

SURFACE CHANGES OF ENAMEL AND DENTIN AFTER TWO DIFFERENT BLEACHING PROCEDURES

Eva Klarić¹, Marijan Marcius², Mira Ristić², Ivan Sever³, Katica Prskalo¹ and Zrinka Tarle¹

¹Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb; ²Rudjer Bošković Institute; ³Institute for Tourism, Zagreb, Croatia

SUMMARY – Bleaching agents have effect on chemical/physical and morphological structure of enamel and dentin that must be taken into account when this therapy is used. The aim of this *in vitro* study was to evaluate the effects of two bleaching agents containing a high concentration of hydrogen peroxide for professional use on human enamel and dentin surface and to evaluate the potential remineralizing effect of amorphous calcium phosphate gel (ACP). Twenty-five human third molars were divided into two groups and dissected in half and both surfaces were bleached with either ZOOM2 or Opalescence BOOST for 3×15 minutes. Vickers microhardness of enamel and dentin was measured before, after the bleaching treatment, and after treatment with artificial saliva and ACP gel or 2-week storage in deionized water. Surface microstructure was evaluated using scanning electron microscopy. The mixed model ANOVA and Wilcoxon Rank Sum test were used. Both bleaching agents showed significant reduction in surface microhardness ($p < 0.001$ for both BOOST and ZOOM2 application). ZOOM2, which had a lower pH value showed greater decrease in surface microhardness ($p = 0.005$) compared to BOOST. Post-treatment with artificial saliva and ACP showed significant increase in surface microhardness ($p < 0.001$). After the bleaching procedure, enamel and dentin surface microstructure showed mild or slight alterations with no loss of superficial structure. In conclusion, both bleaching agents resulted in reduction in surface enamel and dentin microhardness. Treatment with ACP led to increase in surface microhardness, improved surface roughness, and enhanced remineralization of the hard dental tissues.

Key words: *Tooth bleaching agents; Light; Dental enamel – drug effects; Dental enamel – ultrastructure; Dentin – drug effects; Dentin – ultrastructure; Hydrogen peroxide; Amorphous calcium phosphate*

Introduction

An indication for teeth bleaching is change of teeth color, which can be external and internal. The frequently used bleaching techniques are in-office bleaching and at home bleaching¹. Vital tooth bleaching is associated with many unwanted side effects, which include enamel and dentin surface alterations²⁻⁴ and tooth sensitivity, which can be reduced with reminer-

alizing agents^{5,6}. Some studies claim that changes in enamel structure are minor and return to the original level after bleaching is completed, while others declare the opposite. No clinical studies or case reports in the literature have documented macroscopically or clinically visible damage due to vital bleaching or clinically relevant tissue destruction. The presence of saliva, fluorides or other remineralizing solutions is to maintain the balance between the remineralization and demineralization processes⁷. Amorphous calcium phosphate (ACP) is unique in the class of calcium phosphates as a direct precursor of biologic apatite in the biomineralization processes of both vertebrates and invertebrates^{8,9}. ACP can release calcium and phosphate

Correspondence to: *Eva Klarić, DDM*, Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb, Gundulićeva 5, HR-10000 Zagreb, Croatia
E-mail: eklarić@sfzg.hr

Received April 29, 2013, accepted November 20, 2013

ions and therefore maintain a supersaturated mineral environment that can reduce demineralization and improve remineralization of enamel¹⁰. Changes in organic and inorganic content after bleaching treatment may be evaluated by microhardness tests. The Vickers or Knoop microhardness test has been used previously as a parameter to assess a possible demineralization process. *In vitro* evaluations have reported alterations of enamel microhardness after exposure of the surface enamel to hydrogen peroxide³⁻⁵. Some studies showed that more acid bleaching gels could cause more alterations in the enamel surface^{4,11} and low pH of bleaching agents could sometimes be below the critical pH for enamel demineralization¹².

Scanning electron microscopy (SEM) has been extensively used in enamel and dentin surface morphological examinations following bleaching. Many studies have reported pitting, rugosity and increased surface porosity following bleaching treatment, while others report no significant change in surface roughness and a tendency toward a smoother surface¹³⁻¹⁵.

The aim of this *in vitro* study was to evaluate the effects of two bleaching agents containing a high concentration of hydrogen peroxide for professional use on human enamel and dentin surface and to evaluate the potential effect of the ACP remineralizing agent. Null hypotheses were as follows: (1) different bleaching agents with different acidity do not significantly lower the surface microhardness; (2) there is no difference between the bleaching agents used in this study; and (3) post-treatment with ACP and artificial saliva does improve surface microhardness.

Material and Methods

Specimen preparation

Twenty-five freshly extracted intact human third molars were cleaned and stored in 1% chloramine solution. The use of extracted human teeth was approved by the Research Ethics Committee of the School of Dental Medicine, University of Zagreb, Zagreb, Croatia. The root portions of the teeth were sectioned with a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, USA) approximately 2 mm below the cemento-enamel junction perpendicular to the long axis of the teeth, and stored in deionized water until used. Each tooth was free of dental caries or restoration. For the enamel

microhardness measurements, tooth crowns were dissected in half and embedded in acrylic resin (Acry-Fix Kit, Struers, Ballerup, Denmark). Subsequently, the specimen labial or oral surface was polished using water cooled carborundum discs (Water Proof Silicon Carbide Paper, 4000 grit, Buehler, Düsseldorf, Germany) and 1.0 µm, 0.3 µm and 0.05 µm micropolish powder (Buehler, Düsseldorf, Germany) to expose an area standardized in 3x3 mm. As for the dentin microhardness measurements, crowns were sectioned in half, fixed in acrylic resin, and dentin surface was polished as previously described for enamel surface.

