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Chemical Analysis of Enamel and Dentin Following the Application of Three Different At-home Bleaching Systems

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Clinical Relevance

Bleaching procedures with different concentrations of carbamide peroxide may influence the chemical composition of enamel and dentin.

SUMMARY

Purpose: **To determine the change in the chemical composition of enamel and dentin as well as to evaluate the differences in surface texture of the same dental hard tissues following three at-home bleaching systems *in vitro*.**

Methods: **Sixty extracted intact human anterior teeth were used in this study. Thirty teeth were used as samples for enamel, and the buccal surfaces of the remaining 30 teeth were abraded and used as dentin samples. Prior to bleaching treatments, calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), fluoride (F), and oxygen (O) levels of each sample were measured using an energy dispersive spectrometer. The teeth were then randomly allocated into three groups according to the bleaching system used, as follows: GI, 10% carbamide peroxide (CP); GII, 20% CP; GIII, and 35% CP. Following the bleaching treatments, Ca, P, K, Na, Mg, F, and O measurements were repeated. The surface configurations were examined using scanning electron microscopy (SEM). The data were analyzed using Wilcoxon signed rank and Kruskal-Wallis tests followed by the Dunn test.**

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Results: **All three bleaching systems tested caused similar changes in the chemical composition of enamel and dentin. Bleaching systems decreased Ca and K, while F and O levels increased in enamel. In dentin, Ca, P, and K levels decreased; however, Na, F, and O levels increased. SEM observations revealed no deleterious effect on enamel and dentin.**

Conclusion: **The use of home bleaching agents could affect the chemical composition of dental hard tissues, whereas the change in the chemical composition of enamel and dentin was not affected by the CP concentration of the bleaching systems used.**

INTRODUCTION

Teeth are important biological structures in the field of biomineralization. The mineral tissue of a tooth consists of hydroxyapatite (HAP) crystals, $Ca_{10}(PO_4)_6(OH)_2$. The inorganic content of dental hard tissues has been shown[1] to consist of not only calcium (Ca), phosphorus (P), and oxygen (O), as indicated by the formula of calcium HAP, but also carbon (C), magnesium (Mg), sodium (Na), and fluoride (F), as well as large trace elements.

With the increasing demand for treatments to enhance esthetic appearance, tooth bleaching is becoming a more common procedure in dentistry. Contemporary bleaching agents are typically composed of either hydrogen peroxide (HP) or carbamide peroxide (CP). HP is capable of oxidizing a wide range of colored organic and inorganic compounds, causing decolorization and hence bleaching of the substrate. CP is a chemical adduct of urea and HP, which, upon dissolving in water or saliva, disassociates back into HP and urea. Thus, CP can be considered a precursor of the active bleach species HP.[2] Combined with the night-guard bleaching technique, CP has been proven successful in providing a long-lasting whitening effect.[3] At-home bleaching is a method by which the patient fills a custom-designed tray with bleaching material (10% to 35% CP, resulting in 3.35% to 10.6% HP) that is worn for several hours each day for two to eight weeks. The benefits achieved with tray-based systems are widely known[4]: lower incidence of tooth sensitivity or gingival irritation; achievement of the same whitening results offered by higher concentration agents; and safety and efficacy of CP. This corresponds to the fact that macroscopically or clinically visible damage due to vital bleaching has not been described in the literature so far, although it is reported[5] that bleaching of nonvital teeth using the walking bleach technique involves the risk of development of external cervical resorption. Despite the fact that vital bleaching is not regarded as creating macroscopically visible defects, there are numerous studies[6-8] that exhibited microstructural changes of dental hard tissue induced by bleaching agents, especially when peroxides are applied in high concentrations. However, a number of other studies[9-11] showed little or no topographic changes on bleached tooth tissues. While studies on the effects of bleaching on morphological changes to tooth tissue are contradictory, it is generally agreed that peroxides can alter tooth mineral content.[12]

Scanning electron microscopy (SEM) is a rapid and convenient method for qualitatively analyzing the surface morphology of enamel and dentine specimens following bleaching.[2] On the other hand, energy dispersive spectrometry (EDS) provides a specific method for determining the concentration of chemical elements on the substratum.

