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ORIGINAL ARTICLE

Effect of carbamide peroxide and hydrogen peroxide on enamel surface: an in vitro study

Thaer Abouassi · Martin Wolkewitz · Petra Hahn

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Abstract The aim of the study was to investigate changes in the micromorphologyl and microhardness of the enamel surface after bleaching with two different concentrations of hydrogen peroxide (HP) and carbamide peroxide (CP). Bovine enamel samples were embedded in resin blocks, and polished. Specimens in the experimental groups (n=10) were treated with bleaching gels containing 10% CP, 35% CP, 3.6% HP, and 10% HP, respectively, for 2 h every second day over a period of 2 weeks. The gels had the identical composition and pH and differed only in their HP or CP content. The roughness and morphology of the enamel surface were analyzed using laser profilometry and SEM. Microhardness was measured using a Knoop hardness tester. The data were evaluated statistically. Specimens in the 10% HP group showed significantly higher roughness after bleaching compared to the control group (ΔRa, p=0.01). Bleaching with 35% CP showed only a tendency to increase roughness (ΔRa, p=0.06). Application of 10% CP or 3.6% HP had no significant influence on Ra. Enamel microhardness was significantly higher after application of 10% HP compared to the control (ΔMic=8 KHN, p=0.0002) and 35% CP (ΔMic=20KHN, p=0.01) groups. In summary, application of CP and HP showed only small quantitative and qualitative differences. In addition, the influence of bleaching procedure on the morphology and hardness of the enamel surface depended on the concentration of the active ingredients.

Keywords Bleaching · Enamel surface · Roughness · Microhardness · Hydrogen peroxide · Carbamide peroxide

Introduction

The smile is one of the most important factors in interactive social communication between people. Patients' demands for esthetic treatments have increased in recent years. With regard to the discoloration of teeth, bleaching of teeth has proven to be a conservative esthetic solution [1, 2]. Hydrogen peroxide (HP) is used as an active oxidizing agent during tooth bleaching. HP can be used directly or indirectly, produced by a chemical reaction from carbamide peroxide (CP) [3–5]. HP diffuses through the organic matrix of the tooth structure due to its low molecular weight [6, 7]. The mechanism of tooth bleaching and the role of HP are not yet completely clear. However, as its oxidative effect is not specific, bleaching agents may not only oxidize the chromogen but also attack the organic matter of the teeth [7–9]. The result of this attack is a destruction of the long organic chains into colorless short chains by an oxidizing reaction.

High concentrations of CP (35–37%) and HP (30–35%) are used as sources of oxidizing agents for professional use in the office, while at-home bleaching can be achieved by using tooth-bleaching materials containing up to 20% CP and 10% HP.

T. Abouassi · P. Hahn
Department of Operative Dentistry and Periodontology,
University of Freiburg,
Freiburg, Germany

M. Wolkewitz
Institute of Medical Biometry and Medical Informatics,
University of Freiburg,
Freiburg, Germany

T. Abouassi (✉)
Hugstetter Straße 55,
79106 Freiburg, Germany
e-mail: thaer.abouassi@uniklinik-freiburg.de

There is no agreement to date about the morphologic alteration of the enamel surface or microhardness according to the bleaching procedures [5, 8–19]. Sulieman et al. [20] reported that HP itself had no deleterious effects on enamel, but that the pH of the bleaching materials might cause adverse effects during the bleaching procedure. Examination of enamel surfaces after bleaching with 30% CP by atomic force microscopy [21] demonstrated a minimal increase in enamel roughness (from 19±4 to 33±5 nm). This increase in roughness happened during the first hour of bleaching, while no further change was observed over the next 6 h of bleaching. The authors suggested that partial lysis of the enamel matrix proteins might have caused these changes. Faraoni-Romano [22] showed no alteration of bovine enamel concerning microhardness and surface roughness after bleaching with low (10% CP) and relative high (38% HP) concentrated bleaching agents. In addition, tooth bleaching over a period of 21 days with 10% CP and 7.5% HP demonstrated no significant changes in micromorphology or microhardness of the enamel after the treatment [23]. However, an increase in microhardness of the enamel was measured after storing the specimens for 14 days in artificial saliva after the end of the treatment.

