



# Evaluation of the Effect of Different Laser Activated Bleaching Methods on Enamel Susceptibility to Caries; An In Vitro Model

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## Abstract

**Introduction:** Today, bleaching is a routine noninvasive alternative for treatment of discolored teeth. The aim of this study was to determine whether conventional or laser activated bleaching predispose teeth to develop caries or not.

**Methods:** Sixty human molars were mounted on acrylic cylinders and their Knoop microhardness (KHN) as well as DIAGNOdent (DD) values were recorded. They were divided into 4 experimental groups; G1) conventional bleaching with 40% hydrogen peroxide gel, G2) Diode laser assisted bleaching with same gel, G3) Nd:YAG laser assisted bleaching with the same gel, G4) control group. After bleaching, all samples were subjected to a three day pH cycling regimen and then, KHN and DD values were measured.

**Results:** All groups had significant reduction in KHN values. It seems that there is no statistically meaningful difference between changes in enamel microhardness of the sample groups and all groups have changed in a similar amount. Reduction of DD scores were significant in Diode laser and conventional groups, however changes in Nd:YAG laser and control groups were not significant. Changes in DD values have followed a similar pattern among groups, except in G1-G4 and G2-G4 couples. Conventional and diode laser groups had a meaningful difference in reduction of DD values in comparison with the control group.

**Conclusion:** It can be concluded that bleaching whether conventional or laser activated, does not make teeth vulnerable to develop carious lesions.

**Keywords:** Bleaching; Diode laser; Nd:YAG laser, Microhardness; DIAGNOdent.

## Introduction

In recent years, tooth whitening has become one of the most growing oral care sectors, boosted by patients, demanding both healthy mouth and attractive smile.<sup>1</sup> As tooth color relies on the composition of tooth tissues, any chemical, mechanical or biological change can damage the esthetic equilibrium of the smile.<sup>2</sup> Bleaching is a simple, non-restorative and noninvasive treatment for discolored teeth that is capable of satisfaction of the high demanding patients. So it has attracted much interest from the profession.

Bleaching systems today are based on hydrogen peroxide as the active agent and are often activated by heat or light.<sup>3</sup> Hydrogen peroxide acts as a strong oxidizing agent through the formation of free radicals and reactive molecules which attack dark colored macromolecules and break them into smaller and less colored ones.<sup>4</sup> Although other products such as carbamide peroxide or sodium

perborate are available for bleaching, hydrogen peroxide is still considered to be the most effective and popular agent, since it is available in a range of formulations, concentrations and activation modes.<sup>5</sup>

In-office bleaching can provide significant brightness after only one session of treatment, but achievement of optimum results may require multiple sessions or longer time of application of the bleaching agents.<sup>6,7</sup> However, this may increase the risk of tooth sensitivity<sup>8</sup> and cause certain amount of enamel matrix degradation.<sup>9</sup> Acceleration of hydrogen peroxide decomposition can be carried out through energy absorption from external source of energy like heat or laser, providing better patient compliance and may eradicate the side effects of high concentrated hydrogen peroxide.<sup>10,11</sup> Because of tooth temperature concerns, lasers are now more preferred. Lasers such as diode (LED), halogen, Nd:YAG etc can be highlighted.

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Bleaching technique of vital teeth requires direct contact of the whitening gel on the outer enamel surface. Oxidizing reaction for an extended period of time can lead to demineralization effects.<sup>12,13</sup> Demineralization is a process which involves the loss of calcium ions of the surface of calcified dental tissues. Surface morphology of the enamel can be affected, which results in loss of enamel prism peripheries and core.<sup>14</sup>

Some white spots are developed during the whitening process, which disappear after discontinuing the procedure. The concern has been raised whether or not these white spots are precarious lesions.<sup>15,16</sup> Basting et al suggested that bleaching agents may have a possible influence on active caries lesions in enamel and dentin.<sup>17</sup> Only few studies have examined the impact of tooth whitening on the susceptibility of enamel to either a carious or erosive challenge.<sup>9,18-20</sup>

Due to controversies in the existing reports, we aimed to evaluate the influence of hydrogen peroxide bleaching with two different light activation methods on enamel susceptibility to caries development, by assessing enamel microhardness and DIAGNOdent (DD) caries values.