The aim of this study, therefore, was to measure the levels of inorganic elements and to evaluate the differences in surface texture of dental hard tissues as well as to determine the change in the chemical composition of enamel and dentin following three at-home bleaching systems *in vitro*.

MATERIALS AND METHODS

Sixty extracted intact human anterior teeth (noncarious) were stored in distilled water at room temperature until required for analysis. Thirty teeth were used as samples for enamel, and the buccal surfaces of the remaining 30 teeth were abraded with 600-grit and 1000-grit silicone carbide abrasive paper using a mechanical device (Mecapol P230, Presi, France); these teeth were used as dentin samples. Prior to bleach application, the buccal surfaces of each tooth were subjected to EDS analyses to obtain a reference point and the coordinates and snapshots were recorded.

After the application, samples were mounted on the same holders. Recorded coordinates and images aided in location of the reference point. EDS analyses were performed on the combined SEM and EDS (Bruker Axs XFlash 3001 SDD-EDS, Cambridge, UK) in Hacettepe University, Faculty of Engineering, Department of Geological Engineering (Ankara, Turkey). Measurements of Ca, P, potassium (K), Na, Mg, and F were carried out in standardless mode, whereas O contents were calculated stochiometrically. The operating conditions were as follows: 15-Kv accelerating voltage, 10-nA beam current, and 30–45–second counting times with a 10-mm working distance. Under the given electron column conditions, EDS can detect X-rays generated from 1–2 μm of depth from the surface. The teeth were then randomly divided into three groups according to the at-home bleaching systems used (n=10), as follows: GI: Opalescence PF (10% CP; Ultradent Dental GmbH, Salt Lake City, UT, USA); GII: Opalescence PF (20% CP; Ultradent Dental GmbH); and GIII: Opalescence PF (35% CP; Ultradent Dental GmbH). Bleaching procedures were performed following the manufacturer's instructions (Table 1). Bleaching agents were applied onto the labial surfaces of the incisors. All other surfaces were enclosed with plaster. The junction of plaster with bleached surfaces was sealed with wax to prevent the inflow of bleach. The bleaching gel was applied and removed from the tooth surface using a small brush for both enamel and dentin samples. The bleaching agents were applied on the samples and stored at 100% humidity at 37°C. After each treatment, the samples were rinsed with tap water for one minute to remove the bleaching agent and then they were stored in distilled water at 37°C until the next bleaching treatment. At the end of the two-week bleaching period, Ca, P, K, Na, Mg, F, and O measurements were repeated, and the changes in mineral levels were recorded in the same manner. The data were analyzed using Wilcoxon signed rank and Kruskal-Wallis tests (p=0.05).

Two samples from each group were separated for SEM. First they were cleaned with distilled water. Because the vacuum conditions needed to sputter the enamel and dentin surfaces could possibly result in deterioration of surfaces, the images were obtained using a Zeiss EVO 50 EP SEM (Carl Zeiss, Cambridge, UK) in extended variable pressure mode without coating.

RESULTS

All three bleaching systems tested caused similar changes in the chemical composition of enamel and dentin ($p>0.05$; Kruskal-Wallis test).

In the enamel, a significant reduction in Ca and K levels was found following treatment with CP ($p<0.05$). Bleaching systems did not create any change in the levels of P, Mg, and Na in the enamel ($p>0.05$). On the other hand, F and O levels increased significantly following CP treatment ($p<0.05$; Wilcoxon signed rank). Results are shown in Table 2.

In the dentin, the use of bleaching systems did not cause any difference in Mg levels ($p>0.05$), whereas Na, F, and O levels increased ($p<0.05$). A significant reduction in Ca, P, and K levels occurred in the dentin following treatment with CP ($p<0.05$; Wilcoxon signed rank). Results are summarized in Table 3.

SEM observations revealed no deleterious effect on enamel and dentin (Figures 1 and 2). SEM assessments demonstrated that no obvious effect of the bleaching treatments on the chemical composition of teeth was evident.