Bleaching procedure

Teeth were dissected in half to enable separate assessment of enamel and dentin microhardness for two different bleaching agents applied (Table 1). Thus, before dissection they were randomly divided into two groups. Both surfaces were bleached with ZOOM2 (25% HP) in group 1 (n=20) and with Opalescence BOOST (38% HP) in group 2 (n=20) for 3x15 minutes, with the only difference that the ZOOM2 gel was supported by light activation with a lamp of the same manufacturer. Before application of the bleaching agents, teeth were removed from artificial saliva and the enamel and dentin surfaces were dried with cotton tissues. Bleaching gel was applied with a Heideman spatule in 2-mm thick layer. During the bleaching treatment, the specimen was on the cotton pellet soaked with artificial saliva. After the bleaching procedure, the bleaching gel was removed with Heideman spatule and cotton pellet and the surface was cleaned with deionized water, dried with compressed air and cotton tissues, and another layer of bleaching gel was put on the surface.

After the bleaching procedure, enamel and dentin surfaces were cleaned and dried, and the ACP gel (Table 1) was applied on the surface for 20 minutes every day for 14 days. After each ACP gel application, teeth were soaked with artificial saliva (thus protecting the specimens from dehydration), and stored at 37 °C. After the bleaching treatment and microhardness measurements, specimens were transferred to artificial saliva and stored at 37 °C in order to simulate intraoral conditions (Cultura Incubator, Ivoclar Vivadent, Schaan, Liechtenstein). For this experiment, 50 mL of Glandosane spray was used. Since its pH

Table 1. Summarized bleaching products and remineralizing agent (all data corresponding to data given by the manufacturers) including ingredients, application, active bleaching agent and percentage concentration of hydrogen or carbamide peroxide

Product	Manufacturer	Ingredients	Application	Active agent	%
ZOOM2	Discus Dental, Culver City, USA	Water, poloxamer 497, glycerin, propylene glycol, potassium nitrate, potassium hydroxide, <i>Mentha piperita</i> , eugenol, ferrous gluconate, hydrogen peroxide (25%)	In office	Hydrogen peroxide	25
BOOST	Ultradent, South Jordan, UT, USA	Propylene glycol, hydrogen peroxide (38%), 1.1% fluoride, 3% potassium nitrate	In office	Hydrogen peroxide	38
ACP Relief	Discus Dental, Culver City, USA	ACP, 5% potassium nitrate, 0.22% sodium fluoride, water, natural peppermint, calcium nitrate, sodium phosphate, sodium saccharine	In office/at home	Amorphous calcium phosphate	–

ACP = amorphous calcium phosphate

value is 5.23 (Pinnacle 555 pH/Ion meter, Corning, Tewksbury, USA), it was mixed with 6.73 g of 1% NaOH using the magnetic stirrer with hot plate (Cole Parmer, East Bunker Court Vernon Hills, USA) solution to have neutral pH 7.0 (Table 2). During the experiments, the teeth were stored in artificial saliva except for the period of the bleaching procedures and the time required for Vickers microhardness measure-

ments. Artificial saliva was replaced daily. Another half of the specimens were kept in deionized water for 14 days. Deionized water was replaced daily. Additional group (n=10) served as control. Molars in the control group were also dissected in half to enable separate measurements of enamel and dentin and were kept in artificial saliva for two weeks without any bleaching treatment.

Table 2. Artificial saliva made from Glandosane spray and NaOH solution (all data corresponding to data given by the manufacturers except for the pH value) including active ingredients and pH value

Product	Manufacturer	Ingredients	pH
Glandosane spray	Fresenius KABI, Cheshire, UK	Carboxymethylcellulose, sorbitol, potassium chloride, sodium chloride, magnesium chloride, hexahydrate, calcium chloride, potassium monohydrogen phosphate	5.23
NaOH	Kemika, Zagreb, Croatia	1% NaOH	12

Microhardness measurements

Microhardness was determined using a Vickers diamond (Leitz Miniload 2 Microhardness Tester, Leitz, Germany) at a load of 100 g applied for 10 seconds. The Vickers hardness indentations were located with their long axis applied perpendicularly to the surfaces of the enamel and dentin. Three indentations were performed on the central area of each specimen, with a distance of 100 µm between them, and then they were averaged. Indentations were performed before, immediately at the end of the bleaching treatment, and after 2-week storage in deionized water or artificial saliva and ACP application.

pH measurements

For pH measurements, a pH meter (Pinnacle 555 pH/Ion meter, Corning, Tewksbury, USA) was used. The pH meter was initially calibrated. The bleaching gels were 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 gels were in contact with the pH electrode for 20 min at room temperature (24 °C). The electrode was thoroughly washed between samples.

