

The effect of long-term use of tooth bleaching products on the human enamel surface

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Abstract The aim of this in vitro study was to evaluate the long-term effect of bleaching on human enamel. Four groups of enamel specimens were prepared ($n = 20$): group 1: bleaching with Opalescence Boost [40% hydrogen peroxide (H_2O_2), 3×20 min/week]; group 2: control group (the specimens were stored in human saliva); group 3: bleaching with Vivastyle Paint on Plus (6% H_2O_2 , 2×10 min/day), and group 4: bleaching with Opalescence PF 16% [16% carbamide peroxide (CP), 6 h/day]. After each bleaching session the specimens were stored in human saliva. Knoop microhardness and surface roughness were measured: before bleaching, after 2-week and after 8-week bleaching. After 2-week treatment, surface roughness was significantly increased in all experimental groups ($p < 0.05$), while among them no significant difference was found ($p > 0.05$). The roughness changes exerted after 8-week bleaching were not significantly higher than the ones after 2 weeks ($p > 0.05$). After 8-week treatment, the increase in roughness caused by 16% CP was significantly higher ($p < 0.05$) than the one caused by 40% H_2O_2 . Microhardness increased in all groups including control; however, only 40% H_2O_2 increased the microhardness significantly ($p < 0.05$). The effect of bleaching on enamel was not shown to be dependent on the method or the H_2O_2

concentration. Bleaching with CP 16% resulted in higher roughness than bleaching with H_2O_2 , while 40% H_2O_2 caused the higher microhardness increase. The present study showed that in-office bleaching with 40% H_2O_2 seems to be at least as safe as home bleaching as far as their effects on human enamel are concerned.

Keywords Tooth bleaching · In-office · Home bleaching · Roughness · Microhardness

Introduction

Nowadays, an increased demand on aesthetic dental treatment can be recognized. Among the treatments available to improve the tooth aesthetics, tooth bleaching has been established during the last decades as a minimal conservative method to improve human's smile when used as treatment alone or as a pretreatment in combination with restorative techniques.

The efficacy of tooth bleaching, independently of the method used has been widely documented in the literature [1–4]. It has been shown that the available bleaching methods are able to change the tooth color achieving the desired white teeth and satisfying the patients' demands and needs [2–4]. Although tooth bleaching is not new, there are still a few concerns about its safety [5–8]. Especially since the Directive 2011/84/EU [9] concerning cosmetic products, a discussion about the safety of bleaching products containing more than 6% H_2O_2 arose, as their use in the daily dental practice is under question in many countries in Europe. It is important to mention that after the directive went on force, most of the dental companies in Europe have classified their bleaching products into cosmetic and medical bleaching products in order to

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differentiate their clinical indication. Additionally, it is very often suggested that in the case of treating severe tooth discolorations, where usually “power” bleaching would be chosen as a treatment in the past, a long use of home bleaching products containing (up to 6% H₂O₂ or equivalent amount of CP) might bring the desired results without any harmful effects on dental hard tissues. In the case of severe tooth discolorations e.g., caused by genetic defects [10–12] or drug-induced staining [13–18], the use of tooth bleaching is recommended in the literature as the most conservative and appropriate treatment. According to Haywood et al. [19], extended treatment times are necessary when using lower concentrations of H₂O₂ in order to be effective in such cases. In the cases of drug-induced staining, an application time of three or six months was used [14, 15, 17, 20].

The findings in the literature concerning the effect of bleaching products and methods on the dental hard tissues are controversial. Some studies revealed no effects on the mechanical properties of dental hard tissues after tooth bleaching [21–29], while in other studies, different concentrations of CP and H₂O₂ were shown to decrease enamel microhardness values [30–32] and to increase surface roughness post-bleaching [32–34].

Although some data exist concerning the use of bleaching products for a longer period of time [22, 24, 27, 35], most of the studies available evaluated a maximum time of 2, 3, or 4 weeks [22, 24, 35] using products with low concentrations of CP or H₂O₂. In the study of Totoda et al. [27], a time period of 8 weeks was evaluated, however; only one bleaching product was tested (6% hydrogen peroxide). No data exist concerning the effect of long-term use of home bleaching products on human enamel compared to the respective use of in-office products.

The aim of the present in vitro study was to evaluate the effect of different bleaching methods/products on the surface roughness and microhardness of human enamel after simulation of a clinical use for bleaching products up to two months. The hypotheses tested were as follows: (1) home bleaching products cause fewer detrimental effects on the microhardness and roughness of the human enamel, and (2) all bleaching concentrations used can be recommended in a same manner for a long use.

Materials and methods

In the present in vitro study, extracted human third molars were used. The use of extracted human teeth was performed according to the guidelines of the ethics committee of the Medical Center-University of Freiburg. The teeth were evaluated prior the experimental use according to

cracks or other surface defects. The roots were removed and the teeth were cleaned to remove any external discolorations and then immersed in 0.9% NaCl until the beginning of the experiment. Each tooth crown was embedded separately in self-curing acrylic resin [Technovit (Heraeus-Kulzer GmbH, Hanau, Germany)]. The upper and lower surfaces of the acrylic resin blocks were parallelized and the buccal enamel surface was polished up to 4000 grid. Then the teeth samples were randomly divided among four groups of 20 teeth each.

