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Original Article

Microhardness Change of Human Dental Enamel Due to Power Bleaching with Different Gels

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Abstract

Aim: Since the introduction of bleaching treatments in the office, different lights have been suggested to accelerate the bleaching reaction. This study aimed to evaluate the microhardness of tooth enamel after office bleaching using different materials.

Materials and methods: Thirty-three sound human upper premolars were randomly divided into 3 groups as follows (n=11): Group 1: Whitesmile HP40% gel with R&B LED light source with 3 W power output; Group 2: HP 35% Dr Smile gel with a 980 nm diode laser, 2 W power and continuous wavelength; Group 3: HP 40% Ultra boost gel according to factory instructions. Enamel surface microhardness was measured before and after the bleaching procedure in each group using the Vickers microhardness test. One-way ANOVA and Tukey post hoc tests were used for statistical analysis. We used a SEM microscope to examine the surface of one sample from each group and one sample as a negative control.

Results: In group 1, enamel microhardness increased remarkably (p=0.013) whereas in group 2 and group 3 enamel microhardness decreased. Enamel microhardness decreased in group 3 significantly (p=0.00) but its reduction in group 2 was not significant (p=0.833). SEM examination of the enamel surfaces after bleaching revealed erosion and surface porosities in group 1, enamel structure melting, and shallow porosities in group 2, and enamel prism exposure and etching in group 3.

Conclusions: Due to the limitations of the present study, power bleaching with HP40% Whitesmile gel with LED Monitex increases microhardness, so it can have better results for treatment in the clinic. Additionally, using Dr Smile gel with a 980 nm diode laser does not reduce surface microhardness.

Keywords

bleaching, diode laser, hardness, human enamel

INTRODUCTION

The demand for tooth whitening as a non-invasive esthetic dental procedure to improve smile attractiveness has been increasing recently.[1] Hydrogen peroxide is the most common agent used during this treatment and its application on the surface of the tooth is reported to be safe and secure.[2] Oxygen radicals derived from hydrogen peroxide can eas-

ily penetrate the tooth enamel and dentin structure due to their small molecular size and cause oxidation of colored substances that are responsible for tooth discoloration and staining.[2] Following oxidation, the pigments are broken into smaller molecules which reflect light less than the initial compounds, and as a result, tooth color looks brighter and more opaque.[3] Bleaching techniques are divided into two main categories including in-office bleaching and at-home bleaching. Several advantages have been reported for the in-office bleaching approach such as shorter duration, less bleaching materials swallowing and tissue irritation, and no need for fabrication of special trays.[1] Decreasing bleaching duration is preferred as an acidic pH of some bleaching agents induces enamel demineralization.[4] By using a source of energy such as heat during bleaching, the treatment time and chair-side time can be reduced; however, uncontrolled exposure to heat may be harmful and some adverse effects have been reported such as dental pulp necrosis and internal root resorption.[5,6] Light irradiation as a source of energy during power bleaching has been suggested by some studies. Photosensitive dyes added to bleaching gel absorb the light and convert it to thermal energy that accelerates the production of oxygen radicals. Achieving optimal results depends on compatibility between the wavelength of light and the photosensitive dye. Several light sources have been investigated before for power-bleaching including plasma arc, halogen lamp, LED (light-emitting diode), and lasers.[5-8]

Laser-assisted bleaching has been mentioned to be a successful technique as the risk of overheating of the dental pulp is low due to the monochromatic irradiation spectrum of the laser; also, its analgesic and anti-inflammatory impacts will reduce post-bleaching complications such as dental hypersensitivity.[1,6,9] LED devices also can be used during bleaching because of their low cost, availability, and low risk of dental pulp overheating.[8]

There have been some concerns about the adverse effects of the bleaching procedure on enamel structure. Morphological changes, surface porosity, and mild erosion have been reported to follow bleaching.[10] Assessment of microhardness of enamel is mentioned as a useful index to determine enamel mineralization or demineralization.[10,11]

Nemati et al.[11] reported a reduction of enamel microhardness after bleaching of a bovine tooth with plasma arc and GaAlAs diode laser; however, Goharkhay et al.[12] did not observe the change of enamel microhardness after laser-assisted bleaching with diode and Nd:YAG lasers.