Scanning electron microscopy observations

Three specimens of each group were randomly selected and analyzed using SEM (JSM 7000F, JEOL, Japan) before and directly after bleaching treatment, and after 2-week storage in artificial saliva and ACP application. The specimens were dried and fixed in aluminum stubs. The surface morphology of enamel and dentin surfaces was examined using different magnifications up to 5000 magnification and photomicrographs of representative areas were taken. The enamel and dentin changes were classified as no alterations, mild or slight alterations, and altered surfaces (loss of superficial structure) and were modified with morphological alterations reported by Ferreira *et al.*¹⁵.

Statistical analysis

Descriptive statistics in the form of box plot diagrams was used to describe the main features of microhardness change. Data were transformed prior to analysis to enhance variance homogeneity and normality. Box-Cox test indicated that logarithm transformation

was appropriate, which was also advisable because of discrepancies in microhardness values between enamel and dentin. A mixed model ANOVA was applied to the transformed data and used for comparison of repeated measurements (baseline measurements, measurements observed immediately after and 2 weeks after the bleaching). Comparison of averages was based on geometric means. A compound symmetry assuming equal covariances between repeated measurements was selected as appropriate covariance structure on the basis of Akaike information criterion (AIC). Residuals were normally distributed. Deviation from normality was assessed by the Shapiro-Wilk test and normal probability plot. Sample size (n=40) was large enough to detect large effect sizes (according to Cohen's effect size conventions) for both main effects and pair-wise comparisons, with the satisfactory level of power set at 80%. Furthermore, decrease in microhardness observed immediately after the bleaching was analyzed by the exact Wilcoxon Rank Sum test and included comparisons of bleaching gels separately for each surface (enamel and dentin) and *vice versa*.

Given the exploratory nature of this study, significance level was set to 0.10 and data were analyzed without multiplicity adjustment¹⁶. Analysis was conducted using SAS 8.2.

Results

Microhardness analysis

Great differences were detected in baseline values of surface microhardness between enamel and dentin (Fig. 1). The mean baseline microhardness value measured at enamel was 38.20 HV (Vickers Pyramid Number) and at dentin 30.14 HV. Bleaching with either of the two gels tested had detrimental effect on the surface microhardness, which on average dropped by 11.26 HV at enamel and 11.56 HV at dentin after the bleaching treatment ($p < 0.001$) (Table 3). Decrease in values of microhardness observed immediately after the bleaching was different between the two gels applied (Mixed model ANOVA, ratio of means=1.04, $p = 0.005$). On average, application of ZOOM2 led to a greater microhardness decrease at enamel compared to BOOST (Wilcoxon Rank Sum test, $p = 0.003$). Median decrease after bleaching with ZOOM2 was 11.89 HV and 9.95 HV after bleaching with BOOST.

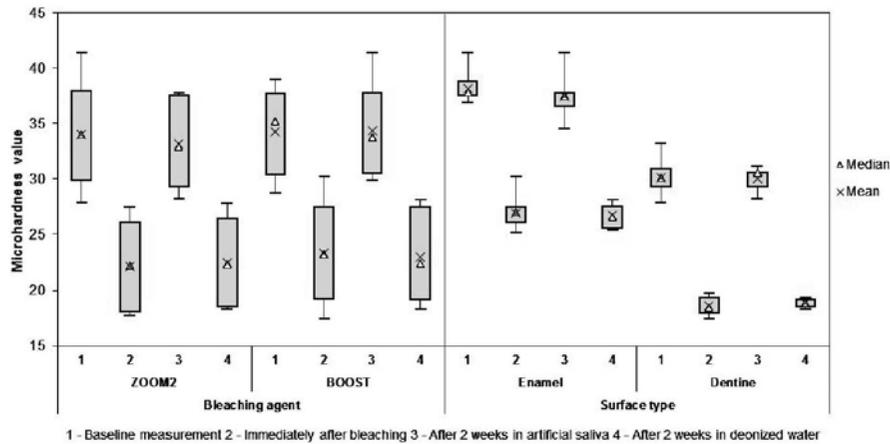


Fig. 1. Box-plot diagrams of microhardness values.

Table 3. Effects of different treatments on surface microhardness – separate results for each surface and gel type

Group	Statistics	Comparison of measurements					
		Base <i>vs.</i> bleach	Base <i>vs.</i> artificial saliva	Base <i>vs.</i> deionized water	Bleach <i>vs.</i> artificial saliva	Bleach <i>vs.</i> deionized water	Artificial saliva <i>vs.</i> deionized water
All	Ratio*	1.52	1.01	1.51	0.66	1.00	1.50
	df	69	69	69	69	69	69
	t-value	63.0	0.9	50.3	-50.0	-0.5	41.3
	p-value	<0.001	NS	<0.001	<0.001	NS	<0.001
Enamel	Ratio*	1.43	1.02	1.43	0.71	1.00	1.41
	df	69	69	69	69	69	69
	t-value	37.9	1.6	31.1	-29.0	0.4	24.6
	p-value	<0.001	NS	<0.001	<0.001	NS	<0.001
Dentin	Ratio*	1.61	1.00	1.59	0.62	0.99	1.60
	df	69	69	69	69	69	69
	t-value	51.2	-0.3	40.0	-41.8	-1.1	33.9
	p-value	<0.001	NS	<0.001	<0.001	NS	<0.001
ZOOM2	Ratio*	1.55	1.02	1.52	0.66	0.98	1.49
	df	69	69	69	69	69	69
	t-value	47.3	1.8	36.4	-36.0	-1.4	28.8
	p-value	<0.001	0.073	<0.001	<0.001	NS	<0.001
BOOST	Ratio*	1.49	0.99	1.50	0.67	1.01	1.51
	df	69	69	69	69	69	69
	t-value	41.9	-0.5	34.7	-34.7	0.7	29.7
	p-value	<0.001	NS	<0.001	<0.001	NS	<0.001

*Ratio of geometric means adjusted for model covariates – gel type and surface type.