This controversy may be due to the different experimental protocols used to test bleaching materials such as differences in the commercial components of the tested products, the pH of these materials, as well as the duration of bleaching.

Therefore, the aim of this study was to investigate (1) the effect of low versus relatively high concentrations of bleaching agents on micromorphology and on microhardness of the enamel surface using the laser profilometer, scanning electron microscope, and Knoop hardness tester and (2) the influence of bleaching with CP compared to HP.

Materials and methods

Central upper bovine incisors were stored in 0.1% thymol solution at room temperature before use. Teeth with any visible cracks or hypoplastic defects were excluded. Enamel samples were prepared from buccal surfaces of the teeth using a diamond bur (Ø=5 mm). They were embedded in acrylic resin blocks (Technovit, Heraeus Kulzer, Wehrheim, Germany), finished with sand papers (1200, 2400, 4000, Sic-paper, Struers, Denmark) and polishing paste 0.1 µm (AP-D Suspension, Struers, Denmark). The enamel surfaces were examined with a light microscope (magnification ×50, Axioskop 2 MAT, Carl Zeiss, Göttingen, Germany) for any defect on the surface after finishing. Samples were randomly divided into five groups ($n=10$). Four experimental bleaching gels were provided by the same manufacturer (Ultradent products, Inc. South Jordan, UT, USA). The gels differed only concerning their content of CP or HP, respectively. Apart from this, all gels contained the same components according to the manufacturer's information: approximately 20% water, less than 3% of carbomer, sodium hydroxide, stabilizers, and buffers. Glycerin was used to complete the volume up to 100%. Specimens from the four experimental groups were treated with 10% CP (Batch No BZ 008A), 35% CP (Batch No BZ 008B), 3.6% HP (Batch No BZ 008D), and 10% HP (Batch No BZ 008C), respectively. The fifth group was used as a negative control (without bleaching). PH values were measured with a digital pH meter (inoLab pH 720, WTW, Weilheim, Germany) after calibrating with two standard buffers at 25°C (pH=7 and pH=4.01; Technical Buffer, WTW, Weilheim, Germany). The enamel specimens were bleached according to the same protocol with the respective bleaching material for every experimental group: 2 h every 2 days, over a period of 2 weeks. According to the manufacturer, the delivered gels were ready for direct application and no extra activation was necessary. Of the gel, 0.1 ml was applied with a graduated syringe and stirred every 10 min ensuring continuous contact of fresh material with the tested surface. The procedure was repeated three times within 2 h. Samples were washed with water for 10 s followed by drying with compressed air for 5 s before application of fresh material. During the experiment, all of the samples were stored in artificial saliva over the course of the experiment. The composition of the saliva was 0.015 g glucose, 0.290 g NaCl, 0.085 g $CaCl_2$, 0.17 g Na_2HPO_4, 0.08 g NH_4Cl, 0.635 g KCl, 0.080 g NaSCN, 0.165 g KH_2PO_4, and 0.1 g urea in 500 ml aqua bidest [24] at room temperature. A dynamically focusing optical profilometer (Mikrofocus, UBM, Type 2010, UBM, Karlsruhe, Germany) with a laser diode and UBSOFT software (UBM Meßtechnik, version Nr. 1.909) were used for measuring the surface roughness at baseline and after the bleaching procedures. This was performed at three different areas (0.7×0.7 mm) for every sample. The measurements were obtained by scanning the surface with a pixel density of 1000 points/mm. Three micro-roughness parameters were used for the description of the recorded profiles: (1) average roughness (Ra, DIN 4287) which reflects the average condition of the roughness profile. Ra is the arithmetic mean of the absolute values of all roughness profile deviations of the centerline within the measured surface. (2) Maximal roughness depth (Rt-Ry, DIN 4762/1E), which is the vertical distance between the highest and lowest point within the scanned area. (3) Roughness average in Z dimension (Rz, DIN 4768/1), which is the arithmetic mean of five depth values in the scanned area.