AIM

Due to the inconsistent results of previous studies, the current study aimed to investigate and compare the effect of power bleaching with a diode laser and LED device on enamel microhardness.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Tehran University of Medical Sciences with an ethical code of IR.TUMS.DENTISTRY.REC.1397.176.

Sample preparation

Thirty-three healthy upper human premolars that were extracted due to orthodontic treatment or periodontal problems and were not extracted for more than three months were selected. After examination of the specimens by a stereomicroscope with a magnification of 10, specimens with external stains, caries, hypoplasia, and enamel cracks were replaced. Samples were kept in 0.1% thymol solution for 24 hours to be disinfected and then stored in normal saline until the study.

The specimens were randomly divided into three groups (11 specimens in each group). First, the tooth roots were cut using a diamond flat-end bur and high-speed handpiece from 2 mm below the CEJ. The crowns of the teeth were embedded in the self-polymerizing acrylic resin (Acropars, Iran). The buccal surfaces of the specimens were flattened with 600, 800, 1200, and 4000 grit abrasive papers by a rotary polishing machine (Isomet 4000 Buehler, USA) with water cooling to obtain an area of 3.3 mm of tooth enamel.

Bleaching treatment

- Group 1: 40% hydrogen peroxide bleaching gel (Power whitening YF, Whitesmile GmbH, Germany) was applied in uniform thickness of 1 mm on the surface of samples which were then irradiated under a LED light source (Whiten MAX-BR800, Monitex, Taiwan) with the blue & red light mode 4 blue LEDs (at 420-490 nm) and 1 red LED (at 620-630 nm), and 3 W power output (high mode) for 20 minutes. Then, the bleaching gel was completely cleaned with cotton rolls; this procedure was repeated one more time. The Whitesmile gel is photosensitive to 465 nm wavelength. All steps were done according to the manufacturer's guidelines.
- Group 2: 35% hydrogen peroxide bleaching gel (LWS Laser Whitening System, Doctor Smile, Italy) was applied in uniform thickness of 1 mm on the surface of samples which were then irradiated with a diode laser (Wiser, Doctor Smile, Italy) at a 980 nm wavelength, 2 W power output, from 1 mm distance for 30 seconds. After one minute delay, the same procedure was repeated. Finally, after the third time of 30-s irradiation, the gel remained for 7 minutes on the surface of samples, then it was completely cleaned and the samples were washed with an air/water spray for 30 seconds and dried with blown air. We used a single tooth head handpiece and the power density was 156.25 J/cm^2. All steps were done according to the manufacturer's instructions.

- Group 3 (control): 40% hydrogen peroxide bleaching gel (Opalescence™ Boost™, Ultradent, USA) was applied in uniform thickness of 1 mm on the surface of samples and left there for 20 minutes. Then the bleaching gel was cleaned by cotton rolls. This procedure was repeated one more time without any time interval. All steps were done according to the manufacturer's instructions.

After bleaching, the samples were stored in artificial saliva containing 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 1 mM KCl (pH 7.0, 37°C) in an incubator for 24 hours.

Microhardness test

Vickers microhardness, at a load of 200 g, with an indention time of 10 seconds, was assessed using a microhardness tester (FM-700, Future-Tech, Tokyo, Japan). Three indentions were carried out on the surface of each sample, with a distance of 50 µm between them and the mean Vickers hardness (VH) was calculated. Microhardness was measured once before the bleaching procedure and once again after bleaching and changes in the microhardness values were determined based on these two measurements in each group **(Fig. 1)**.