Note: results of mixed model ANOVA; NS = nonsignificant.

Gel effect was not significantly different at dentin. Furthermore, change in microhardness immediately after the bleaching indicated significant differences between enamel and dentin only when BOOST was applied (Wilcoxon Rank Sum test; $p=0.012$). Also, the application of BOOST led to a smaller microhardness decrease at enamel (median=9.95) than at dentin (median=11.51).

After two weeks in artificial saliva, microhardness of both enamel (mean=37.50) and dentin (mean=30.05) was restored. However, analysis of ZOOM2 treatment indicated differences between the baseline measurements and measurements observed after 2-week treatment with artificial saliva ($p=0.073$) (Table 3). Nevertheless, this difference was clinically negligible as baseline measurements were on average only 1.02 times higher. The type of bleaching gel had no effect on the change in microhardness value from baseline to 2 weeks after the bleaching when artificial saliva was used as a post-treatment medium (Mixed model ANOVA; ratio of means=1.03; NS).

On the other hand, post-treatment with deionized water did not induce microhardness recuperation and led to a significantly lower surface microhardness when compared to the baseline values ($p<0.001$ for enamel and dentin) (Table 3). Microhardness of both enamel (mean=26.71) and dentin (mean=18.82) after 2 weeks in deionized water remained at the same level as immediately after the bleaching (NS). The type of bleaching gel had no effect on the change in micro-

hardness values when deionized water was used after the bleaching treatment (Mixed model ANOVA; ratio of means=1.01; NS).

Scanning electron microscopy analysis

A representative photomicrograph of polished non-bleached samples presented no significant morphological alterations of enamel (Fig. 2) and dentin (Fig. 3). Both bleaching agents showed alterations in surface smoothness and presented different levels of surface changes like minor alterations and slight irregularities. Also, both hydrogen peroxide bleaching agents promoted dissolution of some enamel superficial areas (Fig. 4) as well as dentin tubules to become opened (Fig. 5). After storage for 14 days in artificial saliva and daily 20-minute application of ACP gel, enamel surface showed mineral deposition on the enamel surface (Fig. 6) with occluded dentinal tubules (Fig. 7).

Discussion

Bleaching agents have effect on chemical and morphological structure of hard dental tissues. Hydrogen peroxide is an oxidative agent and has the ability to produce highly reactive peroxide and superoxide ions. Although bleaching is a complex process, the main reaction is oxidation. As a result of oxidation in the enamel and dentin organic and inorganic substance, change in microhardness and morphological characteristics can be observed¹².

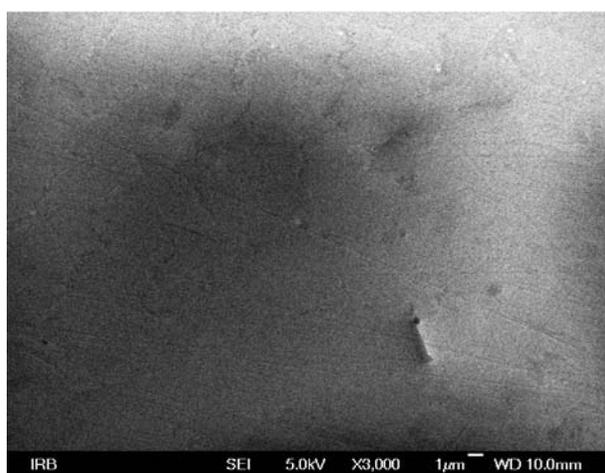


Fig. 2. Enamel presenting smooth surface with a few scratches.

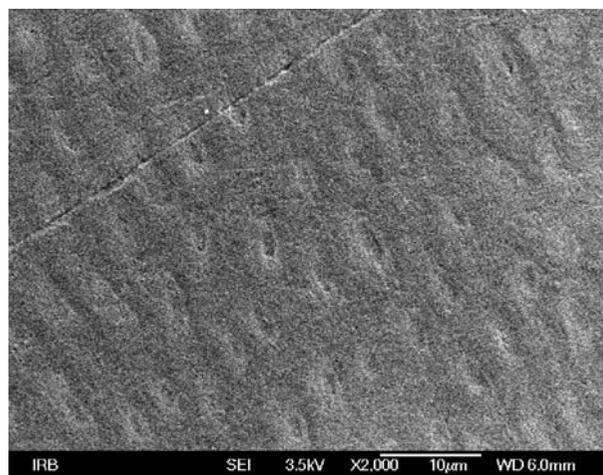


Fig. 3. Dentin presenting smooth surface without opened dentinal tubules.

