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Enamel and Dentin Microhardness and Chemical Composition After Experimental Light-activated Bleaching

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Clinical Relevance

With regard to enamel and dentin microhardness and chemical composition, bleaching lights can be safely used. The adverse effects of peroxide concentration and gel acidity could be reversed after a two-week amorphous calcium phosphate and artificial saliva treatment.

SUMMARY

Objectives: To evaluate 1) the influence of five bleaching agents (with additional light activation) on enamel and dentin surface microhardness and chemical composition and 2) the remineralizing potential of artificial saliva and amorphous calcium phosphate (ACP).

Methods and Materials: The study was conducted on 125 human third molars dissected into quarters for separate enamel and dentin measurements. The bleaching process was performed with 38% and 25% hydrogen peroxide (HP) and 30%, 16%, and 10% carbamide peroxide (CP) gels two times for 15 minutes each time. All bleaching gels were tested alone and in combination with ZOOM2, light-emitting diode (LED), organic LED, and femtosecond laser. A total of 25 bleaching combinations (n=10) were evaluated. Microhardness was measured by a Vickers diamond. Chemical analysis was performed using energy-dispersive X-ray spectroscopy.

Results: Bleaching agents used in the absence of light activation caused a significant reduction in enamel and dentin surface microhardness ($p<0.001$), ranging from 8% for 16% CP to 40% for 25% HP. The effects of different light activations were negligible. After two-week treatment with ACP and artificial saliva, maximum deviation from baseline microhardness

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was just 3%. Such treatment increased the concentrations of calcium, phosphorus, and fluorine.

Conclusions: An increase in peroxide concentration and gel acidity negatively affected microhardness and concentrations of calcium and phosphorus in enamel and dentin. ACP and artificial saliva stimulated the remineralization of hard tissues.

INTRODUCTION

Tooth bleaching is considered the easiest and most cost-effective procedure for treating tooth discoloration. To date, studies on the effects of peroxide- and carbamide-based products on dental ultrastructure remain inconclusive. While some authors have reported no adverse effects, others have claimed that the use of such products can be associated with many side effects, which include enamel and dentin surface alterations such as reduction in surface or subsurface microhardness, reduction in calcium and phosphate ratios with loss of organic components from treated tooth surfaces,[1-3] or tooth sensitivity, which could be reduced with remineralizing agents.[4,5] Bleaching agents have an effect on the chemical and morphological structure of hard dental tissues, while hydrogen peroxide (HP) has the ability to produce highly reactive peroxide and superoxide ions. The main reaction of the bleaching process is oxidation. Reduction of microhardness or changes in the chemical structure are primarily the result of the oxidation process in the enamel and dentin organic and inorganic substances.[6] However, it is highly probable that the low pH of the bleaching agents can also lead to chemical and structural changes in dentin, which was demonstrated for internal dental bleaching.[7] The postoperative sensitivity is usually related to the small microscopic enamel defects and subsurface pores and the ability of bleaching agents, typically hydrogen peroxide, to penetrate into the pulp and cause an irritating effect in the pulp cells. This can sometimes lead to mild reversible pulpitis, which can be manifested as tooth hypersensitivity and intermittent spontaneous pain.[8]In the postbleaching period, the presence of saliva, fluorides, or other remineralizing solutions, such as amorphous calcium phosphate (ACP), may provide a balance between the remineralization and demineralization processes.[9] ACP is a direct precursor of biologic apatite in the biomineralization process. It can release calcium and phosphate ions and maintain a supersaturated mineral environment, which can in turn reduce demineralization and improve remineralization of dental hard tissues.[10]