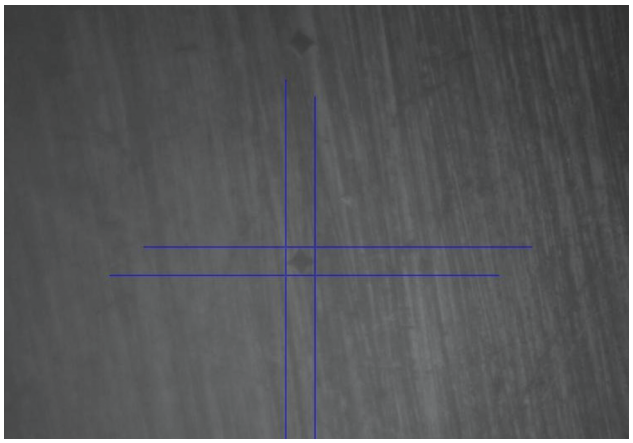

Figure 1. Knoop microhardness indention on enamel surface in a random sample.

SEM microscope assessment

For SEM evaluation, one sample from each group and one not-treated sample as a control were cleaned with ethanol, dried, and coated with a 10-15-nm gold layer in a vacuum. The examination was done using the SEM (FEI Nova Nano-SEM 450, Sydney) at magnification of ×500 and ×1000.

Statistical analysis

All statistical analysis was performed using the SPSS 25 (IBM, Armonk, NY, USA). Descriptive statistics, including the minimum, maximum, mean, and standard deviation values were registered for each group. One-way analysis of variance (ANOVA) and post hoc test were used to compare the changes of microhardness values between groups. The level of significance was set at $p<0.05$.

RESULTS

Descriptive data including means, standard deviation, minimum and maximum values of enamel surface microhardness before and after bleaching in each group are shown in **Table 1**. Enamel surface microhardness decreased in group 2 (diode laser) by 3.18 units and group 3 (conventional) by 15.63 units whereas it increased in group 1 (LED) by 78.37 units.

The results of repeated measure one-way ANOVA analysis were significant ($p=0.002$). **Table 2** shows the changes in enamel surface microhardness were significant in group 1 and group 3, both. Enamel microhardness increased in group 1 (LED light source) remarkably whereas it decreased in group 3 (conventional), significantly. In contrast in group 2 (laser), changes in enamel microhardness were not significant.

To compare the statistical results of the groups after bleaching in pairs in terms of the studied parameter, a post hoc Tukey test was performed, the results of which can be seen in **Fig. 2**. Based on this, it was found that the level of enamel surface hardness in conventional and laser groups

Table 1. Minimum, maximum, mean, and standard deviation changes of enamel surface microhardness values before and after bleaching in different groups

Group		Minimum	Maximum	Mean	Standard deviation
1 (LED)	Before	70.67	449.33	292.96	120.29
	After	161.33	529.33	371.36	116.84
	Changes	-19.00	228.33	78.39	86.79
2 (Laser)	Before	296.33	495.00	411.48	54.91
	After	311.33	464.67	408.30	48.38
	Changes	-53.00	61.00	-3.18	48.76
3 (Conventional)	Before	284.67	390.00	322.57	29.50
	After	265.33	378.33	306.93	31.66
	Changes	-24.67	-2.67	-15.63	7.27

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Table 2. Hardness changes in different experimental groups

	Group	Average	*P*-value (sig)
Hardness (after) Hardness (before)	Laser (group 2)	3.18182	0.833
	LED (group 1)	78.39394	0.013
	Conventional (group 3)	15.63636	0.000

is significantly related (p=0.000). But LED did not have a significant relationship with either laser (p=0.725) or conventional groups (p=0.281).

After evaluating the electron microscope images in the control tooth: **(Fig. 3)** intact surface and parallel lines and perikymata and developmental cavities were observed. In the Dr Smile group, shallow depressions, grooves, and evidence of melting were perceived **(Fig. 4)**. In the Whitesmile group, areas with larger and more porosities and erosion were observed on the surface **(Fig. 5)**. In the opalescence boost group, enamel dissolution and exposure of enamel prisms were perceived **(Fig. 6)**.