## Materials and Methods

### Experimental Design

Sixty erupted human third molars, stored in saturated thymol solution at 20°C for at least 1 month were used in this study. They were wet-polished with 600 and 1200 grit aluminum oxide abrasive papers. Teeth were mounted onto acrylic cylinders, with an angle ensuring that a flat surface is provided by the buccal aspect of the molar tooth. Microhardness and DD values of the teeth were assessed as the baseline. Samples were then divided into 4 groups (n=15): (1) conventional bleaching with 40% hydrogen peroxide (Opalescence Boost: PF 40, Ultradent, USA), (2) laser assisted bleaching with Diode (CHEESE™, GIGAA, China), (3) laser assisted bleaching with Nd:YAG (FIDELIS™, Fotona, Slovenia), and (4) control group. After bleaching was carried out, samples were submitted to artificial early caries solution, during a pH cycling model. After these treatments, Knoop surface microhardness was determined for each group.

### Microhardness and DIAGNOdent Values Measurement

Surface microhardness was determined in samples before the bleaching (baseline), and after the pH cycling model. A Knoop diamond indenter (V-Test, Bareiss Prüfgerätebau GmbH, Germany) was utilized under a load of 50 g for 8 seconds. Three indentations spaced 100 µm from each other were performed. The Knoop hardness indentations were located with their long axis applied perpendicular to the surfaces of the labial enamel. The lengths of the indentations were measured immediately after the indentations had been made.

A DIAGNOdent device (KaVo, Biberach, Germany) was used to assess caries associated with erupted molars. This device uses wavelength of 655 nm and 5 mW power. Degrees of caries are reported in values ranged from

0 to 99. Cut-off values for enamel and dentin caries are described by Lussi, which 0-13 represent healthy tooth substance, 14-20 represent beginning demineralization, 21-29 represent strong demineralization and above 30 represent dentin caries.<sup>21</sup> Device was calibrated at each session to ceramic standard to ensure reliability of the results. Samples were examined once before the bleaching process and also after the pH cycling model, by direct and perpendicular contact of the device tip to the study field of the buccal surface.

### Bleaching Procedure

Teeth were bleached according to their bleaching protocol. Facial enamel surfaces were dried with air spray, before the process. Group 1 received a conventional bleaching regimen which included 3 times application of 40% hydrogen peroxide with gel thickness of 1.5 mm, remaining on the teeth for 20 minutes each time, following manufacturer's instruction. In group 2, teeth were painted with the whitening gel once with gel thickness of 1.5 mm and irradiated with a diode laser (810 nm, continuous mode, 1.5 W) with a 400 µm fiber tip from a 6 mm distance. Laser was irradiated for 3 times, 30 seconds each, with 1 minute interval between sessions. Five minutes after last irradiation, whitening agent was washed away with de-ionized and distilled water after 3 minutes of laser application.

In group 3, teeth were painted with the whitening gel once with gel thickness of 1.5 mm and irradiated with a Nd:YAG laser (1064 nm, pulsed mode, 2.5 W) with a 320 µm fiber tip from a 6mm distance with same protocol as diode laser. Whitening agent was washed away with de-ionized and distilled water after 3 minutes of laser application.

Samples were kept in artificial saliva and stored at 20°C, to avoid dehydration.

### pH-Cycling Process

A 3-day pH-cycling scheme was performed, with an 18-hour demineralization followed by a 6-hour remineralization, at 37°C.<sup>22</sup> The demineralizing solution at pH 4.2 contained 0.05M acetic acid, 2.2mM calcium chloride, 2.2mM monosodium phosphate, 0.1M sodium chloride, 2.3mM sodium fluoride and 0.3M sodium azide. The remineralizing solution at pH 7 contained the same formulation, except citric acid. A 5-minute wash with the de-ionized and distilled water was done between the demineralization and remineralization cycles and at the end of the protocol. Both remineralizing and demineralizing solutions were changed daily.