For acceleration and more effective bleaching, different light sources may be used, such as a quartz-tungsten-halogen lamp, plasma lamps, light-emitting diode (LED), and halogen or laser lights.[11] In this study, bleaching was activated by a ZOOM2 (Discus Dental, Culver City, CA, USA) light source and the following experimental light sources: LED (LED Engin Inc, San Jose, CA, USA) with a center wavelength of 405 nm (LED405), white organic LED (OLED) (PPML, Pescara, Italy), and femtosecond laser (Millenia, Spectra-Physics, Santa Clara, CA, USA). OLED was never tested as a light source for tooth bleaching, but it is often used for widespread illumination.[12] Its organic semiconductor is situated between two electrodes. The organic films consist of a hole-injection layer, a hole-transport layer, an emissive layer, and an electron-transport layer. When voltage is applied to the OLED cell, the injected positive and negative charges recombine in the emissive layer and create electroluminescent light. The experimental setup of the femtosecond laser used in this study consisted of a green pump laser at 532 nm (Millenia, Spectra-Physics, Santa Clara, CA, USA) and a Tsunami oscillator (Ti:sapphire laser, Spectra-Physics, Santa Clara, CA, USA) which generated femtosecond pulses. The function of the pump laser was to stimulate the crystal Ti:sapphire in the oscillator, which in turn generated femtosecond pulses of a wavelength in the range of 700-950 nm. In this study, we used the central wavelength of approximately 770 nm. Moreover, light-accelerated bleaching may lead to considerable heat production,[13] which can cause pulp irritation or even necrosis.

Surface and chemical changes after the bleaching treatment may be evaluated by microhardness tests, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS).[2,3] Surface microhardness measurement is used to determine the mechanical properties of dental hard tissues.[14] SEM and EDS are typically used to characterize the chemical composition after bleaching.

The purposes of this *in vitro* study were to evaluate the effects of peroxide concentration and acidity of bleaching agents and the effects of experimental light sources on the tooth surface and to assess the remineralizing potential of artificial saliva and ACP. The research hypotheses (H) were as follows: H1: Bleaching agents of different concentrations and acidities lead to a significantly different

Table 1: *Bleaching Agents Evaluated in the Study*

Product	Manufacturer	LOT Number	Active Bleaching Agent	Percent
ZOOM2	Discus Dental, Culver City, CA, USA	12 311007	Hydrogen peroxide	25
Boost	Ultradent, South Jordan, UT, USA	B66KD	Hydrogen peroxide	38
Viva Style 30	Ivoclar Vivadent, Schaan, Liechtenstein	B8HW7	Carbamide peroxide	30
Viva Style 16	Ivoclar Vivadent, Schaan, Liechtenstein	RL3318	Carbamide peroxide	16
Viva Style 10	Ivoclar Vivadent, Schaan, Liechtenstein	ML3364	Carbamide peroxide	10

decrease in enamel and dentin surface microhardness. H2: Bleaching agents of different concentrations and acidities have different demineralization effects on enamel and dentin. H3: Additional light activation leads to a significant decrease in enamel and dentin surface microhardness. H4: Additional light activation leads to further demineralization of enamel and dentin. H5: Posttreatment with ACP and artificial saliva restores surface microhardness and stimulates remineralization of enamel and dentin.

METHODS AND MATERIALS

Specimen Preparation

A total of 125 freshly extracted intact human third molars were cleaned and stored in 1% chloramine solution. The root portions of the teeth were sectioned with a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) approximately 2 mm below the cementoenamel junction and stored in deionized water. Tooth crowns were dissected into quarters and embedded in acrylic resin (AcryFix Kit; Struers, Balerrup, Denmark). For enamel measurements, specimen vestibular surfaces were polished using water-cooled carborundum discs (Water Proof Silicon Carbide Paper, 4000 grit; Buehler, Dusseldorf, Germany) and 1.0-μm, 0.3-μm, and 0.05-μm micropolish powder (Buehler) to expose a standardized area of 3 × 3 mm. After the crowns were dissected, lingual parts of the crowns with inner exposed dentin were used for dentin measurements. Dentin samples were polished in the same way as enamel samples.