Additionally, scanning electron microscopy (LEO 435 VP, LEO Elektronenmikroskopie GmbH, Oberkochen, Germany) was used in the study for observation of enamel surface morphology before and after bleaching. One sample from each group was chosen randomly. A Knoop microhardness tester (Leitz Miniload, Ernst Leitz GmbH, Wetzlar, Germany) was used to test the superficial microhardness of the samples with a load of 1.030 mN (loading time, 30 s). Two microhardness measurements were performed on each sample at baseline and after the bleaching procedure. Roughness and microhardness results were statistically analyzed. An analysis of covariance was used. The response variable was the change which was calculated by subtracting the values after bleaching from the baseline values before bleaching (Δ value). The group effect was calculated with controlling for the baseline values as a covariate. Least square means with 95% CI were calculated for each group. Adjustment for multiple comparisons was done by the method of 'Dunnett-Hsu'.

Results

The four experimental bleaching gels had a comparable pH between 6.47 and 6.96 at room temperature. Results of roughness measurements are summarized in Table 1. The data of measured roughness parameters showed a normal distribution. All of the tested parameters were significantly different between the five groups before bleaching (p=0.0001). Therefore, differences in the roughness values after bleaching and baseline values were calculated (ΔRa, ΔRz, ΔRt-Ry). Statistical analysis demonstrated that bleaching procedures in this study had significant effects on Ra (p=0.0002), Rz (p=0.0001), and Rt-Ry (p=0.002). The 10% HP group showed significantly higher roughness after bleaching compared to the control (Ra, p=0.01; Rz, p=0.04). The 35% CP group showed only a tendency for higher Ra values (ΔRa, p=0.056), and no change concerning Rz and Rt-Ry values. However, bleaching with 10% CP resulted in significantly lower Rt-Ry (−70 nm; p=0.05), and Rz (−50 nm; p=0.03). No significant change in the three tested roughness parameters was observed after application of 3.6% HP. Comparison of 3.6% HP vs. 10% CP, and 10% HP vs. 35% CP demonstrated no statistically significant differences concerning ΔRa and ΔRz values. However, ΔRt-Ry showed a significant difference between 3.6% HP and 10% CP (p=0.03). ΔRt-Ry was lower in the 10% CP group (p=0.05). According to the bleaching procedures in this study, enamel microhardness was significantly higher after bleaching, with 10% HP (ΔMic=20 KHN, p=0.0002) and 35% CP (ΔMic=8 KHN, p=0.01) compared to the control. After application of 10% CP or 3.6% HP, no significant change of enamel microhardness was observed (Table 2).

A laser profilometer was used to show the 3D changes in enamel surface in this study (Fig. 1a-e). Scanning electron microscopy (SEM) can provide a 2D view of the enamel surface (Fig. 2a-e). The enamel surface of the control samples appeared smooth in general, with some scattered clear scrapes due to the polishing procedure (Fig.2a). Bleaching with 10% HP resulted in the most pronounced changes in surface morphology compared to the other experimental specimens and the control specimens. The enamel surface had intermittent depressions of various diameters and depths (Fig.2e). After treatment with 3.6% HP, 10% CP, respectively, the enamel surface showed only minimal surface changes compared to the control (Fig.2b, Fig.2d). However, some of the random depression spots became noticeable after application of 3.6% HP.

Table 1 Δ values demonstrate the increase (positive values) or decrease (negative values) of the three tested roughness parameters (Ra, Rz, Rt-R-y) after bleaching with carbamide peroxide (CP) or hydrogen peroxide (HP)

Bleaching gels	ΔRa (μm)	ΔRz(μm)	ΔRt-Ry(μm)
35% CP	0.014	0.12	0.12
10% CP	−0.003	−0.05 (p=0.02)	−0.07 (p=0.05)
10% HP	0.017 (p=0.01)	0.17 (p=0.04)	0.18
3.6% HP	0.002	0.02	0.04

P values of the significant changes are mentioned

Discussion

Bleaching materials come into direct contact with the tooth surface for a rather long time depending on the technique used. Therefore, it is important to know the effect of bleaching on the tooth structure. In spite of the widespread use of a number of different systems for tooth bleaching, there are still controversial results about the effect of bleaching agents on the structure of dental hard tissues. In this study, the experimental set-up was standardized in order to make sure that any change on enamel surface was due to the active ingredients HP or CP, respectively. HP is used in dentistry as a whitening material at various