DISCUSSION

The use of EDS enabled us to determine the mineral content of the enamel and dentin following use of three different concentrations of CP used in at-home bleaching agents. The main advantage of this system is its capability to provide an accurate and nondestructive analysis of the samples. The method is based on bombarding the sample with a high-voltage electron beam, giving characteristic wavelength emission for each mineral. The changes in the wavelengths of the rays emitted from the sample indicate changes in the specific mineral concentration on the sample surface.[13] In dental research, the levels of mineral content in enamel and dentin are a good indication of the demineralization and remineralization processes.[14,15]

Table 1: *Bleaching Agents Used in this Study*

Group	Materials	Manufacturer	Application
I	Opalescence PF (10% carbamide peroxide)	Ultradent Dental GmbH, Salt Lake City, UT, USA	2 weeks (4 hours per day)
II	Opalescence PF (20% carbamide peroxide)	Ultradent Dental GmbH	2 weeks (4 hours per day)
III	Opalescence PF (35% carbamide peroxide)	Ultradent Dental GmbH	2 weeks (30 minutes per day)

Table 2: *Mean and Standard Deviation Values of Element Levels of Enamel Before and After Bleaching*

Elements	Before Bleaching, %			After Bleaching, %		
	Group I	**Group II**	**Group III**	**Group I**	**Group II**	**Group III**
Ca	27.97 ± 1.81	26.96 ± 1.72	26.55 ± 1.63	20.75 ± 0.89	19.82 ± 1.12	20.43 ± 1.39
P	13.90 ± 1.06	13.78 ± 1.07	14.21 ± 1.23	13.21 ± 1.30	13.43 ± 1.39	14.02 ± 0.90
K	1.02 ± 0.09	1.16 ± 0.04	0.94 ± 0.04	0.01 ± 0.01	0.02 ± 0.04	0.00 ± 0.01
Na	0.23 ± 0.32	0.22 ± 0.17	0.51 ± 0.40	0.29 ± 0.27	0.33 ± 0.22	0.58 ± 0.30
Mg	0.20 ± 0.35	0.18 ± 0.13	0.49 ± 0.34	0.19 ± 0.11	0.17 ± 0.10	0.42 ± 0.19
F	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.92 ± 0.00	0.78 ± 0.00	0.83 ± 0.00
O	56.52 ± 3.96	57.11 ± 1.54	56.45 ± 2.84	64.48 ± 3.74	65.12 ± 2.14	64.43 ± 2.20

Abbreviations: Ca, calcium; F, fluoride; K, potassium; Mg, magnesium; Na, sodium; O, oxygen; P, phosphorus.

Table 3: *Mean and Standard Deviation Values of Element Levels of Dentin Before and After Bleaching*

Elements	Before Bleaching, %			After Bleaching, %		
	Group I	**Group II**	**Group III**	**Group I**	**Group II**	**Group III**
Ca	20.45 ± 1.90	22.67 ± 1.86	21.97 ± 1.28	16.73 ± 1.87	17.65 ± 1.81	17.58 ± 1.34
P	12.15 ± 1.29	12.98 ± 2.04	12.04 ± 1.26	9.23 ± 0.70	8.97 ± 1.12	9.29 ± 1.63
K	0.96 ± 0.01	0.87 ± 0.02	0.92 ± 0.00	0.01 ± 0.04	0.00 ± 0.04	0.01 ± 0.06
Na	0.68 ± 0.58	0.51 ± 0.38	0.65 ± 0.39	1.45 ± 0.48	1.15 ± 0.46	1.49 ± 0.61
Mg	0.82 ± 0.52	0.42 ± 0.23	0.52 ± 0.28	0.78 ± 0.30	0.35 ± 0.28	0.45 ± 0.33
F	0.08 ± 1.41	0.02 ± 0.00	0.02 ± 0.00	0.71 ± 1.34	0.31 ± 0.90	0.21 ± 1.76
O	64.76 ± 3.13	64.48 ± 2.24	64.78 ± 2.75	68.15 ± 1.78	66.91 ± 2.04	66.31 ± 2.93

Abbreviations: Ca, calcium; F, fluoride; K, potassium; Mg, magnesium; Na, sodium; O, oxygen; P, phosphorus.