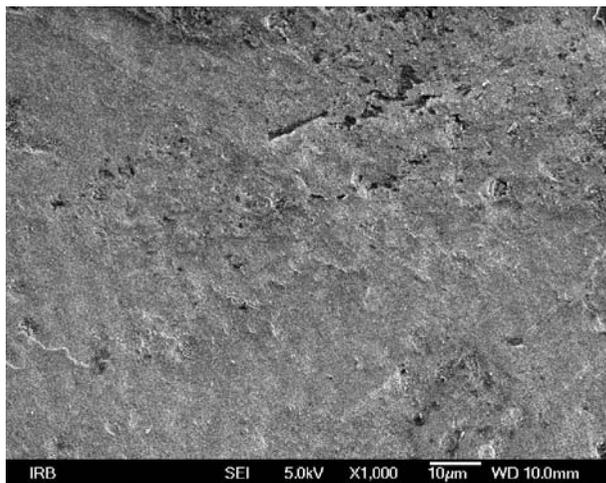


Fig. 4. Enamel surface presenting slight irregularities.

In our study, both bleaching agents showed significant reduction in surface microhardness of enamel and dentin, so our first null hypothesis that different bleaching agents do not decrease surface microhardness was rejected. Several studies estimated the relationship between concentrations of hydrogen peroxide or carbamide peroxide and the decrease in enamel and dentin microhardness^{2,5,17-20}. The present study revealed a significantly greater reduction in enamel and dentin microhardness for both groups containing 25% and 38% HP. The impact of bleaching agents on the possible side effects also depends on pH of the agent as well as on the quality of dental hard tissues. Bleach-

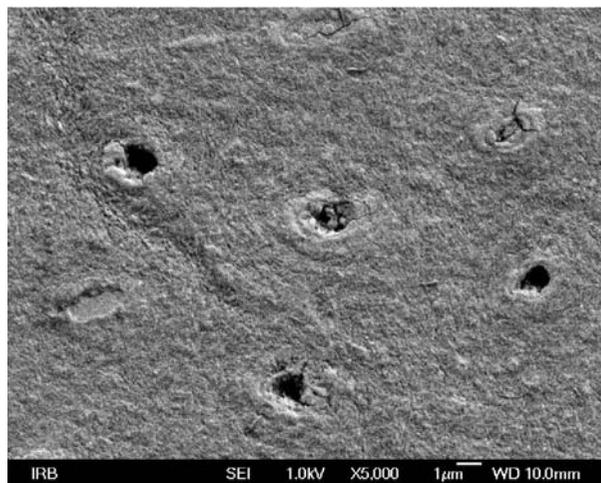


Fig. 5. Dentin surface presenting slight irregularities with opened dentinal tubules.

ing agents with higher acidity can produce more alterations of the enamel structure and reduce enamel microhardness^{3,11,22}. ZOOM2, which had a lower pH value (pH=3.20) showed greater decrease in surface microhardness compared to BOOST (pH=6.75) immediately after the bleaching procedure, so the second null hypothesis was rejected. The acid pH measured for ZOOM2 was below the critical level for enamel, which is in the range of 4.5-5.5 and it can cause demineralization. This can be attributed to the low concentrations of calcium and phosphate and high concentrations of sodium and chloride in bleaching

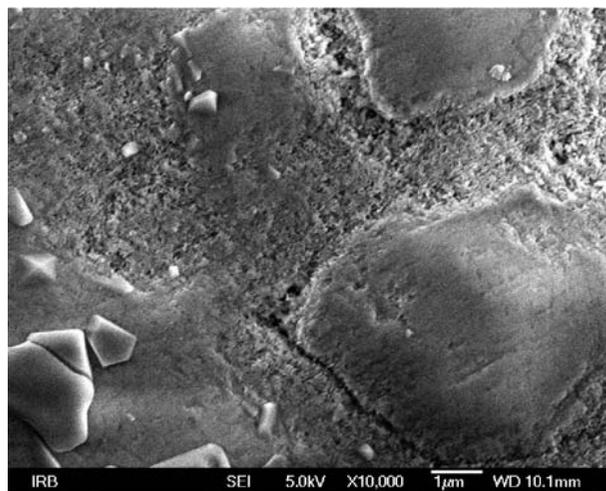


Fig. 6. Enamel surface presenting deposition of amorphous calcium phosphate.

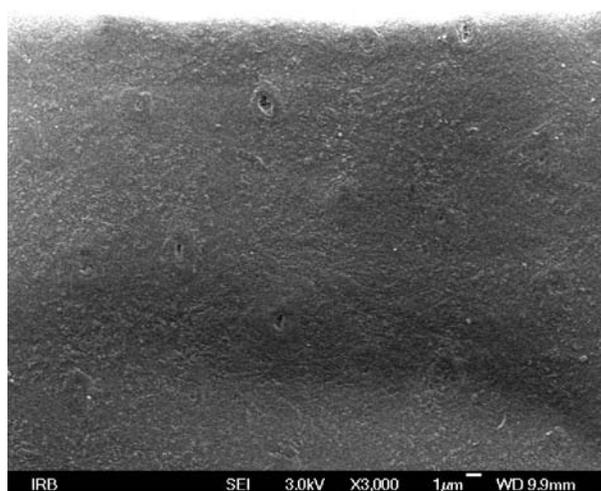


Fig. 7. Dentin surface presenting deposition of amorphous calcium phosphate and closed dentinal tubules.

gels, which can cause under-saturation with respect to hydroxyapatite³. BOOST had pH over this critical value, but it also caused hard tissue demineralization. Sa *et al.* also confirmed that neutral 30% HP had the same efficiency in tooth bleaching and it caused less deleterious effects on enamel than acidic 30% HP²².