Table 2 Δ Mic demonstrate the increase (positive values) or decrease (negative values) of the enamel microhardness (Mic) after bleaching with carbamide peroxide (CP) or hydrogen peroxide (HP)

Bleaching gels	35% CP	10% CP	10% HP	3.6% HP
ΔMic (KHN)	8.4 (p=0.01)	−3.4	20 (p=0.0002)	−7.5

P values of the significant changes are mentioned

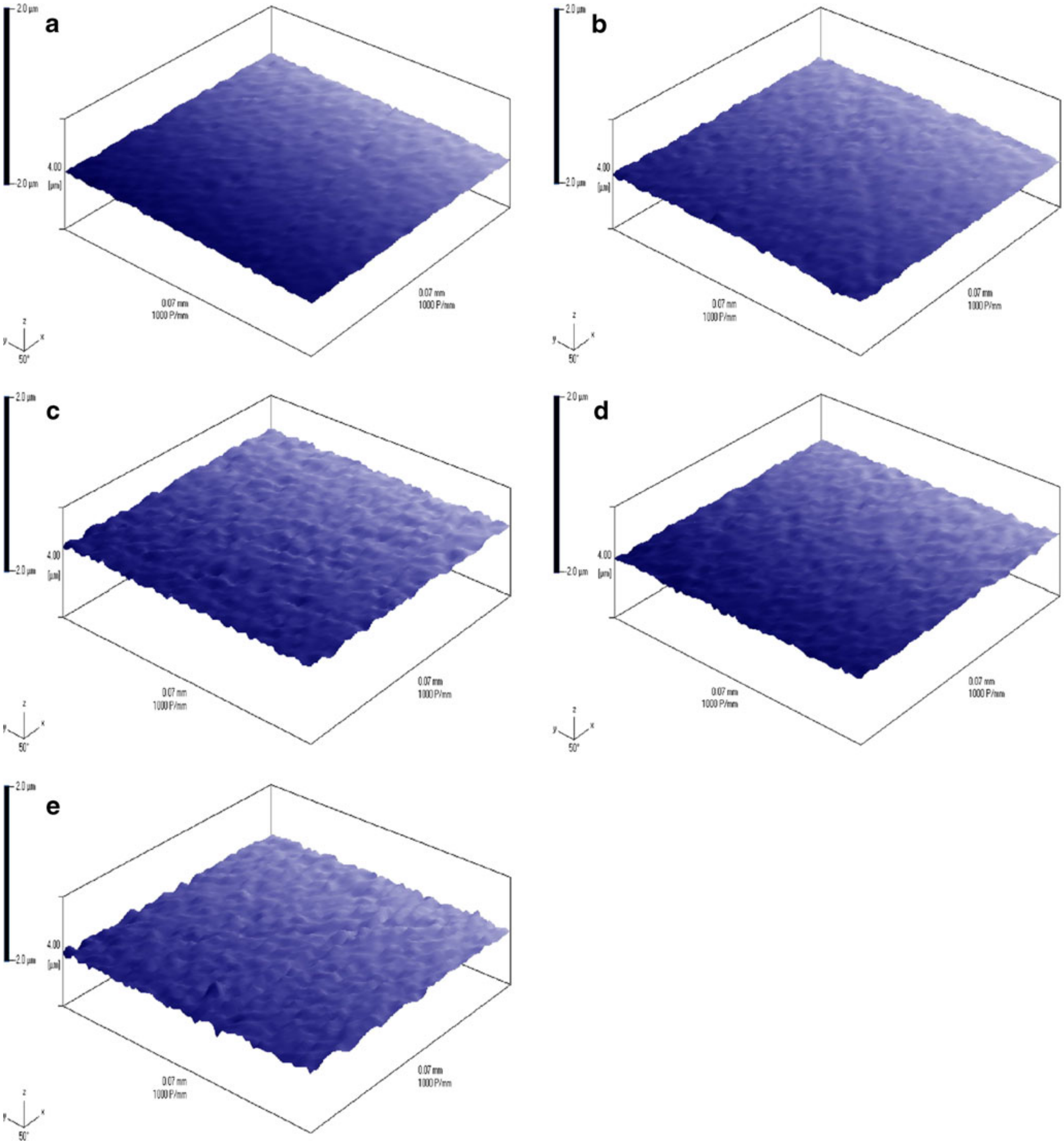

Fig. 1 **a** 3D view of enamel surface morphology (0.7×0.7 mm) before bleaching scanned with a laser profilometer with 1000 points/mm as a pixel density. The figure shows the enamel surface morphology after polishing procedures. The acceptable surface roughness was within ±0.03 µm. **b** 3D view of enamel surface morphology (0.7×0.7 mm) after bleaching with 10% CP scanned with a laser profilometer with 1000 points/mm as pixel density. Qualitatively the figure shows no distinguishable changes in surface morphology compared to the control group. **c** 3D view of enamel surface morphology (0.7×0.7 mm) after bleaching with 35% CP scanned with a laser profilometer with 1000 points/mm as pixel density. Qualitatively the figure shows a minimal increase in surface roughness compared to the control group. **d** 3D view of enamel surface morphology (0.7×0.7 mm) after bleaching with 3.6% HP, scanned with a laser profilometer with 1000 points/mm as pixel density. Qualitatively the figure shows no distinguishable changes in surface morphology compared to the control group. **e** 3D view of enamel surface morphology (0.7×0.7 mm) after bleaching with 10% HP, scanned with a laser profilometer with 1000 points/mm as pixel density. Qualitatively the figure shows an increase in surface roughness compared to the control group