McCracken and Haywood[16] reported Ca loss from human enamel exposed to 10% CP. Using electron probe microanalysis, Potocnik and others[17] demonstrated that 10% CP decreased the concentration of Ca as well as the average Ca:P value of all bleached enamel samples. Lee and others[18] reported a decrease in the Ca:P ratio of bleached bovine enamel after application of 30% HP. In the present study, all concentrations of CP significantly reduced the Ca levels in enamel and dentin but did not significantly affect the P levels for enamel, whereas there was a significant decrease in P levels in dentin. The reduction of mineral element (Ca) could be attributed to the dissolution of this element by bleaching agents.

At present, the role of K is not known sufficiently. K levels decreased both in enamel and dentin

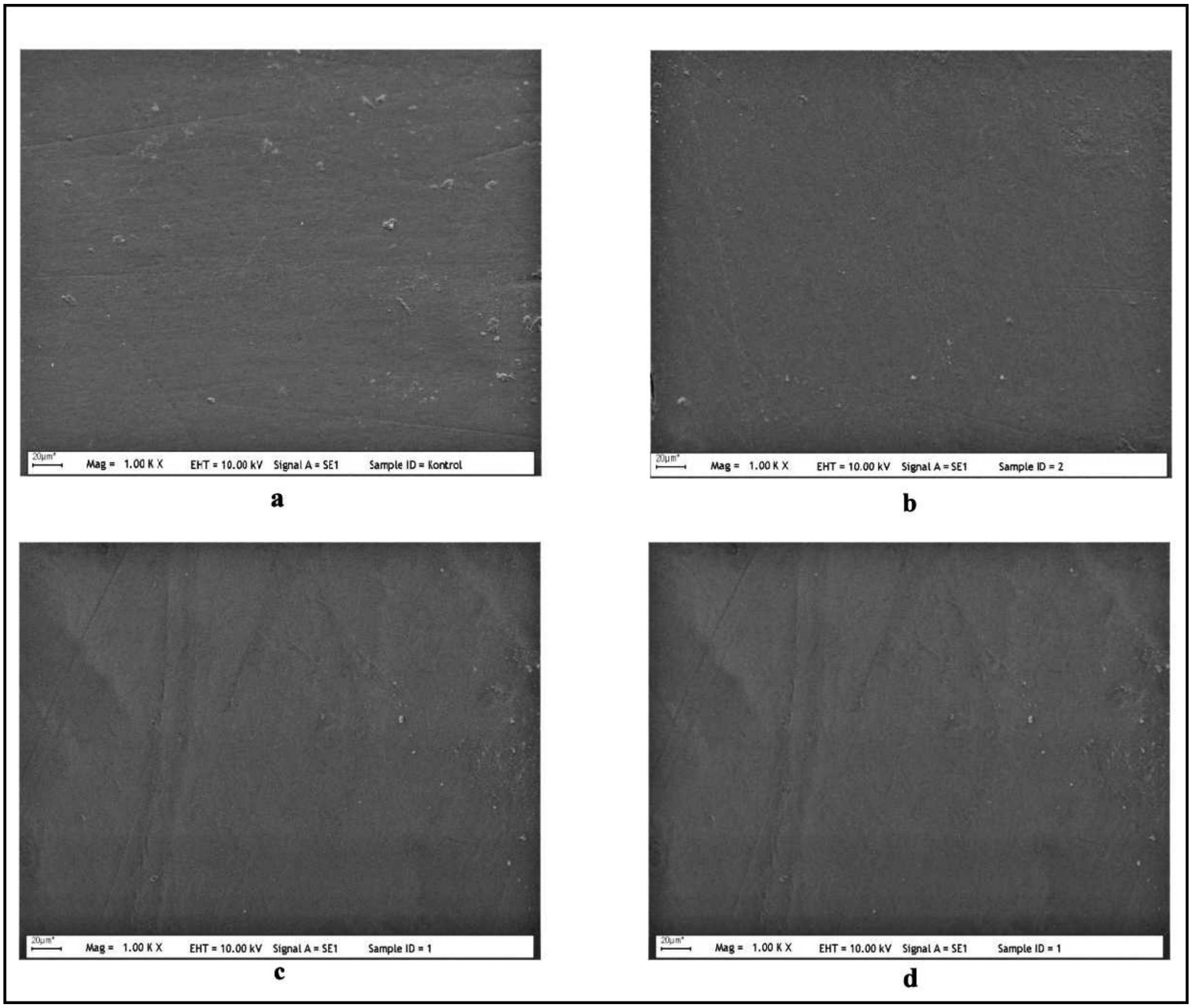

Figure 1. *SEM photomicrographs of enamel (a) before application of CP agents, (b) after application of 10% CP bleaching agent (group I), (c) after application of 20% CP bleaching agent (group II), and (d) after application of 35% CP bleaching agent (group III).*

following bleaching treatments. This result was compatible with the findings of Rotstein and others[19] that the bleaching agents caused a reduction in the organic components of dental tissues. In the present study, the loss of Mg was not statistically significant; however, a relatively large amount of Mg loss may be due to the fact that Mg is among the first elements to be dissolved during the demineralization process. Thus, the loss of Mg could be the first sign of demineralization.[20]

In this study, F levels increased both on enamel and dentin after bleaching. The possible explanation for this increase may be the use of three recently developed at-home bleaching agents containing fluoride to prevent either hypersensitivity or demineralization during bleaching treatments. However, controversial results exist on the topic since no supporting evidence regarding the influence of fluoride-containing bleaching gels on the remineralization has been documented. An *in vitro* study[21] reported that CP combined with F or Ca did not prevent the reduction in the surface microhardness of enamel. Contrarily, an X-ray photoelectron spectroscopy study[22] revealed that the additional sodium fluoride in HP bleaching agents generates fluoridated HAP and calcium fluoride crystals on enamel surfaces. This mechanism is believed to accelerate the remineralization process on the tooth surface. Fluoridated bleaching agents are expected to reduce the adverse effects of tooth whitening.[4] However, this needs to be verified in future investigations. In this study we preferred to use recently developed bleaching agents that represent the latest trend in cosmetic dentistry.

Souza and others[23] determined that the percentage of O was comparable between both halves in the