### Statistical Analysis

In the descriptive analysis, means and standard errors of microhardness and DIAGNOdent measurements were computed at baseline (before) and directly after the pH cycling procedure (after). Paired *t* test was used to compare after and baseline situation of each group. Analysis of variance (ANOVA) followed by Tukey test

for pair wise comparison was utilized for comparison of each 2 group. Commercially available statistical software (SPSSWIN, release 18.0; SPSS, Chicago, IL, USA) was used for all computing.

## Results

Table 1 presents the mean Knoop surface microhardness (KHN) values and standard errors for the experimental groups, before and after the bleaching process. The highest reduction in microhardness of the enamel ( $-77.97 \pm 18.76$ ) was in conventional group and was followed by diode laser and control groups. The least reduction in enamel microhardness was seen in Nd:YAG laser group ( $-48.19 \pm 14.68$ ). All groups had significant reduction in microhardness values.

DD values and standard errors of the study groups can be seen in Table 2. Mean DD value changes are also noted. The highest reduction in DD values was seen in diode laser group ( $-1.6 \pm 0.66$ ), followed by conventional group and Nd:YAG laser. It was revealed that control group developed some increase in DD values. Reduction of DD scores was significant in diode laser and conventional groups ( $P=0.03$ ,  $P=0.02$ ), however changes in Nd:YAG laser and control groups were not significant ( $P=0.17$ ,  $P=0.15$ ).

KHN changes and DD changes of the study group during the treatment are compared in Table 3. It seems that there is no statistically meaningful difference between changes in enamel microhardness of sample groups, and all groups have changed in a similar amount. Changes in DD values

have followed a similar pattern among groups, except in G1-G4 and G2-G4 couples. Conventional and diode laser groups had a significant difference in reduction of DD values in comparison with control group ( $P=0.02$ ,  $P=0.01$ ).

## Discussion

Oxidizing reactions during the bleaching process lead to brightening of teeth by decomposition of peroxides into free radicals. Some reports suggest that these oxidation reactions cause enamel structural alterations.<sup>23-25</sup> These alterations include surface modifications, increases in surface roughness, an increase in the fracture susceptibility, a loss of strength and higher solubility of enamel.<sup>15</sup> However, all the mentioned topics are on debate. Short time usage of the bleaching agents with lower peroxide concentrations is suggested to be safer compared to the application of high concentration bleaching agents with prolonged contact time. It seems that after a critical concentration, enamel destruction would not be increased anymore and is dependent on application time.<sup>26</sup>

Laser activated bleaching provides improvements in effectiveness, short impact time, and enamel surface protection.<sup>10,11</sup> The use of high intensity and amplified light has been indicated to accelerate the rate of chemicals involved in bleaching. Zhang et al reported a better bleaching outcome with brighter shades with a safer pulpal temperature rise.<sup>27</sup> de Magalhães et al<sup>28</sup> reported no change in enamel microhardness after bleaching with hydrogen peroxide gel activated with laser.

**Table 1.** Mean Knoop Surface Microhardness Values ( $\pm$ SE) and Mean KHN Changes for Experimental Groups (n = 15)

Group	n	Period	Mean KHN $\pm$ SE	Mean KHN Changes $\pm$ SE	P Value
1- Conventional	15	Before	262.35 $\pm$ 34.95	- 77.97 $\pm$ 18.76	<0.001
		After	184.38 $\pm$ 21.56		
2- Diode laser	15	Before	325.17 $\pm$ 28.48	- 73.06 $\pm$ 16.32	<0.001
		After	252.11 $\pm$ 27.10		
3- Nd:YAG laser	15	Before	275.63 $\pm$ 24.63	- 48.19 $\pm$ 14.68	0.005
		After	227.44 $\pm$ 22.9		
4- Control	15	Before	266.33 $\pm$ 27.25	- 60.64 $\pm$ 14.79	0.001
		After	205.68 $\pm$ 21.83		

Abbreviations: KHN, Knoop surface microhardness.