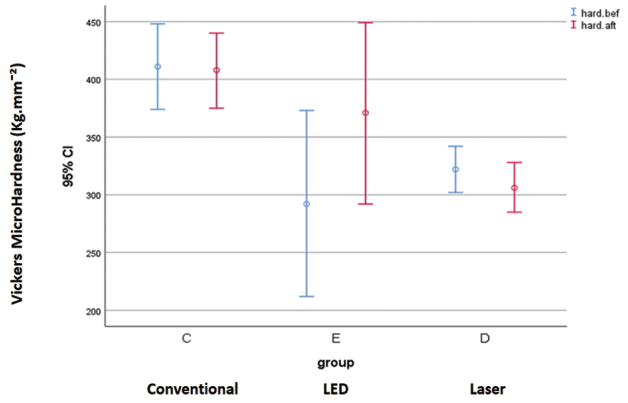

Figure 2. The error bar of means and 95% confidence interval (CI) of the mean change of enamel microhardness values in different groups.

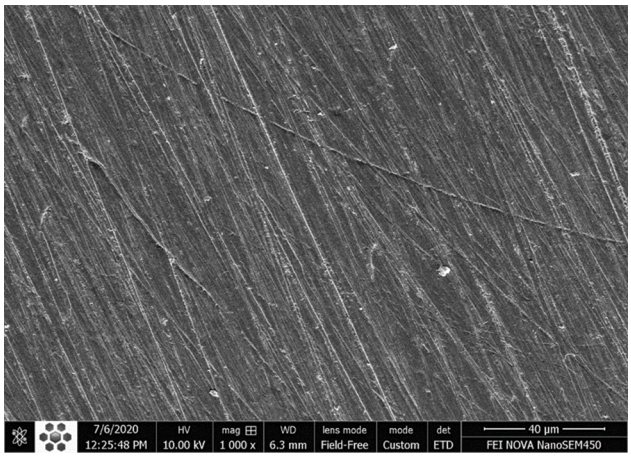

Figure 3. SEM image of the control group (×1000 magnification).

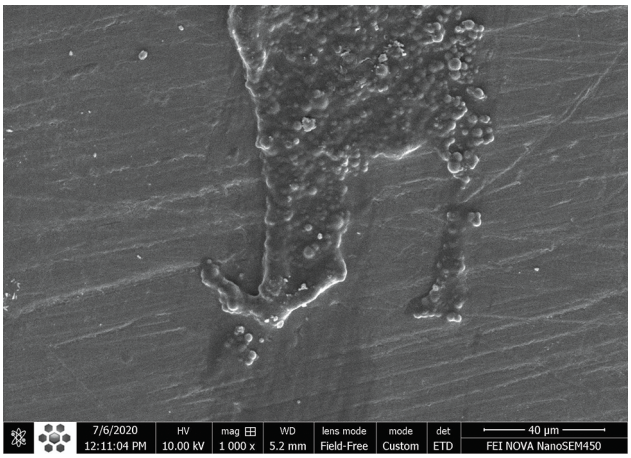

Figure 4. SEM image of Dr Smile group (×1000 magnification).

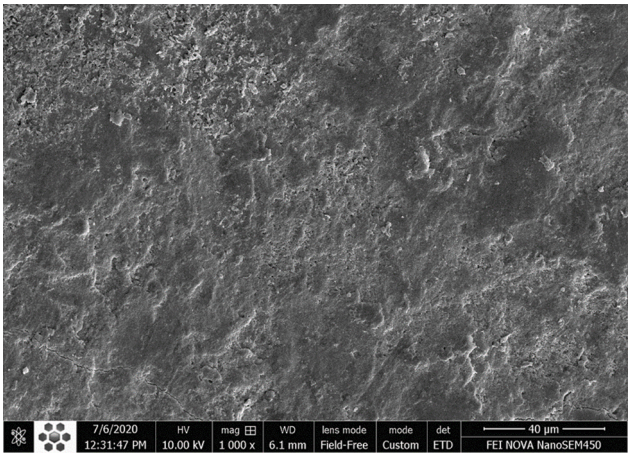

Figure 5. SEM image of Whitesmile group (×1000 magnification).