Neither the buffering potential of the saliva nor its specific remineralization effect can be ignored. The composition of saliva is variable during the day making difficult to simulate physiological conditions in the experimental setup²³. In order to simulate the clinical situation concerning salivation and to standardize the experimental conditions at the same time, the samples were stored in artificial saliva before, in-between, and for 2 weeks after bleaching treatments. Amorphous calcium phosphate gel like ACP, CPP-ACP is known to be an important factor for enamel remineralization. Amorphous calcium phosphate helps restoring the necessary mineral balance in the mouth in an easy and efficient way and decrease adverse side effects from tooth bleaching²³⁻²⁵. SEM analysis of post-treatment nano-carbonate apatite (n-CAP) usage showed that n-CAP particles were deposited regularly on the damaged surface²⁶. Also, adding ACP CPP to carbamide peroxide bleaching agents can increase the bleached enamel's microhardness²⁷. In many *in vitro* studies, artificial saliva served as a good medium for possible remineralization of hard dental tissues, especially enamel. It can regain the mineral loss or increase disrupted surface microhardness^{23,28}. In our study, post-treatment with artificial saliva and ACP showed significant increase in surface microhardness compared to post-treatment with deionized water, so the third null hypothesis was not rejected. Two-week storage in artificial saliva with daily ACP treatment showed both agents to have a potential remineralization effect. Since there was no significant difference between the baseline and post-treatment values, microhardness returned after a period of remineralization, which was an indication that enamel and dentin were completely 'recovered'. Reaching the initial values of microhardness can be explained by the remineralization effect of the saliva. Although microhardness values after ACP and saliva treatment increased, the time period was maybe not long enough to repair the considerable microstructural enamel surface defects observed on SEM. Dentinal tubules were oc-

cluded, which was a good sign. Da Costa Soares *et al.* showed that the morphology and microhardness recovery did not occur 14 days after treatment with a sodium fluoride gel or application of a nanohydroxyapatite-based agent²⁹. Results from our study are partially consistent to the study by De Abreu *et al.*, which showed that hydrogen peroxide bleaching agents and bleaching agents with ACP caused enamel microhardness decrease during bleaching treatment, while post-treatment phase with artificial saliva after 7 and 14 days recovered mineral content and microhardness values were statistically similar to baseline values³⁰. Borges *et al.* showed that acid bleaching gel significantly reduced enamel microhardness, while the application of fluoride gel and a combination of calcium and fluoride gel with artificial saliva significantly enhanced the microhardness of the bleached enamel²¹. Generally, a combination of hydroxyapatite or fluoride and calcium with hydrogen peroxide can reduce microhardness loss^{31,32}. ACP Relief gel used in this study also includes sodium fluoride as its active ingredient. The results of the current study are partially in agreement with the results of Lewinstein *et al.*, since they report a significant reduction of enamel microhardness after treatment with 35% HP or CP, which fully recovered after the application of 0.05% fluoride solution³³. Chen *et al.* noted that adding fluoride as a protective ingredient in bleaching agents may raise concerns about adverse interactions between CP and fluoride because the bleaching efficiency of CP may be impaired by the calcium fluoride layer, while the remineralization potential of fluoride may be hampered by CP³⁴.

Many studies evaluated the effects of bleaching agents in the original protocol on the surface micro-morphology of dental structure, showing that both enamel and dentin are permeable by hydrogen peroxide and carbamide peroxide. SEM analysis showed changes in enamel such as the presence of erosions and porosities that could be justified by an extended time of contact between bleaching agents and dental structure, which can be related to the byproducts, mainly urea and oxygen³⁵. Our study showed that after the bleaching procedure (3x15 minutes), enamel and dentin surface microstructure had mild or slight alterations with no loss of superficial structure but it was difficult to determine whether they are micro-

scopically reversible. The results of the current study are partially in agreement with the study by D'Amario *et al.*, in which SEM analysis showed no relevant alteration on the enamel surfaces when the bleaching protocol was applied once or twice, but important alterations of the prismatic structure occurred when the bleaching protocol was applied three or four times³⁶. In our study, storage for 14 days in artificial saliva and daily 20-minute application of ACP gel on enamel surface showed mineral deposition on the enamel surface with occluded dentinal tubules. The same was found for fluoride ingredient, which may generate calcium fluoride deposits that modify the bleaching effect and impair penetration of peroxide but this could not be confirmed by SEM. The specimens treated with fluoridated bleaching agents showed only minor erosive patterns and no crystal deposition on enamel surfaces³³. Sasaki *et al.* showed improved surface micromorphological characteristics of dental structures through deposition of calcium fluoride crystals³⁷. Gels containing ACP affected remineralization patterns of pre-demineralized bovine enamel better than fluoridated bleaching agents³⁸. In the study by de Vasconcelos *et al.*²⁴, ACP CPP in combination with peroxides showed increased hardness and roughness, which were also associated with mineral deposition.