Bleaching Procedure

The tooth surface was bleached with either 25% HP, 38% HP, 10% carbamide peroxide (CP), 16% CP, or 30% CP gel two times for 15 minutes each time (Table 1). Bleaching gel was applied in a 2-mm-thick layer and removed after 15 minutes using a Heidemann spatula. The tooth surface was rinsed with deionized water and dried with compressed air and cotton tissues before applying another layer of bleaching gel to the surface. Bleaching was performed in two modes: with bleaching gel alone (ie, without the light activation) and with bleaching gel activated by one of the following light sources: ZOOM2 light source, LED405, OLED, and femtosecond laser (Table 2).

A total of 500 specimens were divided into two main (enamel/dentin) groups and 25 subgroups (ie, treatment groups) defined by 25 different bleaching combinations, differing in bleaching gel and/or light source treatments (Table 3). The specimens were randomly assigned to each treatment group (n=10) separately for enamel and dentin measurements. Before randomization, they were aligned and appointed a unique identification number. Simple randomization of 250 specimens into 25 treatment groups was done in the procedure PROC PLAN of the SAS System software (SAS Institute Inc, Cary, North Carolina, USA). During the bleaching, each specimen was placed on a cotton pellet soaked in artificial saliva. Afterwards, enamel and dentin surfaces were cleaned and dried. ACP gel (ACP Relief, Discus Dental, Culver City, CA, USA) was applied to the surface of half of the specimens for 20 minutes every day for 14 days. After each ACP gel application, teeth

Table 2: *Light Sources Evaluated in the Study*

Product	Manufacturer	Type of Light	Power, mW/cm^2
ZOOM2	Discus Dental, Culver City, CA, USA	Mercury metal halide light λ 350-400 nm	2.000
LED	LED Engin Inc, San Jose, CA, USA	LED λ 405 nm	400
PPML OLED KIT engineering prototype	PPML, Pescara, Italy	OLED λ 400-760 nm	200
Millenia	Spectra-Physics, Santa Clara, CA, USA	Femtosecond laser λ 770 nm	800

Abbreviations: LED, light-emitting diode; OLED, white organic light-emitting diode.

Table 3: *Treatment Group Assignments with Corresponding Group Labels*

Bleaching Gel/Light Source Tested	Light Source (Application Time: 2 × 15 min)				
	None (Control)	ZOOM2	LED405	OLED	Femtosecond Laser
Bleaching gel (application time: 2x15 minutes)					
25% HP	25HPnone	25HPZOOM	25HPLED	25HPOLED	25HPfemt
38% HP	38HPnone	38HPZOOM	38HPLED	38HPOLED	38HPfemt
10% CP	10CPnone	10CPZOOM	10CPLED	10CPOLED	10CPfemt
16% CP	16CPnone	16CPZOOM	16CPLED	16CPOLED	16CPfemt
30% CP	30CPnone	30CPZOOM	30CPLED	30CPOLED	30CPfemt

Abbreviations: CP, carbamide peroxide; HP, hydrogen peroxide.

were soaked in artificial saliva (to protect the specimens from dehydration) and stored at 37°C in order to simulate intraoral conditions (Cultura Incubator, Ivoclar Vivadent, Schaan, Liechtenstein). The artificial saliva was replaced daily. The other half of the specimens were kept in deionized water for 14 days, which was also replaced daily.

The pH of the bleaching gel was measured using a pH/Ion meter (Pinnacle 555 pH/Ion meter, Corning, Tewksbury, MA, USA), which was initially calibrated. Each bleaching gel was placed in 30-mL graduated plastic cups. The pH electrode was immersed inside the gel to allow uniform contact with the electrode tip. The bleaching gel was in contact with the pH electrode for 20 minutes at room temperature (24°C). The electrode was thoroughly washed between samples. ZOOM2 gel had a pH of 3.20 and BOOST gel had a pH of 6.75, while all VivaStyle gels had a pH of 7.00.

For this experiment 500 mL of Glandosane spray (Fresenius KABI, Cheshire, UK) was used. Since its pH value is 5.23 (Pinnacle 555 pH/Ion meter, Corning), it was mixed with 67.3 g of 1% NaOH solution (Kemika, Zagreb, Croatia) using the magnetic stirrer with a hot plate (Cole Parmer, East Bunker Court, Vernon Hills, USA) to obtain a neutral pH of 7.0.