The four groups were treated as follows:

- Group 1: In-office bleaching with Opalescence Boost (40% H₂O₂, Ultradent Products, Inc, South Jordan, UT, USA). Each treatment session included 3 × 20 min application of the bleaching product, as it is recommended by the manufacturers. This session (3 × 20 min) represented the bleaching treatment of this group per week. Among the applications, the teeth were washed with running tap water and after the end of each session, the teeth were stored in human saliva for the rest of the week. This group represents the positive control, as this product is the one with a higher concentration of H₂O₂ in the market.
- Group 2: In this group, the specimens were stored only in human saliva (control group).
- Group 3: Home bleaching with Vivastyle Paint on Plus (Ivoclar Vivadent AG, Schaan, Liechtenstein). This product contains 6% H₂O₂. It was applied twice a day for 10 min according to the manufacturers’ instructions. After each application, the teeth were washed under running tap water and then they were stored in human saliva. The total application time each week was 1.4 h.
- Group 4: Home bleaching with Opalescence PF 16% (16% CP, Ultradent Products, Inc, South Jordan, UT, USA). This product was applied 6 h per day (as it is recommended by the manufacturer). The total application time was 42 h per week. After the 8 h application, the teeth were washed with running tap water and were stored in human saliva for the rest of the day until the next application.

The whole tooth bleaching procedure was repeated for 8 weeks for each tested group during a time period of 8 weeks. The saliva used was given by one person, collected by chewing paraffin. Informed consent from the volunteer was taken. Saliva was centrifuged twice for 10 min at 15000 rpm (Hettich, Tuttlingen, Germany). The resulting supernatant was decanted into sterile cups and was kept at –8 °C until use. The saliva was renewed once a week during the experiment. In the present study, surface hardness and microhardness were determined at three different time points: before treatment (baseline), after

2 week's treatment, and after 8-week treatment. Specimens of each group underwent evaluation of the surface roughness using a focusing optical profilometer (Mikrofocus, UBM, Type 2010, UBM, Karlsruhe, Germany) with a laser diode and UBSOFT software (UBM Meßtechnik, version Nr. 1.909). For the estimation of the surface roughness, three measurements (0.5 mm × 0.5 mm) were performed at the surface of each sample. For the estimation of microhardness five measurements were performed on the surface of each sample using a knoop microhardness device (Leitz Miniload, Ernst Leitz GmbH, Wetzlar, Germany) with a load of 1.030 mN and a loading time of 30 s.

Statistical analysis

A linear mixed model was fitted with random intercepts for each sample to evaluate time and material effects on response variables. Samples are considered as clusters since data are collected at several time points. This was done separately for each response variable (Ra, KHN). Furthermore, pairwise comparisons between different materials and time points were performed. Therefore, the method of "Tukey" was applied to correct for the multiple testing problem (adjustment of p values). The calculations were made with the statistical software STATA 13 and SAS 9.2.

Results

For a descriptive analysis, mean, median, and standard deviations were computed. Tables 1 and 2 show the mean values and the standard deviations (SD) of the surface roughness and knoop microhardness numbers for all the groups and each tested time period. A one-way ANOVA revealed a significant difference among the Ra-values ($p = 0.0297$) and the microhardness values ($p = 0.0001$) among the groups at baseline. Therefore, in order to evaluate the effect of the different methods on the human enamel, the differences to the baseline values were calculated for each group and at each tested time period. Figs. 1 and 2 present the boxplots for the differences to baseline

for roughness and microhardness for each tested time point. In Table 3, the findings of the pairwise analysis for the differences to baseline for each group and each tested time for surface roughness and microhardness are presented.

Surface roughness

A first analysis using a linear mixed model showed that the surface roughness of the samples bleached with in-office bleaching (group 1) was significantly higher after 2- and 8-week bleaching compared to baseline ($p < 0.05$). In this group, the difference in roughness to baseline was not significantly influenced by the time of the bleaching ($p = 0.154$), meaning that the change of enamel roughness after bleaching for 8 weeks with 40% H₂O₂ (compared to baseline) was not significantly different than the one caused after 2 weeks of bleaching. In group 3, where the specimens were bleached with 6% H₂O₂, the surface roughness after 2- and 8-week bleaching was significantly higher than the one at baseline ($p < 0.05$). According to the statistical analysis, the differences in roughness compared to baseline was significantly different among the tested time periods ($p = 0.027$), meaning that the surface roughness was increased at each tested time period. In group 4 (home bleaching with 16% CP), the surface roughness after 2 and 8-week bleaching was significantly higher than the one at baseline ($p < 0.05$). The differences in roughness compared to baseline differed significantly differently among the two tested time periods ($p = 0.024$), meaning that the surface roughness was increased significantly at each tested time period.