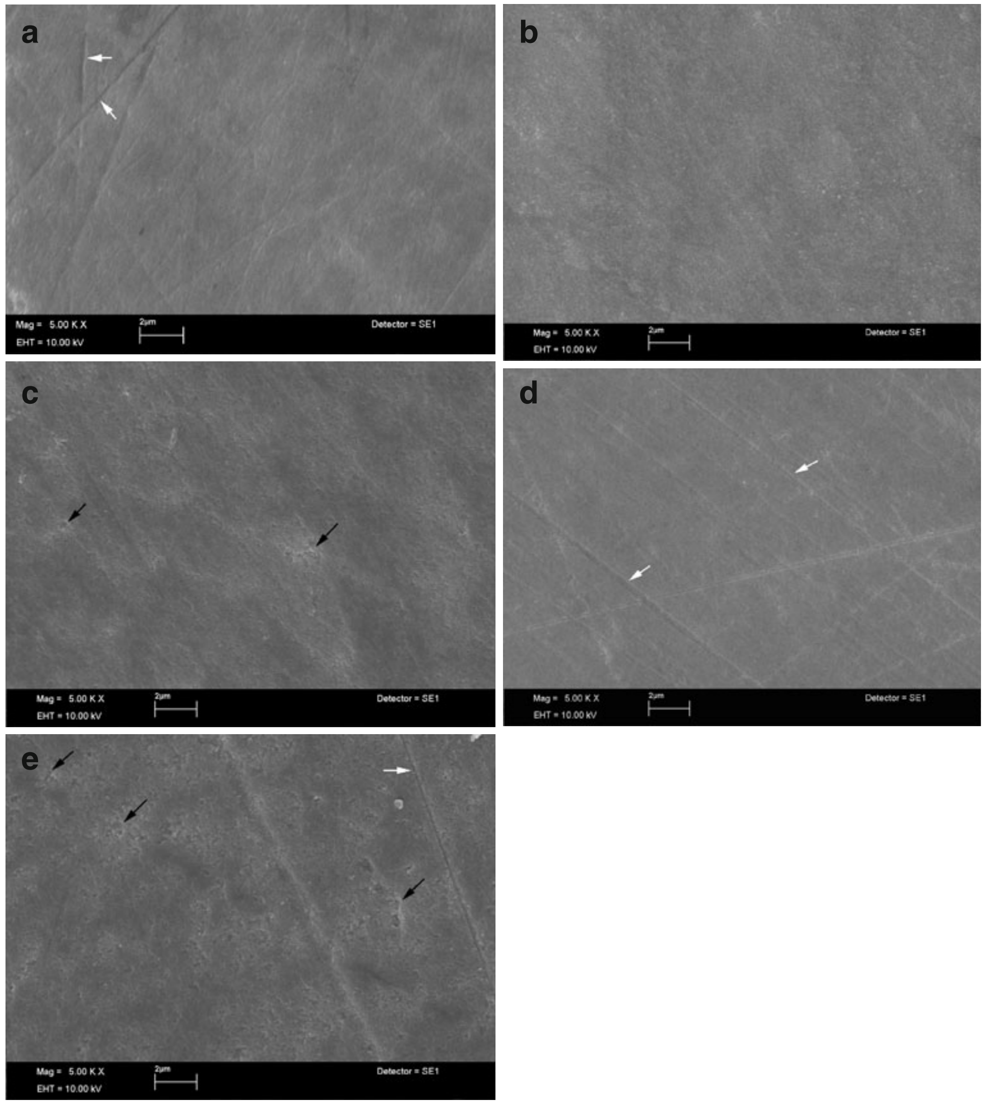

Fig. 2 **a** 2D view of enamel surface before bleaching, scanned with SEM (magnification ×5,000). The figure shows scrapes due to polishing procedures (*white arrows*) within smooth enamel surface. **b** 2D view of enamel surface after bleaching with 10% CP, scanned with SEM (magnification ×5,000). No surface defects were observed after bleaching. **c** 2D view of enamel surface after bleaching with 35% CP, scanned with SEM (magnification ×5,000). Some scattered defects were observed after bleaching (*black arrows*). **d** 2D view of enamel surface after bleaching with 3.6% HP and scanned with SEM (magnification ×5,000). The figure shows scattered clear scraps due to polishing procedures (*white arrows*) within smooth enamel surface. **e** 2D view of enamel surface after bleaching with 10% HP scanned with SEM (magnification ×5,000). Many defects were observed after bleaching (*black arrows*)

concentrations [25]. The chemical reaction of 10% and 35% CP releases about 3.6% and 10% HP, respectively. Therefore, CP or HP was used with the corresponding concentrations in the present study in order to compare their effect on the enamel surface. The time required for bleaching depends on the bleaching technique and the concentration of the materials used. In the home-bleaching technique, 10% CP is utilized for up to 6 h per day over a period of 6 weeks [26]; whereas in the office, the application of 35% CP is suggested as an initial treatment in order to accelerate the bleaching results [27]. In this study, all of the experimental groups tested were submitted to the same time protocol in order to avoid any effect of bleaching time on the surface roughness between the groups. Bleaching materials can cause erosive effects on enamel according to their low pH [20], and some studies demonstrated that even tooth brushing during the bleaching procedure could significantly increase the roughness of the enamel surface [28, 29]. In order to avoid any erosive effect, all of the tested materials had neutral pH