Microhardness and EDS Measurements

Microhardness was determined using a Vickers diamond (Leitz Miniload2 Microhardness Tester, Leitz, Germany) at a load of 100*g* applied for 10 seconds. The Vickers hardness indentations were performed on the central area of each specimen, with a distance of 100 μm between them, and were averaged.

Three specimens in each group were randomly selected and analyzed using EDS (JSM 7000F, JEOL, Japan). The specimens were dried and fixed on aluminum stubs. Elemental analysis and precise chemical characterization of a sample were performed at 10,000 keV.

Measurements were performed just before and immediately after the bleaching and after two-week storage in artificial saliva and ACP or deionized water.

Statistical Analysis

Analysis was performed separately for enamel and dentin. Descriptive statistics were presented graphically in the form of medians and interquartile ranges (IQs). Microhardness data were positively skewed and were thus log-transformed prior to further analysis. A mixed effects analysis of variance model was applied. Random effect parameters represented general variability among specimens due to the heterogeneous characteristics of teeth related to their color, composition, size, and shape. Different treatment levels were specified as fixed effects. Covariance structure among repeated measurements on the same specimen was further modeled by selecting appropriate residual covariance structure based on minimizing the information criterion (AIC). A normal probability plot indicated that model residuals were approximately normally distributed.

Log-transformed data were transformed back to the original scale for reporting, so differences between treatment groups were expressed as ratios of geometric means, interpreted as a percent change in surface microhardness. The significance level was set at 0.05. *p*-values were adjusted for multiple comparisons according to the Bonferroni-Holm method. Analysis was performed in SAS 8.2 (SAS Institute Inc, Cary, North Carolina, USA).

RESULTS

Microhardness Analysis

Median baseline enamel microhardness was 37.81 (IQ range: 37.01-38.92), and dentin microhardness

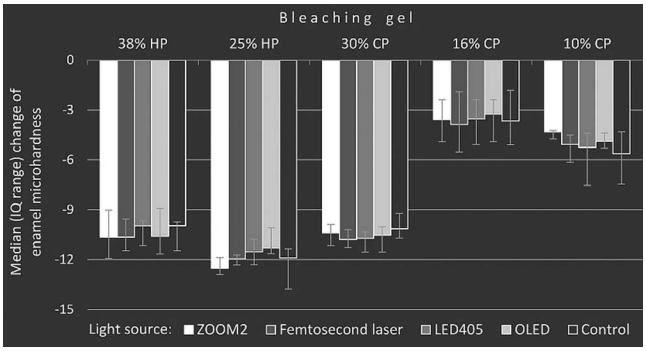

Figure 1. *Effects of different bleaching treatments on enamel surface microhardness.*

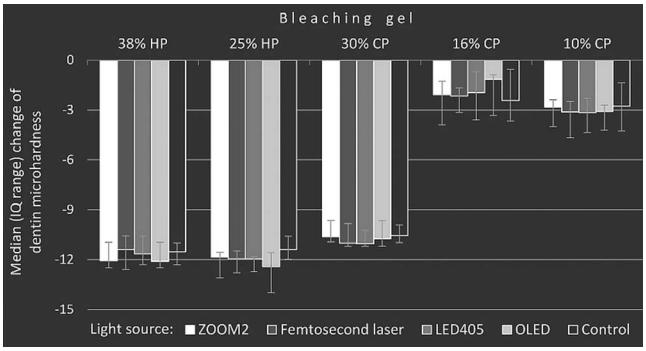

Figure 2. *Effects of different bleaching treatments on dentin surface microhardness.*

measured 29.70 (IQ range: 28.55-30.29). As suggested in Figures 1 and 2, the type of light source or light activation itself was not a significant factor for microhardness change during bleaching. The effects of 25HPZOOM, 25HPLED, 25HPOLED, and 25HPfemt were on average similar (not significantly different, $p>0.05$) to those of the 25HPnone treatment group. The same was observed for comparisons of 38HPZOOM, 38HPLED, 38HPOLED, and 38HPfemt with 38HPnone, as well as for comparisons of 10CPZOOM, 10CPLED, 10CPOLED, and 10CPfemt with 10CPnone; for comparisons of 16CPZOOM, 16CPLED, 16CPOLED, and 16CPfemt with 16CPnone; and for comparisons of 30CPZOOM, 30CPLED, 30CPOLED, and 30CPfemt with 30CPnone. Therefore, the analysis focused on the effects of different bleaching gels, which are described in more detail below.