After evaluating each group separately, the differences caused in roughness after 8-week bleaching did not differ significantly compared to the changes caused after the first 2 weeks. As far as the changes among after the first 2 weeks of bleaching are concerned, the pairwise analysis showed that the differences achieved by all bleaching groups were statistical higher compared to control group ($p < 0.05$). No significant difference ($p > 0.05$) in roughness was found among the bleached groups after 2-week treatment. The pairwise analysis showed that the differences caused by the two home bleaching methods did not

Table 1 Mean values of surface roughness (Ra) and standard deviations (SD) for all tested groups and evaluated time periods

	Before bleaching	After 2-week bleaching and saliva	After 8-week bleaching and saliva
Ra_values (MW ± SD) µm			
In-office bleaching (Opalescence Boost: 40% H ₂ O ₂)	0.08 ± 0.03	0.11 ± 0.03	0.10 ± 0.02
Control/without bleaching	0.08 ± 0.03	0.08 ± 0.04	0.09 ± 0.03
Home bleaching (6% H ₂ O ₂)	0.06 ± 0.02	0.09 ± 0.02	0.10 ± 0.03
Home bleaching (16% CP)	0.07 ± 0.03	0.11 ± 0.03	0.12 ± 0.02

Table 2 Mean values of surface microhardness (KHN) and standard deviations (SD) for all tested groups and evaluated time periods

	Before bleaching	After 2-week bleaching and saliva	After 8-week bleaching and saliva
KHN_values (MW ± SD)			
In-office bleaching (Opalescence Boost: 40% H ₂ O ₂)	296.99 ± 31.14	314.79 ± 32.12	334.63 ± 32.69
Control/without bleaching	309.31 ± 29.62	331.99 ± 24.63	326.70 ± 30.59
Home bleaching (6% H ₂ O ₂)	314.91 ± 32.20	318.60 ± 40.88	330.56 ± 35.03
Home bleaching (16% CP)	320.67 ± 32.25	326.66 ± 35.24	327.05 ± 41.46

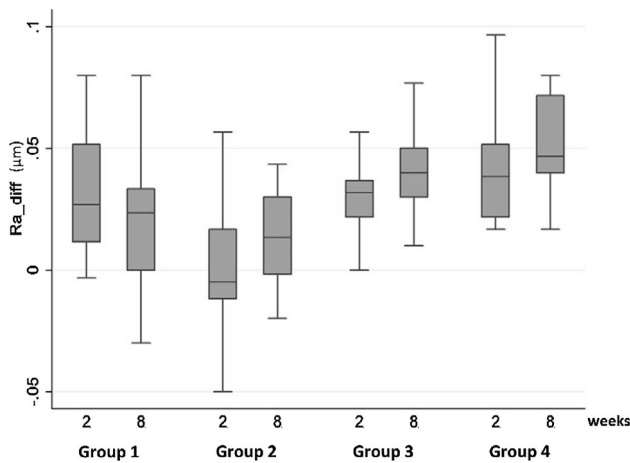


Fig. 1 Differences of surface roughness related to baseline values in all tested groups and all evaluated time periods. Box plot illustration (Group 1: 40% H₂O₂, Group 2: control, Group 3: 6% H₂O₂, Group 4: 16% CP)

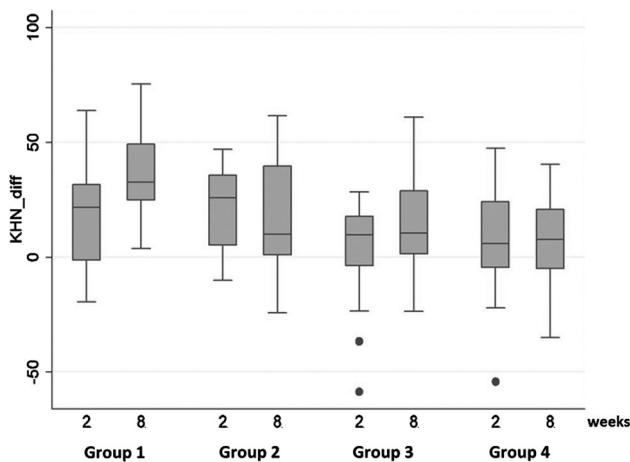


Fig. 2 Differences of surface microhardness related to baseline values in all tested groups and all evaluated time periods. Box plot illustration (Group 1: 40% H₂O₂, Group 2: control, Group 3: 6% H₂O₂, Group 4: 16% CP) (dots present outliers)

differ significantly after 8-week treatment ($p < 0.05$), while both were significantly higher compared to control group ($p < 0.05$). As far as the in-office bleaching is

concerned, the difference caused in roughness did not differ compared to control group and group 3, but it was significantly lower than the ones caused in group 4.

Knoop microhardness

The microhardness of the samples of group 1 was significantly increased after 2- and 8-week bleaching compared to baseline ($p < 0.05$). In the control group (group 2), microhardness after 2- and 8-week storage in saliva was significantly higher compare to baseline ($p < 0.05$). In group 3, the microhardness after 2-week bleaching was similar to baseline ($p = 0.433$), while after 8-week bleaching, the microhardness was significantly higher compared to baseline ($p = 0.001$). In group 4, the microhardness after 2 ($p = 0.222$)- and 8 ($p = 0.136$)-week bleaching was similar to baseline.