In clinical situation, tooth surfaces are protected by the enamel pellicle and saliva, so the demineralized enamel and dentin after bleaching can undergo possible recalcification. In *in vitro* conditions, ACP post-treatment can improve surface roughness and enhance remineralization of the hard dental tissues by providing calcium and phosphate ions and should be used after bleaching treatment.

Conclusion

Under the limitations of this *in vitro* study, it can be concluded that both types of bleaching agents with different concentration of hydrogen peroxide have a significant influence on the surface microhardness of human enamel and dentin. Application of ACP remineralizing agents in combination with saliva can cause increase in surface microhardness. Both in-office bleaching agents should be used under strict manufacturer's directions. Further studies with different concentrations of bleaching agents and possible remineralizing agents are required.

Acknowledgments

This study was supported by the Ministry of Science, Education and Sports, Republic of Croatia (project no. 065-0352851-0410) and Evaluation of New Bioactive Materials and Procedure in Restorative Dental Medicine, Croatian Science Foundation.

References

- LIMA DA, De ALEXANDRE RS, MARTINS AC, *et al.* Effect of curing lights and bleaching agents on physical properties of a hybrid composite resin. *J Esthet Restor Dent* 2008;20(4):266-73.
- ATTIN T, VOLLMER D, WIEGAND A, *et al.* Subsurface microhardness of enamel and dentin after different external bleaching procedures. *Am J Dent* 2005;18:8-12.
- MAGALHÃES JG, MARIMOTO AR, TORRES CR, *et al.* Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity. *Acta Odontol Scand* 2012;70:122-6.
- 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.
- KLARIĆ E, PAR M, PROFETA I, *et al.* Postoperative sensitivity after two in-office bleaching methods. *Acta Stomatol Croat* 2011;45:100-9.
- MARKOWITZ K, PASHLEY DH. Discovering new treatments for sensitive teeth: the long path from biology to therapy. *J Oral Rehabil* 2008;35:300-15.
- CHEN HP, CHANG CH, LIU JK, *et al.* Effect of fluoride containing bleaching agents on enamel surface properties. *J Dent* 2008;36:718-25.
- DOROZHKIN SV. Amorphous calcium (ortho)phosphates. *Acta Biomater* 2010;6:4457-75.
- TAO J, PAN H, ZENG Y, *et al.* Roles of amorphous calcium phosphate and biological additives in the assembly of hydroxyapatite nanoparticles. *J Phys Chem B* 2007;111:13410-8.
- MANTON DJ, WALKER GD, CAI F, *et al.* Remineralization of enamel subsurface lesions *in situ* by the use of three commercially available sugar-free gums. *Int J Paediatr Dent* 2008;18:284-90.
- SUN L, LIANG S, SA Y, *et al.* Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. *J Dent* 2011;39:686-92.
- RODRIGUES JA, BASTING TR, SERRA MC, RODRIGUES AL. Effect of 10% carbamide peroxide bleaching materials on enamel microhardness. *Am J Dent* 2001;14:67-71.
- MORAES RR, MARIMON JLM, SCHNEIDER LFJ, *et al.* Carbamide peroxide bleaching agents: effects on surface roughness of enamel, composite and porcelain. *Clin Oral Investig* 2006;10:23-8.