A significant microhardness decrease was observed during applications of all bleaching gel types (Table 4; $p<0.001$ in all bleaching gel groups—25HPnone, 38HPnone, 10CPnone, 16CPnone, and 30CPnone). Enamel surface microhardness was most severely affected during bleaching with 25% HP, which on average caused a 31% decrease in enamel microhardness (corresponding to a geometric mean ratio of 0.69). Treatment with 30% CP and 38% HP resulted in 28% and 27% decreases, respectively. On the other hand, adverse effects of 16% CP and 10% CP were substantially smaller, with an average decrease of 9% and 15%, respectively. A similar pattern was observed with dentin. Bleaching treatment with 25% HP had the largest detrimental effect, demonstrating an average 40% decrease in dentin surface microhardness. Somewhat smaller decreases of 38% and 36% were observed during bleaching with 38% HP and 30% CP, respectively. Again, bleaching with 16% CP and 10% CP was less detrimental, resulting in 8% and 11% average decreases in dentin microhardness, respectively.

The restoration of surface microhardness was not stimulated by two-week storage in deionized water (Table 4). Microhardness remained at a similar level, on average deviating less than 6% and 4% from the enamel and dentin microhardness values recorded immediately after the bleaching process. However, using artificial saliva and ACP as a postbleaching treatment medium restored the enamel and dentin microhardness. After two-week storage in this medium, enamel and dentin microhardness values were significantly higher ($p<0.001$ in all bleaching gel groups—25HPnone, 38HPnone, 10CPnone, 16CPnone, and 30CPnone) than immediately after the bleaching and approximately equal to the baseline values for all bleaching gels tested. Although differences between baseline microhardness values were still significant for some bleaching treatments, the maximum average drop from baseline values was just 3% in enamel and 1% in dentin.

EDS Analysis

Various structural chemical elements were detected in enamel and dentin—calcium (Ca), carbon (C), fluorine (F), magnesium (Mg), oxygen (O), phosphorus (P), and sodium (Na). Detected concentrations of Mg and Na were generally low (median values below 1.0). Summary statistics for concentrations of other elements are presented in Figures 3 and 4. Bleaching had an almost negligible effect on concentrations of C, O, and F, regardless of the bleaching gel applied. However, different effects of bleaching gels were apparent for concentrations of Ca and P. While concentrations of these elements remained at approximately the same level after bleaching with 10% CP and 16% CP, gels containing higher concentrations of peroxide—30% CP, 25% HP, and 38% HP—noticeably reduced the concentrations of Ca and P in

Table 4: *Microhardness Summary Statistics for Different Treatments of Enamel and Dentin*[a]