The statistical analysis showed that the changes in microhardness were statistical different among the tested time periods for group 1 ($p < 0.0001$) and group 3 ($p = 0.037$). For Group 2 (control group) ($p = 0.299$) and group 4 ($p = 0.940$), no significant effect was found among the tested time periods concerning the progress of microhardness.

In Table 3, the results of the pairwise analysis of the microhardness’ differences to baseline achieved at each tested time point and each group are presented. This analysis showed no significant difference ($p > 0.05$) among the groups concerning the differences caused in the microhardness values after 2- and 8-week treatment. Only after bleaching with 40% H₂O₂ (group 1) for 8 weeks, it was found that the microhardness change relation to baseline was significantly higher than the one after 2 weeks ($p = 0.029$).

Discussion

In the present study, the safety of using bleaching products up to two months was evaluated as far as their effect on surface enamel roughness and microhardness is concerned. Although there are several studies in the literature

Table 3 Comparison of the different groups (differences to baseline) (significance at the level of $p < 0.05$)

Pairwise analysis (linear mixed model adjusted by the method of Tukey–Kramer)		
Compared pairs (group/time period of difference to baseline vs. group/time period of difference to baseline)	Roughness (adjusted p values)	KHN (adjusted p values)
group_1/time_2 weeks vs group_1/time_8 weeks	0.8107	0.0290
group_1/time_2 weeks vs group_2/time_2 weeks	0.0003	0.5119
group_1/time_2 weeks vs group_3/time_2 weeks	0.9589	0.9993
group_1/time_2 weeks vs group_4/time_2 weeks	0.9871	1.0000
group_1/time_8 weeks vs group_2/time_8 weeks	0.8396	0.4270
group_1/time_8 weeks vs group_3/time_8 weeks	0.6155	0.6239
group_1/time_8 weeks vs group_4/time_8 weeks	0.0028	0.1494
group_2/time_2 weeks vs group_2/time_8 weeks	0.7372	0.9883
group_2/time_2 weeks vs group_3/time_2 weeks	0.0209	0.1764
group_2/time_2 weeks vs group_4/time_2 weeks	<0.0001	0.7428
group_2/time_8 weeks vs group_3/time_8 weeks	0.0330	1.0000
group_2/time_8 weeks vs group_4/time_8 weeks	<0.0001	0.9971
group_3/time_2 weeks vs group_3/time_8 weeks	0.8240	0.5078
group_3/time_2 weeks vs group_4/time_2 weeks	0.4883	0.9822
group_3/time_8 weeks vs group_4/time_8 weeks	0.3885	0.9792
group_4/time_2 weeks vs group_4/time_8 weeks	0.7372	1.0000

evaluating the effect of bleaching on the dental hard tissues, most of them use extreme single application times or they are not performed according to the recommendations of the manufacturers. Hegedüs et al. [36] found severe study surface alterations after treatment with 10% CP and 30% H₂O₂, revealing deeper grooves after bleaching. However, 30% H₂O₂ was applied on the samples for 28 h, representing an application time far away from the daily clinical practice. Azrak et al. [37] used a bleaching time of 10 h. Surface alterations and porosity of enamel were also observed in the study of Kwon et al. [38] after immersing the samples for 3 days in 30% H₂O₂. In the present study, three different categories of bleaching products and methods were evaluated after simulating 8-week treatment. Such long evaluation periods were chosen to simulate the treatment of severe tooth discolorations. In such cases, usually in-office products with high H₂O₂ concentration were used in the past. However, after the Directive 2011/84/EU [9] went on force, in a lot of European countries, products with high concentration in H₂O₂ are no longer used. In these cases, only bleaching products containing H₂O₂ up to 6% (or 16% CP) are available and longer application periods are necessary in order to treat severe tooth discolorations. In literature [39, 40] tooth bleaching with CP has been recommended to treat severe discolorations by increasing the length of the application to several weeks. These products are used mainly for home bleaching procedures. However, it is not clear if they are safer than in-office bleaching as far as the effect on the dental hard tissues is concerned. The 2-week bleaching

period simulates a usual bleaching treatment period and it is very interesting to compare these effects with those caused after 8-week treatment. The application times used were according to the manufacturers' instructions. Between the bleaching sessions, the samples were stored in human saliva in order to simulate physiological oral conditions.

As far as the surface roughness is concerned, all bleaching methods resulted in increased values after treatment. This is in agreement with several other studies having shown an increase in enamel roughness after tooth bleaching [32, 34, 41–45]. The main change in roughness caused by the in-office bleaching was observed after the first 2 weeks, while a further bleaching treatment up to 8 weeks did not affect the surface roughness significantly. As far as the home bleaching products are concerned, it was found that the treatment time had a significant effect on the increase of surface roughness. Our findings indicate that a long-term use of in-office or home bleaching products lead to similar changes in surface roughness after long-term use. Therefore, the first hypothesis made at the beginning of the study, cannot be accepted, as far as the effect of the bleaching products on the surface roughness is concerned. In-office bleaching resulted in higher increase of roughness after the first 2 weeks, while the home bleaching products resulted in a gradual increase of the surface roughness. These findings imply no negative conclusions for using the in-office technique for longer time. A short-term use of bleaching products resulted in similar effects on human enamel, while after 8-week bleaching the treatment with CP 16% caused significantly rougher