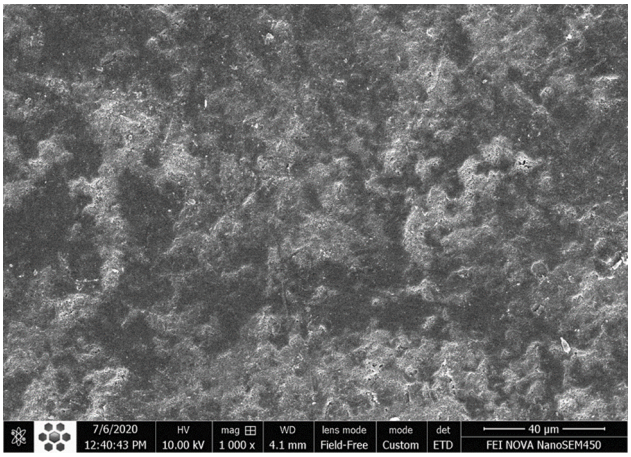

Figure 6. SEM image of opalescence boost group (×1000 magnification).

DISCUSSION

Bleaching is an effective esthetic dental procedure for whitening teeth and improving smiles that is more conservative than alternative treatment options for changing tooth colors such as crowns or porcelain laminates; however, some studies have pointed out its adverse effects on enamel structure.[13,14]

As demineralization or remineralization of the enamel surface results in changing its microhardness, measuring enamel surface microhardness can be a valuable method to determine the effect of bleaching on the structure of enamel.[15] Vickers microhardness test is mentioned as a suitable laboratory test for assessing surface microhardness of dental materials.[16]

In this study, sound human teeth were used and this may cause some inconsistency in the results with some previous studies in this field that used bovine teeth.[17]

The results of the current study indicated the greatest reduction in enamel microhardness after bleaching in group 3 (Opalescence Boost bleaching gel). In group 2 (Dr smile bleaching gel combined with a 980 nm diode laser), no significant change in enamel microhardness was observed. But in group 1 (White Smile bleaching gel combined with LED) enamel microhardness increased after bleaching.

In the study by Magalhaes et al.[18], it was shown that the greatest reduction in microhardness of enamel occurred after bleaching with Opalescence Xtra gel with pH=4.3 which has the lowest pH compared to other bleaching materials that were used. Klaric et al.[19] reported the greatest reduction in enamel microhardness after application of 25% hydrogen peroxide which had the lowest pH (3.2) compared to other tested bleaching materials. It can be stated that the low bleaching agent pH or its higher acidity causes more reduction of enamel microhardness. In the present study, a significant reduction in microhardness was observed in group 3 (Opalescence Boost PF). The pH of this substance after mixing in a concentration of 38% is equal to 7. Thus, considering the neutral pH of this bleaching agent, it can be concluded that some other factors except bleaching materials' acidity are responsible for microhardness reduction after bleaching. The oxidation of the mineral matrix of enamel by bleaching materials reduces the microhardness of enamel.[18] The reduction of surface microhardness of enamel after bleaching in this group also might be related to demineralization due to low concentration of phosphorus and calcium ions and high concentration of sodium and chloride in bleaching gel, which causes less hydroxyapatite saturation and formation.[19]

Dionysophoulos et al.[20] concluded that a significant reduction of microhardness of enamel following bleaching with 40% hydrogen peroxide was due to mineral matrix oxidation of enamel. Mushashae et al.[21] and Kabbach et al.[22] mentioned the duration of exposure to bleaching agent as a paramount factor in the reduction of enamel microhardness in addition to other factors such as the bleaching agent pH and concentration, so it seems that the higher concentration of hydrogen peroxide and longer exposure duration in group 3 compared to other groups is another cause for reduction of microhardness. In contrast to the current study, Saati et al.[23] reported that Vickers microhardness of enamel after bleaching with Opalescence Boost PF did not change significantly compared to the other group that laser-assisted bleaching was done by using a diode laser. Using bleaching gel with high concentrations of hydrogen peroxide (35% to 50%) combined with a light source such as QTH lamp, diode laser, argon laser, or LED during office bleaching is suggested to decrease bleaching time.[24-26]