values in the present study [30], and no brushing of samples was applied. Additionally, a non-contact optical profilometer was used, which is described as being an effective method for 3D analysis of the surface morphology of the tooth surface [31]. The laser light sensor has advantages compared to mechanical profilometers which use a contact needle and risk causing mechanical defects on the measured surface. Kocher et al. [31] reported that Ra value is highly dependent upon the size of the field to be scanned, increasing linearly with the measured area. Therefore, all areas measured in the current study were investigated according to the same protocol with respect to field size and pixel density. Ra is the most frequently reported parameter to measure surface roughness within dental studies. The additional roughness parameters allow further description of surface quality [32]. Therefore, in addition to Ra, Rz, and Rt-Ry were estimated in the present study. The results from laser profilometry demonstrated that Ra and Rz were significantly higher compared to the control after application of 10% HP, while application of 35% CP had only a tendency to increase roughness (ΔRa, $p=0.056$). The SEM pictures supported these results, showing a qualitative increase of roughness. In contrast to our results, Sulieman et al. [20] reported no changes to the enamel surface after applying 35% HP using a profilometer. They used coarse paper for finishing the surface (800 grit), and their mean surface roughness was within ±0.3 µm at baseline. In our study, enamel surfaces were polished with ultra-fine wet grinding paper and polishing diamond paste. The baseline surface roughness was within ±0.03 µm, and therefore, very small changes could be detected. The application of lower concentrations of bleaching agents (10% CP or 3.6% HP) revealed no significant increase concerning roughness parameters when compared to the baseline. The enamel surface was even smoother after bleaching with 10% CP. Rt-Ry and Rz were significantly lower compared to the baseline.