enamel surfaces than the other two methods. In-office bleaching with 40% H₂O₂ and home bleaching with 6% H₂O₂ caused similar changes. According to the present findings, the application time is an important parameter concerning the effect on enamel roughness and among the kind of bleaching agents use, CP was found to exert more effects. Mondelli et al. [46] showed also that the application time is an important factor concerning the effect of bleaching agents on enamel. They found that after treatment of the enamel samples with 16% CP, the surface roughness was significantly higher than the changes caused by the other products containing H₂O₂. According to Bistey et al. [47], the effects caused on enamel due to bleaching are time dependent suggesting that an application time higher than 60 min can cause considerable effects. Although it is always thought that the H₂O₂ concentration is responsible for exerting effects on dental hard tissues and restorative materials, a lot of studies have shown that several other parameter play important role [33, 45, 48, 49]. Bleaching products with similar concentration in H₂O₂ might cause different effects on enamel [48]. Özkan et al. [45] could show that tooth bleaching with 10% H₂O₂ and 10% CP resulted in similar increase of roughness values after the 4 weeks. This is in accordance with our findings. The same authors [45] suggested that the mechanical brushing procedures and not the bleaching treatment are more important factors affecting the human enamel. In the present study, no brushing of the samples was performed in order to evaluate solely the effect of bleaching on the enamel surface.

According to a literature review of Bollen et al. [50], a roughness value of 0.2 µm is thought as a limit concerning bacterial accumulation. The values in the present study after bleaching were below this threshold roughness independent of the kind of method and time.

Concerning the effect of the bleaching methods/products on enamel microhardness, an increase was observed in all treated groups, including the control group. The highest increase was found after bleaching with 40% H₂O₂. Several previous studies have shown a decrease of enamel microhardness after bleaching [32, 49, 51, 52]. The fact that the samples were stored in human saliva between the applications of the bleaching products, and the fact that the application of the bleaching products was not performed cumulative at once, but in clinically realistic intervals, might have influenced the present findings. The increase of enamel microhardness at the 8-week period does not really indicate a negative effect of bleaching. According to this finding, the part of the first hypothesis concerning the effect of in-office bleaching on microhardness cannot be accepted either. Previous studies [49, 52–54] have used saliva in order to store the samples between the applications of the bleaching agents, like it was also performed in our study.

Borges et al. [53] could show that saliva exerts a remineralizing effect. Lia Mondelli et al. [52] showed that after 7 days remineralization in artificial saliva, the hardness values came back to normal. In their control group stored in artificial saliva, an increase of the microhardness was also observed. Previous studies [54, 55] suggested that the effects of bleaching on microhardness are reversible due to remineralization potential of the saliva that can replace the lost calcium and phosphate ions. According to Borges et al. [54], exposure of the enamel samples to human saliva after bleaching for 1 and 7 days, resulted in increase of the surface microhardness, suggesting that the remineralization action of the human saliva might have a positive effect on the enamel microhardness after bleaching. Abouassi et al. [41] observed in their study an increase in enamel microhardness compared to control after bleaching with 10% H₂O₂, while treatment with 10% CP reduced the enamel microhardness. In the study of Elfallah et al. [30], a reduction in hardness took place after bleaching with CP and H₂O₂. However, 16% CP was applied for 80 min daily for 14 days and 35% H₂O₂ was applied for 3–4 times of 10 min each and the tests were performed after 24 h. Moreover, Hank's balanced salt solution was used instead of saliva. The lack of extra remineralization time might be the reason for the low hardness values in this study.

The pH value of the bleaching gels has been assigned to be responsible for the effects on the enamel structure. The optimal pH for hydrogen peroxide in order to act as a bleaching agent is 9.5–10.8 [56]. The different pH values can cause surface changes in the enamel surface such decalcification, porosity, and surface roughness [33]. Previous studies [57, 58] suggested that the demineralization of the enamel takes place by pH values under 5.2. Sa et al. [48] showed that under in vitro conditions, bleaching products with low pH exert surface alteration of the enamel, but under in situ conditions no changes were observed. According to the findings of Abe et al. [49] the bleaching material with the lowest pH value was the only one that decreased the microhardness of the enamel immediately after bleaching. The pH values of the products used in the present study were almost neutral. Therefore, it is not conceivable that pH might have caused the different effects on enamel. According to the present findings, taking in consideration the limitations of an in vitro study, both in-office and home bleaching methods showed moderate effects on the human tooth structure, being able to be recommended for long-term use in a same way, independently of the contained amount of H₂O₂. Therefore the second hypothesis made at the beginning of the study can be accepted.

Changes in enamel surface might indicate alterations in the mineral and organic part. According to Jiang et al. [59], the organic matrix of enamel can be affected by the

oxidation reaction of hydrogen peroxide. Mahringer et al. [44] suggested that the increased enamel roughness after bleaching might be due to the modification of the enamel's organic matrix due to bleaching. Elfallah et al. [30] concluded that the changes occurred on the human enamel are regardless of the type of bleaching gel. They assumed that the destruction or denaturation of the matrix proteins through bleaching result in decrease of the mechanical properties of the enamel. The dissolution of the organic phase of the enamel might result in higher microhardness. However, the fact that the increase in microhardness in the present study was observed also in the control group does not support this assumption.

