from 100°C to 650°C ⁽²⁷⁾. Enamel crystals melting, fusion and recrystallization were observed when the temperature reached 1200 °C ⁽²⁸⁾. Further studies are required to disclose the

exact mechanism of how diode laser affecting the microhardness of enamel.

In the current study, Vickers hardness test was chosen over Knoop hardness test because the square shaped indentation obtained in Vickers hardness test was easier and more accurate to measure. Even small changes in diagonal length of square indentation can be detected; Whereas Knoop hardness test gave rhomboid shaped indentation with parallel opposing surfaces which made detecting the error more difficult ⁽²⁹⁾.

The null hypotheses of this in vitro study were rejected as both dental bleaching techniques and post bleaching diode laser irradiation caused significant changes in microhardness of enamel.

CONCLUSIONS

Within the limitations of the current in vitro study, it can be concluded that laser-assisted in-office bleaching had the same deleterious effect on the microhardness as conventional bleaching but within shorter period. Post bleaching diode laser irradiation had a positive effect on microhardness of bleached enamel which may indicate a promising post bleaching treatment modality for patients lacking the compliance. Further studies are needed to investigate the clinical significance of this treatment.

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