**Table 2.** Mean DIAGNOdent Values (DD $\pm$ SE) and Mean DIAGNOdent Score Changes for Experimental Groups (n=15)

Group	n	Period	Mean DD Scores $\pm$ SE	Mean DD Changes $\pm$ SE	P Value
1- Conventional	15	Before	7.2 $\pm$ 0.76	- 1.53 $\pm$ 0.59	0.02
		After	5.66 $\pm$ 0.42		
2- Diode laser	15	Before	7.46 $\pm$ 0.96	- 1.6 $\pm$ 0.66	0.03
		After	5.86 $\pm$ 0.56		
3- Nd:YAG laser	15	Before	7.06 $\pm$ 0.62	- 0.66 $\pm$ 0.46	0.17
		After	6.4 $\pm$ 0.64		
4- Control	15	Before	7.13 $\pm$ 0.63	+ 1.13 $\pm$ 0.74	0.15
		After	8.2 $\pm$ 0.67		

Abbreviation: DD, DIAGNOdent.

**Table 3.** Comparison of KHN and DD Changes Among Experimental Groups, During the Treatment

Group Comparison	Difference in KHN changes $\pm$ SE	P Value	Difference in DD changes $\pm$ SE	P Value
G1-G2	-4.9 $\pm$ 22.94857	0.99	0.06 $\pm$ 0.88425	1.00
G1-G3	-29.77 $\pm$ 22.94857	0.56	-0.86 $\pm$ 0.88425	0.76
G1-G4	-17.32 $\pm$ 22.94857	0.87	-2.66 $\pm$ 0.88425	0.02
G2-G3	-24.86 $\pm$ 22.94857	0.71	-0.93 $\pm$ 0.88425	0.71
G2-G4	-12.41 $\pm$ 22.94857	0.94	-2.73 $\pm$ 0.88425	0.01
G3-G4	+12.45 $\pm$ 22.94857	0.94	-1.8 $\pm$ 0.88425	0.18

Abbreviations: KHN, Knoop surface microhardness; DD, DIAGNOdent; G1, conventional bleaching; G2, laser assisted laser bleaching (diode); G3, laser assisted bleaching (Nd:YAG); G4, control group.

Yet, it is a question of whether tooth bleaching and subsequent mineral loss of enamel would hasten caries formation process and make the tooth more susceptible to acidic challenges or not. Some studies reported that the bleaching agents develop microstructural and chemical changes in enamel, similar to initial carious lesions.<sup>15,16</sup> Besides, there is a growing trend in laser activated bleaching among clinicians.

In the present study, the effect of different bleaching activation methods including conventional and two laser activated methods on enamel microhardness, after a cariogenic simulator, was assessed. Baseline microhardness and DD values were measured, and after completing the bleaching process, teeth were submitted to pH cycling regimen. This cycle, included an 18-hour immersion in a demineralizing solution with pH of 4.2, containing calcium, phosphate and fluoride ions. It was followed by a 6-hour remineralizing solution with pH of 6.75. This regimen almost inducted a model of carious challenge. Neutral remineralizing solution was supposed to mimic saliva, providing mineral content needed for repair of initial demineralized areas. Afterwards, microhardness and DD values were reassessed. Although the microhardness test does not provide specific information on changes that happen to a material, it is commonly used to measure changes in tooth structures after experiments involving demineralization and remineralization.<sup>29</sup> Changes in enamel can be efficiently studied by this test.

Our results suggest that the bleaching does not make teeth vulnerable to caries. There was no significant difference between the control group and any of the bleached groups. All groups exhibited a significant change in enamel microhardness after cariogenic challenge and no significant difference was seen in magnitude of these changes. The findings of this study are in accordance with the results of de Menezes et al<sup>20</sup> study, in which they assessed the effect of different concentrations of carbamide peroxide gel on enamel susceptibility to caries like lesions. They also used similar pH cycling regimen to induce caries-like lesions, involving 4 cycles of demineralizing solution with pH of 5 and remineralizing solution with pH of 7. de Menezes et al found that bleaching does not seem to pose any extra risk to caries lesion formation in enamel. Furthermore, Burgmaier et al<sup>30</sup> assessed the effect of an acidic challenge on 10% carbamide peroxide