Among the parameters being responsible for the effect of the bleaching products on dental hard tissue, other ingredients besides H₂O₂, like carbopol, have been identified that might cause changes on enamel microhardness [60] and roughness [61]. Carbopol stabilizes CP [62], meaning that it ensures that carbamide stays longer active in gel form. This might be a parameter explaining the findings for CP.

The load used in the in vitro studies might also influence the findings. Elfallah et al. [30] used a 10 mN load for the hardness evaluation of enamel, while Abe et al. [49] performed their measurements with a load of 100 mN. A relationship is supposed to exist between hardness and indentation depth of crystalline tissues, and hardness level decreases as indentation depth increases [63]. In the present study a load of about 1 mN was used. It can be assumed that using lower loads changes in the more superficial layer of the enamel can be measured. The modification of the organic matrix of the enamel might explain the harder surface identified by our measurements. Increased enamel hardness could mean that the enamel would be more friable and susceptible to crack formation. However, the microhardness' increase was the same occurred in the enamel samples of the control group which were stored solely in human saliva, meaning that this increase has not clinical negative effects and is probably due to the storage in saliva.

The difference of the findings among the studies is thought to be due to the different study designs used, e.g., different application times, substrates used for samples, preparation and polishing of the samples, and especially the kind of materials used. Several studies [38, 45, 64] have used chemical solutions and diluted them in order to get the desired H₂O₂ concentration; however, this does not represent the real clinical conditions.

Besides the effects of bleaching materials on the dental tissues, also the effects on gingiva and tooth sensitivity, have to be kept in mind. In the literature, home bleaching was found to cause tooth sensitivity [65–67] and in some of the studies it has been reported that the sensitivity was higher compared to the use of in-office bleaching products

[65, 66]. Türkün et al. [67] showed that the use of a non-custom-fit tray home bleaching product with 28% CP gel resulted in less tooth sensitivity than the custom-fit tray with 10% CP, probably due to the reduced contact time of the bleaching agent.

Within the limitations of the present study, it can be concluded that in the case of a long use of tooth bleaching agents, the amount of the hydrogen peroxide used does not seem to be the most important factor concerning alterations of enamel surface properties. Instead of this, the application time of the bleaching agent used for each method seems to play an important role. In the case of a correct use of bleaching products, the in-office bleaching method seems to be as safe as the home bleaching procedures.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Attin T, Paqué F, Ajam F, Lennon AM. Review of the current status of tooth whitening with the walking bleach technique. *Int Endod J*. 2003;36:313–29.
2. Carey CM. Tooth whitening: what we now know. *J Evid Based Dent Pract*. 2014;14(Suppl):70–6.
3. Joiner A. The bleaching of teeth: a review of the literature. *J Dent*. 2006;34:412–9.
4. Sulieman MA. An overview of tooth-bleaching techniques: chemistry, safety and efficacy. *Periodontol*. 2000;2008(48): 148–69.
5. Goldberg M, Grootveld M, Lynch E. Undesirable and adverse effects of tooth-whitening products: a review. *Clin Oral Investig*. 2010;14:1–10.
6. Goldstein GR, Kiremidjian-Schumacher L. Bleaching: is it safe and effective? *J Prosthet Dent*. 1993;69:325–8.
7. Minoux M, Serfaty R. Vital tooth bleaching: biologic adverse effects—a review. *Quintessence Int*. 2008;39:645–59.
8. Walsh LJ. Safety issues relating to the use of hydrogen peroxide in dentistry. *Aust Dent J*. 2000;45:257–69.
9. European Union Medical Device Directive (93/42/EEC). <http://www.emergogroup.com/files/EUROPE-CONSOLIDATED-MDD-93-42-EEC.pdf>. Accessed 12 Nov 2013.
10. Bidra AS, Uribe F. Successful bleaching of teeth with dentinogenesis imperfecta discoloration: a case report. *J Esthet Restor Dent*. 2011;23:3–10.
11. Croll TP, Sasa IS. Carbamide peroxide bleaching of teeth with dentinogenesis imperfecta discoloration: report of a case. *Quintessence Int*. 1995;26:683–6.
12. Nathwani NS, Kelleher M. Minimally destructive management of amelogenesis imperfecta and hypodontia with bleaching and bonding. *Dent Update*. 2010; 37:170–2, 175–6, 179.
13. Bodden MK, Haywood VB. Treatment of endemic fluorosis and tetracycline staining with macroabrasion and nightguard vital bleaching: a case report. *Quintessence Int*. 2003;34:87–91.
14. Fiedler RS, Reichl RB. Combined professional and home care nightguard bleaching of tetracycline-stained teeth. *Gen Dent*. 2000;48:257–61.