14. METZ MJ, COCHRAN MA, MATIS BA, *et al.* Clinical evaluation of 15% carbamide peroxide on the surface microhardness and shear bond strength of human enamel. *Oper Dent* 2007;32:427-36.
15. FERREIRA SS, ARAÚJO JL, MORHY ON, *et al.* The effect of fluoride therapies on the morphology of bleached human dental enamel. *Microsc Res Tech* 2011;74:512-16.
16. BENDER R, LANGE S. Adjusting for multiple testing – when and how? *J Clin Epidemiol* 2001;54:343-9.
17. 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.
18. MAJEED A, GROBLER SR, MOOLA MH, *et al.* Effect of four different opalescence tooth-whitening products on enamel microhardness. *SADJ* 2008;63:282-4, 286.
19. RODRIGUES JA, MARCHI GM, AMBROSANO GM, *et al.* Microhardness evaluation of *in situ* vital bleaching on human dental enamel using a novel study design. *Dent Mater* 2005;21:1059-67.
20. BARROS-MATOSO F, De SOUZA-GABRIEL AE, FURTADO-MESSIAS DC, *et al.* Microhardness of intracoronal dentin exposed to bleaching and fluoride treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 201;112:1-5.
21. BORGES AB, YUI KC, D'AVILA TC, *et al.* Influence of remineralizing gels on bleached enamel microhardness in different time intervals. *Oper Dent* 2010;35:180-6.
22. SA Y, SUN L, WANG Z, *et al.* 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.
23. ATTIN T, BUCHALLA W, GOLLNER M, HELLWIG E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. *Caries Res* 2000;34:48-52.
24. De VASCONCELOS AA, CUNHA AG, BORGES BC, *et al.* Enamel properties after tooth bleaching with hydrogen/carbamide peroxides in association with a CPP-ACP paste. *Acta Odontol Scand* 2012;70:337-43.
25. BAYRAK S, TUNC ES, SONMEZ IS, *et al.* Effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application on enamel microhardness after bleaching. *Am J Dent* 2009;22:393-6.
26. KIM YS, KWON HK, KIM BI. Effect of nano-carbonate apatite to prevent re-stain after dental bleaching *in vitro*. *J Dent* 2011;39:636-42.
27. BORGES BC, BORGES JS, De MELO CD, *et al.* Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel. *Oper Dent* 2011;36:521-8.
28. MEYER-LUECKEL H, UMLAND N, HOPFENMULLER W, KIELBASSA AM. Effect of mucin alone and in combination with various dentifrices on *in vitro* remineralization. *Caries Res* 2004;38:478-83.
29. Da COSTA SOARES MU, ARAÚJO NC, BORGES BC, *et al.* Impact of remineralizing agents on enamel microhardness recovery after in-office tooth bleaching therapies. *Acta Odontol Scand* 2013;71:343-8.
30. De ABREU DR, SASAKI RT, AMARAL FL, *et al.* Effect of home-use and in-office bleaching agents containing hydrogen peroxide associated with amorphous calcium phosphate on enamel microhardness and surface roughness. *J Esthet Restor Dent* 2011;23:158-68.
31. JIANG T, MA X, WANG Z, *et al.* Beneficial effects of hydroxyapatite on enamel subjected to 30% hydrogen peroxide. *J Dent* 2008;36:907-14.
32. BORGES AB, SAMEZIMA LY, FONSECA LP, *et al.* Influence of potentially remineralizing agents on bleached enamel microhardness. *Oper Dent* 2009;34:593-7.
33. LEWINSTEIN I, FUHRER N, CHURARU N, CARDASH H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. *J Prosthet Dent* 2004;92:337-42.
34. CHEN HP, CHANG CH, LIU JK, *et al.* Effect of fluoride containing bleaching agents on enamel surface properties. *J Dent* 2008;36:718-25.
35. MARKOVIC L, JORDAN RA, LAKOTA N, GAENGLER P. Micromorphology of enamel surface after vital tooth bleaching. *J Endod* 2007;33:607-10.
36. D'AMARIO M, D'ATTILIO M, BALDI M, *et al.* Histomorphologic alterations of human enamel after repeated applications of a bleaching agent. *Int J Immunopathol Pharmacol* 2012;25:1021-7.
37. SASAKI RT, ARCANJO AJ, FLÓRIO FM, BASTING RT. Micromorphology and microhardness of enamel after treatment with home-use bleaching agents containing 10% carbamide peroxide and 7.5% hydrogen peroxide. *J Appl Oral Sci* 2009;17:611-6.
38. TSCHOPPE P, NEUMANN K, MUELLER J, KIELBASSA AM. Effect of fluoridated bleaching gels on remineralization of predemineralized bovine enamel *in vitro*. *J Dent* 2009;37:156-62.

Sažetak

PROMJENE NA POVRŠINI CAKLINE I DENTINA NAKON DVA RAZLIČITA POSTUPKA
IZBJELJIVANJA*E. Klarić, M. Marcius, M. Ristić, I. Sever, K. Prskalo i Z. Tarle*

Sredstva za izbjeljivanje zubi mogu utjecati na kemijska i fizička svojstva, kao i na mikromorfološku strukturu cakline i dentina, na što se mora pripaziti prilikom provođenja ovakve terapije. Svrha ovoga istraživanja je bila procijeniti učinak dvaju sredstava za izbjeljivanje koja sadrže visoke koncentracije vodikovog peroksida na površinu cakline i dentina, kao i potencijalni remineralizacijski učinak gela amorfnog kalcijevog fosfata (ACP). Dvadesetpet ljudskih trećih molara podijeljeno je u dvije skupine, raspiljeno napola i obje površine su zatim tretirane gelom ZOOM2 ili Opalescence BOOST u trajanju od 3×15 minuta. Vickersova mikrotvrdoća cakline i dentina izmjerena je neposredno prije, nakon postupka izbjeljivanja i nakon tretmana umjetnom slinom i gelom ACP-a kroz 2 tjedna ili nakon držanja u deioniziranoj vodi kroz isto razdoblje. Površinske promjene su promatrane SEM mikroskopom. Korišteni su Mixed model ANOVA i Wilcoxon Rank Sum test. Oba gela za izbjeljivanje pokazala su značajno smanjenje u površinskoj mikrotvrdoći cakline i dentina ($p < 0,001$ za BOOST i ZOOM2). ZOOM2 koji je imao nižu pH vrijednost je pokazao veće smanjenje površinske mikrotvrdoće ($p = 0,005$) u odnosu na BOOST. Naknadna obrada umjetnom slinom i pripravkom ACP-a pokazala je značajno povećanje površinske mikrotvrdoće ($p < 0,001$). Nakon izbjeljivanja površine cakline i dentina su pokazale blage ili umjerene promjene bez gubitka površinske strukture. U zaključku, oba sredstva za izbjeljivanje dovela su do smanjenja površinske mikrotvrdoće. Tretman ACP-om nakon izbjeljivanja doveo je do povišenja mikrotvrdoće i smanjio površinska oštećenja poboljšavši remineralizaciju tvrdih zubnih tkiva.

Ključne riječi: Zubi, sredstva za izbjeljivanje; Svjetlo; Zubna caklina – djelovanje lijekova; Zubna caklina – ultrastruktura; Dentin – djelovanje lijekova; Dentin – ultrastruktura; Vodikov peroksid; Amorfni kalcijev fosfat