Enamel microhardness increased in group 1 (32% hydrogen peroxide gel combined with LED light), significantly after bleaching, which was similar to the results of the study by Kutuc et al.[33] This increase may be due to the pH of this substance which is around 7.9-8, and its non-acidity did not cause enamel surface erosion; also, all samples stored in artificial saliva for 24 hours before the microhardness test might also restore the condition of the mouth[26] and induced ionic changes and increased mineral reabsorption to compensate for mineral deficiencies during treatment because saliva is a major reason of remineralization[22]. In this study, LED (Monitex) in 4 blue LEDs (with a wavelength of 420-490 nm) and 1 red LED (with a wavelength of 620-630 nm) as are in the Monitex device catalog, was also used as an optical activator for Whitesmile bleaching agent which leads to better bleaching efficiency and reduces the possibility of post-treatment hypersensitivity.[27] Similar to the results of the present study, Gomes et al.[28] in their study concluded that optical activation with plasma arc or LED has no significant relationship with the reduction of microhardness. The reason for utilizing blue LEDs is that they work beneath an approximate 420 nm wavelength and it is hypothesized that its radiation presents the same absorbance peak of pigments on the enamel surface, causing a photolytic impact.[29] The reason for using red LED is that the wavelength of red LED lights (620-630 nm) are near to the low-level lasers' wavelength (660 nm) and studies say that low-level lasers may be capable of reducing the irritation and damage initiated by in-office bleaching products in the pulp tissue, and in this way, can conceivably diminish the hazard and intensity of tooth hypersensitivity from bleaching.[30] In this way, we attempted to use this red LED to reduce the post-treatment teeth hypersensitivity. Due to above-mentioned reasons, the combination of blue and red LED was used in the present study.

The purpose of laser-assisted bleaching is to use a very effective source of energy to reduce time and prevent any side effects resulting from the bleaching process.[31] Azarbayejani et al.[32] in their study showed that the laser-assisted bleaching approach restricted the energy to the surface of the enamel and prevented etching and surface roughness of the enamel and the penetration of the bleaching material was reduced, while in the conventional group, penetration of active component of the bleaching substance presented both at the surface and at depth. In the study by Son et al.[33] SEM images showed a decrease in the thickness

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of the demineralized enamel layer as a result of increasing laser irradiation time. These observations clearly showed that laser irradiation during whitening not only improved the brightness of the tooth surface but also prevented the enamel from changing its shape and structure.

In the present study, Dr Smile bleaching agent activated by the 980-nm diode laser group, the microhardness of enamel did not decrease significantly. This lack of reduction in microhardness can be due to less exposure time to the bleaching agent because of the accelerating role of the laser. Son et al.[33] stated that 3 minutes of laser radiation caused the formation of a protective layer that could prevent the invasion of hydrogen peroxide to the surface of the enamel. Moreover, the chemical structure of the bleaching gel could alter during contact with laser radiation, which entailed hydrogen peroxide to penetrate the enamel surface only in the early stages, and then due to the changed structure of the bleaching gel, it was not able to penetrate further. In a study by Ahrari et al.[34] it was believed that the thermal effect of the laser increased the rate of peroxide decomposition and the formation of free radicals, and therefore the bleaching effect of the materials was achieved in a shorter period. In this study, a continuous-wavelength diode laser was used according to the manufacturer's instructions. A laser non-contact mode handpiece was also used to scatter the laser beam and creates a relatively large area on the specimen to reduce thermal damage to the tooth structure. Similar to the present study, Ahrari et al. found that laser-assisted whitening caused a slight decrease in the mineral content of the tooth, which was not statistically significant.[34] In the study by Polydorue et al.[35] during long-term use of bleaching agents, the most important role was related to exposure time and the role of laser on microhardness changes was not significant.

Araujo et al.[26] reported that power bleaching with a combination of 35% hydrogen peroxide and various light sources including laser did not change the microhardness of enamel, which is consistent with the results of the present study. Whereas Kabbach et al.[22] reported enamel microhardness reduction following power bleaching with lights; light irradiation might cause changes in the surface properties of the enamel, also samples that received laser-assisted bleaching showed a lesser amount of remineralization. Saati et al.[23] reported a reduction of enamel microhardness after laser-assisted bleaching with 810 nm and 980 nm diode lasers. The results of that study were in contrast to the present study.