15. Leonard RH Jr, Haywood VB, Eagle JC, Garland GE, Caplan DJ, Matthews KP, Tart ND. Nightguard vital bleaching of tetracycline-stained teeth: 54 months post treatment. *J Esthet Dent*. 1999;11:265–77.
16. Leonard RH Jr. Long-term treatment results with nightguard vital bleaching. *Compend Contin Educ Dent*. 2003;24:364–74.
17. Matis BA, Wang Y, Eckert GJ, Cochran MA, Jiang T. Extended bleaching of tetracycline-stained teeth: a 5-year study. *Oper Dent*. 2006;31:643–51.
18. Tsubura S. Clinical evaluation of three months' nightguard vital bleaching on tetracycline-stained teeth using Polanight 10% carbamide gel: 2-year follow-up study. *Odontology*. 2010;98:134–8.
19. Haywood VB, Robinson FG. Vital tooth bleaching with nightguard vital bleaching. *Curr Opin Cosmet Dent*. 1997;4:45–52.
20. Kugel G, Gerlach RW, Aboushala A, Ferreira S, Magnuson B. Long-term use of 6.5% hydrogen peroxide bleaching strips on tetracycline stain: a clinical study. *Compend Contin Educ Dent*. 2011;32:50–6.
21. Cakir FY, Korkmaz Y, Firat E, Oztas SS, Gurgan S. Chemical analysis of enamel and dentin following the application of three different at-home bleaching systems. *Oper Dent*. 2011;36:529–36.
22. Faraoni-Romano JJ, Da Silveira AG, Turssi CP, Serra MC. Bleaching agents with varying concentrations of carbamide and/or hydrogen peroxides: effect on dental microhardness and roughness. *J Esthet Restor Dent*. 2008;20:395–402 (**discussion 403–4**).
23. Joiner A, Thakker G, Cooper Y. Evaluation of a 6% hydrogen peroxide tooth whitening gel on enamel and dentine microhardness in vitro. *J Dent*. 2004;32(Suppl 1):27–34.
24. Maia E, Baratieri LN, Caldeira de Andrada MA, Monteiro S Jr, Vieira LC. The influence of two home-applied bleaching agents on enamel microhardness: an in situ study. *J Dent*. 2008;36:2–7.
25. Polydorou O, Hellwig E, Auschill TM. The effect of different bleaching agents on the surface texture of restorative materials. *Oper Dent*. 2006;31:473–80.
26. Polydorou O, Mönning JS, Hellwig E, Auschill TM. Effect of in-office tooth bleaching on the microhardness of six dental esthetic restorative materials. *Dent Mater*. 2007;23:153–8.
27. Totoda M, Philpotts CJ, Cox TF, Joiner A. Evaluation of a 6% hydrogen peroxide tooth-whitening gel on enamel microhardness after extended use. *Quintessence Int*. 2008;39:853–8.
28. Türker SB, Biskin T. The effect of bleaching agents on the microhardness of dental aesthetic restorative materials. *J Oral Rehabil*. 2002;29:657–61.
29. Unlü N, Cobankara FK, Altinöz C, Ozer F. Effect of home bleaching agents on the microhardness of human enamel and dentin. *J Oral Rehabil*. 2004;31:57–61.
30. Elfallah HM, Bertassoni LE, Charadram N, Rathsam C, Swain MV. Effect of tooth bleaching agents on protein content and mechanical properties of dental enamel. *Acta Biomater*. 2015;20:120–8.
31. Magalhães JG, Marimoto AR, Torres CR, Pagani C, Teixeira SC, Barcellos DC. Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity. *Acta Odontol Scand*. 2012;70:122–6.
32. Pinto CF, Oliveira RD, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. *Braz Oral Res*. 2004;18:306–11.
33. Cavalli V, Giannini M, Carvalho RM. Effect of carbamide peroxide bleaching agents on tensile strength of human enamel. *Dent Mater*. 2004;20:733–9.
34. Markovic L, Jordan RA, Lakota N, Gaengler P. Micromorphology of enamel surface after vital tooth bleaching. *J Endod*. 2007;33:607–10.
35. Zantner C, Beheim-Schwarzbach N, Neumann K, Kielbassa AM. Surface microhardness of enamel after different home bleaching procedures. *Dent Mater*. 2007;23:243–50.
36. Hegedüs C, Bistey T, Flóra-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent*. 1999;27:509–15.
37. Azrak B, Callaway A, Kurth P, Willershausen B. Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens. *J Esthet Restor Dent*. 2010;22:391–9.
38. Kwon YH, Huo MS, Kim KH, Kim SK, Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. *J Oral Rehabil*. 2002;29:473–7.
39. Haywood VB. Current status of nightguard vital bleaching. *Compend Contin Educ Dent*. 2000;21(Suppl):S10–7.
40. Loyola-Rodriguez JP, Pozos-Guillen Ade J, Hernandez-Hernandez F, Berumen-Maldonado R, Patiño-Marin N. Effectiveness of treatment with carbamide peroxide and hydrogen peroxide in subjects affected by dental fluorosis: a clinical trial. *J Clin Pediatr Dent*. 2003;28:63–7.
41. Abouassi T, Wolkewitz M, Hahn P. Effect of carbamide peroxide and hydrogen peroxide on enamel surface: an in vitro study. *Clin Oral Investig*. 2011;15:673–80.
42. Anaraki SN, Shahabi S, Chiniforush N, Nokhbatolfighahaei H, Assadian H, Yousefi B. Evaluation of the effects of conventional versus laser bleaching techniques on enamel microroughness. *Lasers Med Sci*. 2015;30:1013–8.
43. Dominguez JA, Bittencourt B, Michel M, Sabino N, Gomes JC, Gomes OM. Ultrastructural evaluation of enamel after dental bleaching associated with fluoride. *Microsc Res Tech*. 2012;75:1093–8.
44. Mahringer C, Fureder M, Kastner M, Ebner A, Hinterdorfer P, Vitkov L, Hannig M, Kienberger F, Schilcher K. Examination of native and carbamide peroxide-bleached human tooth enamel by atomic force microscopy. *Ultrastruct Pathol*. 2009;33:189–96.
45. Özkan P, Kansu G, Özak ST, Kurtulmuş-Yılmaz S, Kansu P. Effect of bleaching agents and whitening dentifrices on the surface roughness of human teeth enamel. *Acta Odontol Scand*. 2013;71:488–97.
46. Mondelli RF, Azevedo JF, Francisconi PA, Ishikiriama SK, Mondelli J. Wear and surface roughness of bovine enamel submitted to bleaching. *Eur J Esthet Dent*. 2009;4:396–403.
47. Bistey T, Nagy IP, Simó A, Hegedus C. In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. *J Dent*. 2007;35:325–30.
48. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, Wang Y. Effects of two in-office bleaching agents with different pH on the structure of human enamel: an in situ and in vitro study. *Oper Dent*. 2013;38:100–10.
49. Abe AT, Youssef MN, Turbino ML. Effect of bleaching agents on the nanohardness of tooth enamel, composite resin, and the tooth-restoration interface. *Oper Dent*. 2016;41:44–52.
50. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dent Mater*. 1997;13:258–69.
51. China AL, Souza NM, Gomes Ydo S, Alexandrino LD, Silva CM. Effect of fluoride gels on microhardness and surface roughness of bleached enamel. *Open Dent J*. 2014;8:188–93.
52. Lia Mondelli RF, Garrido Gabriel TR, Piola Rizzante FA, Magalhães AC, Soares Bombonatti JF, Ishikiriama SK. Do different bleaching protocols affect the enamel microhardness? *Eur J Dent*. 2015;9:25–30.
53. Borges AB, Torres CR, de Souza PA, Caneppele TM, Santos LF, Magalhães AC. Bleaching gels containing calcium and fluoride: effect on enamel erosion susceptibility. *Int J Dent*. 2012;2012:347848.