bleached enamel samples and found no significant difference in surface microhardness between bleached and control samples. Moreover, Pretty et al<sup>18</sup> found that bleaching with 10%-22% carbamide peroxide does not make enamel or dentine more susceptible to acid erosion or caries lesion formation, by using quantitative light-induced fluorescence and transverse microradiography. In addition, Al-Qunaian (19) supported the idea that bleaching does not make tooth more vulnerable to caries formation via in vitro microbial caries model, after bleaching with 10% and 20% carbamide peroxide and 35% hydrogen peroxide. This finding was confirmed in rat molars with 10% carbamide peroxide, by Kraigher et al.<sup>31</sup>

However, regarding the application time of the bleached groups, a relative but not definite pattern in KHN reduction could be found. Following manufacturers' protocol, hydrogen peroxide gel had a direct contact with enamel surface for 60 minutes. Laser treated group had a contact time of 9 minutes. Regarding KHN values, the highest reduction was seen in conventional therapy (-77.97 $\pm$ 18.76) and laser treated group had less subsidence of KHN during the bleaching process. Nd:YAG laser had the least reduction of microhardness among the experimental groups, even less than the control group which did not received any bleaching treatment. As differences of KHN changes had no statistically significant difference (Table 3), no reliable inferences could be drawn. de Menezes et al<sup>20</sup> also found that after caries induction phase, an experimental group bleached with 16% carbamide peroxide exhibited higher KHN values compared to unbleached groups.

Some limitations could explain the non-statistically significant results among the groups. The number of sample to thoroughly investigated the effect of bleaching methods on caries susceptibility was relatively small (n=15). There was also a large variability among the samples. This variation could be related to different race, age or area of habitance of teeth donors. This diversity in baseline KHN demanded at least 90 samples per group which could not be covered by this study. Higher number of samples with more unified values of initial KHN, might result in a reliable pattern of KHN changes and therefore, the effects of each intervention could be compared precisely. Also, the demineralizing solution used in this study might have developed an aggressive mineral loss

and also impaired surface mineral recovery. Thus, the enamel prisms could be disorganized and disrupted, as reported by several authors.<sup>32-35</sup>

DIAGNOdent is a device which measures caries level of teeth by emitting a laser beam and analyzing the reflected light, while directly in contact with the enamel surface. Data acquired by this device are proportional and not pure values. It was assumed that induction of caries would result in a general increase in DD values. Surprisingly, the DD values were increased in all bleached groups of this study. Highest reduction was seen in conventional bleaching group ( $P=0.02$ ). In contrast, the control group showed increased DD values. Comparing DD changes among the groups, only conventional and diode laser groups had a meaningful difference in reduction of DD compared to the control group. Obviously, higher decrease in caries values is not expected after the oxidizing challenge exerted during the bleaching process. The underlying mechanism of such finding is that DIAGNOdent device detects caries levels by measuring fluorescent molecules in the decayed teeth. Bleaching targets those fluorescent molecules and decreases fluorescence values of the teeth. However decayed structures remain unaffected. This finding is in accordance with previous studies which concluded that fluorescence level of the teeth decrease or remain stable during the bleaching process, and DIAGNOdent as a caries measurement device would bring unreliable values for recent bleached teeth.<sup>36-38</sup> However, an additional measurement of DD values, after bleaching and prior to pH cycling could better illustrate the precise effect of bleaching on DD values.

Further studies are needed to clarify the clinical relevance of the findings of this invitro study. Invitro studies cannot be fully representative of the oral cavity as continuous supply of saliva containing various minerals, oral hygiene situation and changes in saliva flow might interfere with this simulation.

### Conclusion

Concerning limitation of this study, it can be concluded that bleaching whether conventional or laser activated method, does not make teeth vulnerable to develop carious lesions.

### Ethical Considerations

Not applicable.

### Conflict of Interests

The authors have no conflict of interest to declare.

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