Bleaching Gel	Treatment			
	Baseline G. Mean (95% CI)	Bleaching G. Mean (95% CI)	Deionized Water G. Mean (95% CI)	Artificial Saliva + ACP G. Mean (95% CI)
Enamel				
38% HP	38.0 (37.73-38.28) A	27.7 (27.46-28.03) B	26.9 (26.55-27.21) C	37.5 (36.82-38.29) A
25% HP	38.1 (37.76-38.49) A	26.2 (26.03-26.43) B	26.4 (26.08-26.69) B	37.0 (36.57-37.42) C
30% CP	38.2 (37.93-38.51) A	27.7 (27.57-27.85) B	27.6 (27.03-28.09) B	38.1 (37.52-38.77) A
16% CP	37.9 (37.63-38.21) A	34.3 (33.91-34.79) B	34.5 (34.05-34.97) B	37.2 (36.74-37.71) C
10% CP	36.8 (36.50-37.15) A	31.4 (31.07-31.77) B	29.6 (29.15-29.97) C	35.6 (35.36-35.80) D
Dentin				
38% HP	30.9 (30.52-31.19) A	19.0 (18.89-19.19) B	18.9 (18.80-19.04) B	30.6 (30.26-30.94) A
25% HP	30.3 (30.01-30.58) A	18.2 (18.01-18.34) B	18.5 (18.24-18.67) C	29.9 (29.63-30.17) D
30% CP	29.2 (28.97-29.46) A	18.7 (18.56-18.88) B	19.1 (18.94-19.33) C	29.2 (28.74-29.56) A
16% CP	29.2 (28.97-29.39) A	26.9 (26.48-27.33) B	27.9 (27.60-28.28) C	29.3 (28.81-29.70) A
10% CP	28.2 (27.94-28.51) A	25.0 (24.79-25.25) B	24.1 (23.85-24.36) C	28.1 (27.65-28.43) A

Abbreviations: ACP, amorphous calcium phosphate; CI, confidence interval; CP, carbamide peroxide; HP, hydrogen peroxide.
[a] *Geometric means and 95% confidence intervals presented in the table; different letters in each row represent statistically significant differences among treatments.*

enamel and dentin. Performances within these two groups were similar, regardless of the specific gel applied. Therefore, only the effects of bleaching with 10% CP and 38% HP are presented in Figures 3 and 4.

Artificial saliva and ACP treatment clearly affected enamel and dentin concentrations of C, O, F, Ca, and P. After two-week storage in this medium, the concentration of C in enamel increased, and its concentration in dentin decreased. The same treatment increased the concentrations of Ca, F, and P and reduced the concentration of O in both enamel and dentin.

DISCUSSION

Light-activated bleaching enhances the decomposition of HP for improved bleaching results. When the bleaching agent is induced by light, some amount of its energy is absorbed, and the result is heat production, which can be observed as a possible side effect during this type of tooth bleaching. Light sources may have photothermal effects, which are then associated with the chemical effect of the bleaching materials.[11] Although the light sources used in this study can lead to temperature rise[13] or cause possible pulp damage or tooth hypersensitivity,[4] this study demonstrated that the tested light sources do not stimulate a reduction in enamel and dentin surface microhardness or a change in their chemical composition. Araujo and others[15] assessed microhardness change after application of 35% HP in combination with LED, halogen lamps, and an argon laser and concluded that the application of bleaching gel leads to a decrease in microhardness after 14 days, while the light activation does not affect this value. Gomes and others[16] demonstrated that the use of 35% HP gel alone leads to a

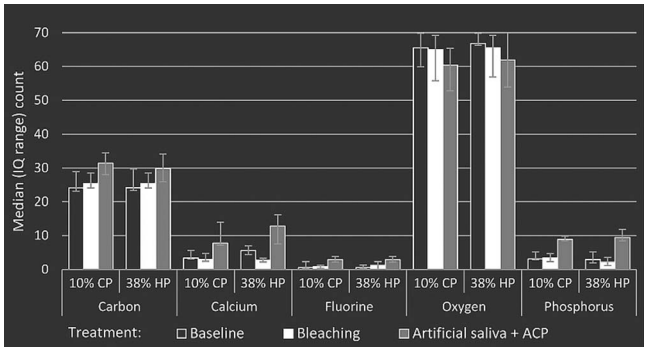

Figure 3. *Effects of bleaching (10% CP and 38% HP presented) and artificial saliva/ACP treatments on enamel concentrations of carbon, calcium, fluorine, oxygen, and phosphorus.*