54. Borges AB, Guimarães CA, Bresciani E, Ramos CJ, Borges AL, Rocha GTC. Effect of incorporation of remineralizing agents into bleaching gels on the microhardness of bovine enamel in situ. *J Contemp Dent Pract.* 2014;15:195–201.
55. Araujo Fde O, Baratieri LN, Araújo E. In situ study of in-office bleaching procedures using light sources on human enamel microhardness. *Oper Dent.* 2010;35:139–46.
56. Frysh H, Bowles WH, Baker F, Rivera-Hidalgo F, Guillen G. Effect of pH on hydrogen peroxide bleaching agents. *J Esthet Dent.* 1995;7:130–3.
57. Joiner A. Review of the effects of peroxide on enamel and dentine properties. *J Dent.* 2007;35:889–96.
58. Shannon H, Spencer P, Gross K, Tira D. Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. *Quintessence Int.* 1993;24:39–44.
59. Jiang T, Ma X, Wang Z, Tong H, Hu J, Wang Y. Beneficial effects of hydroxyapatite on enamel subjected to 30% hydrogen peroxide. *J Dent.* 2008;36:907–14.
60. Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, Pimenta LA. Microhardness evaluation of in situ vital bleaching on human dental enamel using a novel study design. *Dent Mater.* 2005;21:1059–67.
61. Melo CF, Manfroi FB, Spohr AM. Microhardness and roughness of enamel bleached with 10% carbamide peroxide and brushed with different toothpastes: an in situ study. *J Int Oral Health.* 2014;6:18–24.
62. Haywood VB. Overview and status of mouthguard bleaching. *J Esthet Dent.* 1991;3:157–61.
63. Zhou J, Hsiung LL. Depth-dependent mechanical properties of enamel by nanoindentation. *J Biomed Mater Res A.* 2007;81:66–74.
654. Borges AB, Zanatta RF, Barros AC, Silva LC, Pucci CR, Torres CR. Effect of hydrogen peroxide concentration on enamel color and microhardness. *Oper Dent.* 2015;40:96–101.
65. Jorgensen MG, Carroll WB. Incidence of tooth sensitivity after home whitening treatment. *J Am Dent Assoc.* 2002;133:1076–82.
66. Matis BA, Cochran MA, Wang G, Eckert GJ. A clinical evaluation of two in-office bleaching regimens with and without tray bleaching. *Oper Dent.* 2009;34:142–9.
67. Türkün M, Celik EU, Aladağ A, Gökay N. One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique. *J Esthet Restor Dent.* 2010;22:139–46.