Jiang et al. [33] stated that HP is able to penetrate enamel structure and suggested that the organic material in enamel could be affected by the oxidation reaction of HP. The spots with morphological changes that were observed on the enamel surface after bleaching probably were caused by dissolution of the organic portion of the low mineralized spots on the surface and between enamel prisms. Oltu et al. [34] found similar results observing alterations on the enamel surfaces produced by 35% CP using infrared absorption spectroscopy and X-ray diffraction analysis.

Urea is also capable of penetrating into the inter-prismatic region of enamel [35]. Decomposition of CP produces urea that decomposes to carbon dioxide and ammonia. Thus, it might raise the pH value of bleaching materials and reduce any adverse effects due to its alkaline property [36]. The present measurements resulted in less change in the roughness of the enamel surface after CP bleaching compared to HP bleaching with regard to Rz values as well as qualitative analysis with SEM. These results suggest that using 10% HP through chemical reaction from CP might be safer than direct application of 10% HP on enamel. However, the clinical relevance of this difference is probably low.

It has been suggested that the chemical reaction during the bleaching process does not adversely attack the inorganic tissues [37]. This assumption is supported by the fact that microhardness did not decrease but actually increased in the present study. Hardness is generally associated with a salivary remineralization process, and remineralization potential exists in saliva substitutes that contain calcium and phosphate [38], as in this study. Therefore, the artificial saliva used as a storing solution is able to repair possible demineralization that can take place during the bleaching procedure [39]. In comparison to the control group, a significantly higher value of enamel microhardness was observed after bleaching with 10% HP and 35% CP. According to the conditioning effect of weak acids (bleaching materials) the enamel surface is possibly activated, comparable to the conditioning of enamel during adhesive procedures. It has been speculated that this might result in a better remineralization reaction during storage in artificial saliva.

Additionally, the dissolution of the organic phase of enamel by the peroxide-containing bleaching agent [37] may contribute to the increase of microhardness. The resulting defects between the enamel prisms may be repaired by remineralization in the storing solution. These considerations are corroborated by the findings of Mahringer et al. [21]. They showed that the main morphological change which was due to the bleaching treatment occurred in the first hour of bleaching and no significant change was observed after the next 6 h of bleaching treatment. This bleaching effect on the enamel surface probably concerned mainly the organic part of the enamel. As the samples were stored in artificial saliva after every bleaching treatment cycle, calcium-phosphate precipitation occurred inside the porous enamel, a phenomenon that leads eventually to a re-hardening of the enamel surface. Hence, a remineralization supposed to compensate the surface changes of the enamel surface occurs [21]. Borges et al. [10] investigated the potential protective effect of calcium within 35% HP bleaching gel. They found that the bleaching group with the calcium-added agent exhibited an increase in enamel microhardness after 30 min of bleaching. In the present study, the storing solution was the source of calcium as a remineralizing agent.

It must be emphasized that this study was performed in vitro and that other important factors exist in the oral cavity. Antioxidant enzymes and organic component of the neutral saliva may affect the present results.

Conclusion

Within the limitations of this study, the relationship between chemical form, concentration of bleaching materials, and enamel surface alterations could be elucidated.

Minimal morphological changes were registered after bleaching with high concentrations of bleaching agents. Surfaces treated with carbamide peroxide showed somewhat less alterations compared to hydrogen peroxide. The results of the microhardness test showed that there was no decrease concerning enamel microhardness in any of the bleaching groups. The main effect of bleaching on the morphology of enamel surface is thought to relate to the oxidation and subsequent partial lysis of organic material within the enamel. However, the differences between CP and HP, and their effects on the enamel surface are probably clinically irrelevant. These findings resulted in the conclusion that bleaching procedures are minimally invasive treatments that are safe for the enamel surface.

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

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