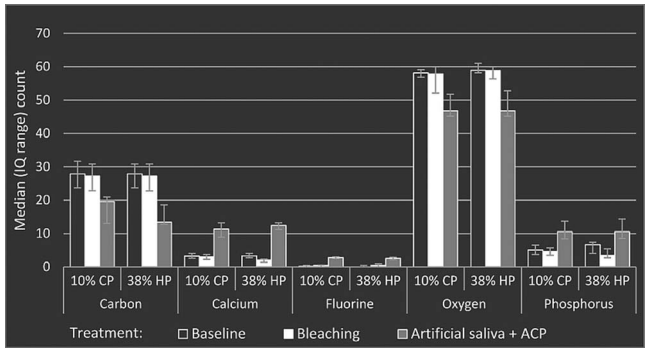

Figure 4. *Effects of bleaching (10% CP and 38% HP presented) and artificial saliva/ACP treatments on dentin concentrations of carbon, calcium, fluorine, oxygen, and phosphorus.*

microhardness reduction of 15%, while light activation by plasma arc and LED light source did not contribute significantly to a reduction of microhardness. Marcondes and others[17] have shown that HP gel combined with an LED light source or Nd:YAG laser causes no greater decrease in enamel surface microhardness, while Zhang and others[18] indicated that LED and diode laser alone do not affect enamel microhardness. Majeed and others[19] showed that 38% HP with and without light activation led to a similar reduction of dentin microhardness. The data for OLED and femtosecond laser are not available in the existing literature, but the general findings of this study are in line with the current research.

On the other hand, bleaching gels affect the chemical and morphological structure as a result of oxidation of the organic and inorganic substances in enamel and dentin.[20] This indicates that both enamel and dentin are permeable to reactive oxygen species released by decomposition of HP and CP. A number of studies[1,15,19,21] assessed the relationship between concentrations of HP or CP and the changes in enamel and dentin microhardness. In this study, HP and CP gels showed significant and detrimental effects on enamel and dentin surface microhardness. The bleaching treatments containing higher concentrations of peroxide—25% HP, 38% HP, and 30% CP—demonstrated a significantly greater reduction in enamel and dentin microhardness than did 10% CP and 16% CP. Furthermore, application of 25% HP gel, which had a lower pH value (pH=3.20) than 38% HP (pH=6.75) and all of the CP gels (pH=7.00), led to the largest decrease in surface microhardness. The acid pH measured for 25% HP was below the critical level for enamel, which is between 4.5 and 5.5 and can cause hard tissue demineralization. Demineralization could be attributed to the low concentrations of calcium and phosphate ions and high concentrations of sodium and chloride ions in bleaching gels, which can cause undersaturation with respect to hydroxyapatite.[2] Thirty-eight percent HP and 30% CP bleaching gels had pHs over this critical value, but they also caused hard tissue demineralization. This suggests that the acidic properties of the bleaching agent also contribute to the change in the mineral content of hard dental tissues. High acidity may cause a further decrease of microhardness.[22,23] Sun and others[24] demonstrated that neutral 30% HP had the same efficiency in tooth bleaching and caused fewer deleterious effects on enamel than did acidic 30% HP. Therefore, it can be concluded that bleaching agents with greater concentrations and acidity are potentially more harmful for the surface microhardness and can produce more alterations of the enamel and dentin structure and reduce their microhardness.

The buffering potential and remineralization effect of saliva are well documented.[9,10,21] Furthermore, ACP gels alone or in combination with casein phosphopeptide or nano-carbonate apatite are known factors for the remineralization of hard dental tissues.[25-28] Our results indicate that postbleaching treatment with artificial saliva and ACP significantly increases and restores enamel and dentin surface microhardness, which was not demonstrated for postbleaching treatment in deionized water. This result is in accordance with the findings of De Abreu and others.[29] Two-week storage in artificial saliva with everyday ACP treatment showed that both agents have potential remineralization effects and cause possible mineral precipitation of calcium and phosphate ions. In addition, this treatment can improve the surface microhardness of the hard dental tissues. Therefore, such treatment should be used after the bleaching process. Separating the remineralization effects of artificial saliva alone from those of artificial saliva in combination with ACP is one interesting venue for future research.