In general, in the present study, it was concluded that the levels of enamel surface microhardness in the conventional and laser groups were significantly different. In other words, the enamel microhardness decreased in the conventional group, but in the laser group, there was no significant difference with the time before the bleaching treatment. The LED group was not significantly different from either laser or conventional groups. In the study by Azarbayejani et al., it was shown that the use of diode laser compared to the conventional method (opalescence boost) might reduce the surface changes of enamel.[32] In the study of Kabbach et al.[22], the same results were obtained and both studies agree with the results of the present study.

SEM examination showed deeper and more obvious enamel surface porosity in group 3 (conventional) as in this group higher concentration of hydrogen peroxide was used with longer exposure time. In group 2 (laser) with a shorter exposure time to the bleaching agent, slight surface changes were observed. Park et al.[25] explained the SEM results are inconsistent with the results of the microhardness test, because the SEM observations depended on the resolution and magnification of the device, and in most cases, the magnification was not sufficient to observe microscopic changes. Also, in the study by Branco et al.[36], it was stated that surface porosity is unrelated to the pH of the bleaching agents. The results of the current study confirmed it and the White Smile group which has higher pH and lower concentration than the laser group showed more surface roughness and depression.

By the results obtained in this study, it seems that studies on changes in other characteristics (such as surface roughness) can be helpful.

CONCLUSIONS

Due to the limitations of the present study, the results of the enamel microhardness test after power bleaching with different materials showed that:

1. Power bleaching using Whitesmile HP32% gel with LED MONITEX increases the microhardness of enamel.
2. Bleaching with Ultra boost HP40% gel reduces the microhardness of enamel
3. Power bleaching with Dr Smile HP35% gel with a 980-nm wavelength diode does not reduce the microhardness of the enamel.

Acknowledgments

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Competing Interests

The authors have declared that no competing interests exist.

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Изменение микротвёрдости зубной эмали человека при клиническом отбеливании различными гелями

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Резюме

Цель: С момента введения кабинетных отбеливающих процедур предлагалось использовать различные источники света для ускорения реакции отбеливания. Целью данного исследования была оценка микротвёрдости эмали зубов после кабинетного отбеливания различными материалами.

Материалы и методы: Тридцать три здоровых верхних премоляра человека были случайным образом разделены на 3 группы следующим образом (n=11): Группа 1: гель Whitesmile HP40% со светодиодным источником света R&B мощностью 3 W; Группа 2: гель HP 35% Dr Smile с диодным лазером 980 nm, мощностью 2 W и непрерывной длиной волны; Группа 3: Гель HP 40% Ultra Boost в соответствии с заводскими инструкциями.

Микротвёрдость поверхности эмали измеряли до и после процедуры отбеливания в каждой группе с помощью теста на микротвёрдость Виккерса. Для статистического анализа использовали однофакторный дисперсионный анализ и апостериорные тесты Тьюки. Мы использовали СЭМ-микроскоп для исследования поверхности одного образца из каждой группы и одного образца в качестве отрицательного контроля.

Результаты: В 1-й группе заметно увеличилась микротвёрдость эмали (p=0.013), тогда как во 2-й и 3-й группах микротвёрдость эмали уменьшилась. Микротвёрдость эмали достоверно снизилась в 3-й группе (p=0.00), но её снижение во 2-й группе было недостоверным (p=0.833). СЭМ-исследование поверхности эмали после отбеливания выявило эрозию и поверхностную пористость в группе 1, плавление структуры эмали и неглубокую пористость в группе 2, обнажение эмалевой призмы и травление в группе 3.

Заключение: Из-за ограничений настоящего исследования, энергетическое отбеливание гелем HP40% Whitesmile с LED Monitex увеличивает микротвёрдость, поэтому может иметь лучшие результаты при лечении в клинике. Кроме того, использование геля Dr Smile с диодным лазером 980 nm не снижает микротвёрдость поверхности.

Ключевые слова

отбеливание, диодный лазер, твёрдость, эмаль человека