The ACP Relief gel used in this study contained sodium fluoride. da Costa and others[30] found no microhardness recovery after treatment with sodium fluoride gel or nanohydroxyapatite materials, indicating that the observed effect of microhardness recovery recorded in this study could be attributable to the ACP activity. On the other hand, Borges and others[22] suggested that application of fluoride gel in combination with calcium and artificial saliva enhances the microhardness of bleached enamel. Lewinstein and others[31] reported a significant reduction in enamel microhardness after the bleaching, which recovered after the application of 0.05% fluoride solution. Furthermore, Chen and others[9] noted that fluoridated bleaching agents produced less demineralization of surface morphology and microhardness, while the addition of fluoride did not impede the whitening effect. Therefore, HP in combination with hydroxyapatite, fluoride, and calcium could reduce microhardness loss and tooth sensitivity.[32-35]

The bleaching process, regardless of whether or not it was light activated, led to relevant changes in the chemical composition of enamel and dentin. A greater decrease in the concentrations of Ca and P was observed during treatment with gels containing

higher concentrations of peroxide—30% CP, 25% HP, and 38% HP, in comparison to 10% CP and 16% CP. In addition, a possible decrease in ion concentrations can be attributed to the lower pH values of bleaching gels such as 25% HP (pH=3.20) and 38% HP (pH=6.75). Acidic properties of the bleaching gels can lead to a reduction in the mineral content of enamel and dentin. While Amaral and others[36] concluded that bleaching gels do not alter concentrations of Ca and P on the enamel surface, Rotstein and others[37] found that the ratio of Ca to P significantly decreased after using 30% HP and 10% CP. McCracken and Haywood[38] noted that six-hour exposure to CP can result in an average Ca loss of 1.06 $\mu g/mm^2$. However, this loss is clinically negligible. For comparison, one glass of cola drink causes a Ca loss of about 1 $\mu g/mm^2$. Cakir and others[39] suggested that 10%, 20%, and 35% CP decrease the levels of Ca, P, and K and increase the amount of Na, F, and O. Such transient loss of minerals is considered reversible after a few days in artificial saliva, fluoride-containing gels, or other means of remineralization. Gjorgievska and Nicholson[40] and others have shown that application of remineralizing paste after bleaching with 16% CP leads to an increase in the quantity of Ca and F. This study showed that 14 days of artificial saliva and daily ACP treatment increased the concentrations of Ca, P, and F ions. The precipitation of salivary components, such as calcium and phosphates, either from artificial saliva or from remineralizing preparation could contribute to a significant recovery of hard dental tissue after bleaching.[41,42] The observed effect can be seen as a potential caries-protective action.

This study has some limitations. Under these clinical conditions, the tooth surface is protected by the saliva and enamel pellicle, so after bleaching the demineralized enamel and dentin can undergo possible remineralization and recalcification. For a simulation of clinical conditions, the samples were stored in artificial saliva before and for two weeks after the bleaching treatment. However, variations in salivary composition throughout the day cannot be simulated in an experimental setup.[25,43] Nevertheless, this *in vitro* study has demonstrated that artificial saliva and ACP treatment can improve surface microhardness, microstructure, and chemical composition as well as enhance the remineralization of the hard dental tissues by providing calcium and phosphate ions. For full and prompt recovery of enamel and dentin, such treatment is strongly recommended.

CONCLUSION

The microhardness and chemical composition of the hard dental tissues were adversely affected by the concentration of peroxide in the bleaching agent and its acidity while applied experimental light sources had a negligible effect. Postoperative treatment with ACP and artificial saliva restored microhardness and positively affected concentrations of Ca, F, and P in enamel and dentin. With regard to enamel and dentin microhardness and chemical composition, the bleaching lights could be safely used.

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Human Subjects Statement

The use of extracted human teeth was approved by the Research Ethics Committee of the School of Dental Medicine, University of Zagreb, Croatia. This study was conducted at the School of Dental Medicine, Department of Endodontics and Restorative Dentistry, in Gunduliceva 5 in Zagreb, Croatia.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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