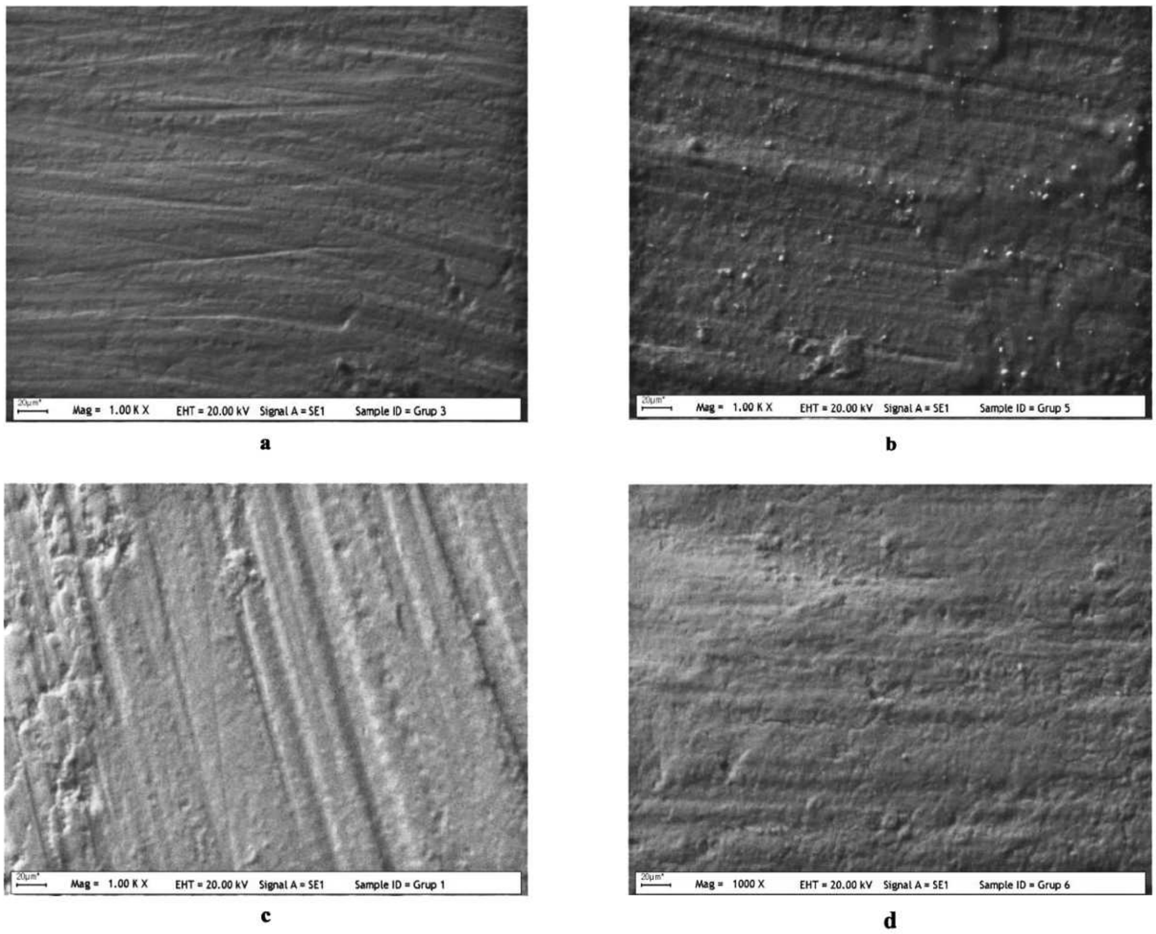

Figure 2. *SEM photomicrographs of dentin (a) before application of CP agents, (b) after application of 10% CP bleaching agent (group I), (c) after application of 20% CP bleaching agent (group II), and (d) after application of 35% CP bleaching agent (group III).*

control and the bleached groups. Contrary to their results, in the present study, an increase was also observed on O levels with the use of all three bleaching systems· Since the amounts of all elements were presented as mass percentage (%) in this study, this could be a relative increase that might be influenced by the decrease of the other elements.

In clinical situations, the enamel surfaces are fully exposed to the bleaching agents, whereas dentin exposure occurs via peroxide diffusing through the enamel to reach the enamel-dentin junction before reaching the dentin regions.[24] In cases of defective restorations and nonvital bleaching procedures the dentin may directly be exposed to the bleaching agent. For this reason, it is important to examine the effect of the oxidizing agents on dentin as well as on enamel.

The oral environment provides conditions for enamel remineralization, and demineralized enamel is more susceptible to remineralization.[25] When a bleaching agent causes demineralization in the enamel, ionic changes are induced, increasing mineral uptake, which replaces the mineral lost during treatment. For this reason, in order to standardize the sole effect of bleaching agents on enamel and dentin we stored the samples in distilled water instead of artificial saliva.[26]

The mineral content of enamel and the concentration of minor component change from surface to interior. For instance, Ca, P, and F concentrations were shown to decrease, whereas Mg, carbonate, and chlorine concentrations were found to increase from the surface to the interior of the enamel.[27] In the present study, one standard point was selected on the buccal surface of each tooth, and all measurements were performed at the same point.

The majority of studies[28-30] that investigated the surface morphology using SEM found no significant changes following bleaching. In contrast, using SEM, Covington and others[31] reported that changes occurred in the surface morphology of the bleached enamel, but with no major changes to the composition of the enamel. Confocal laser scanning microscopy results showed that enamel, subsurface enamel, dentin, and the dento-enamel junction DEJ structure were unchanged during bleaching procedures.[32] With the limitation of this study, two specimens per group were examined under SEM and revealed no deleterious effects on enamel and dentin after bleaching.

Three at-home bleaching agents with different CP concentrations (10%, 20%, and 35%) were used in this study. According to the manufacturer's instructions, 10% and 20% CP concentrated bleaching agents were applied for four hours per day, whereas 35% CP was applied for thirty minutes per day. All three bleaching agents caused similar changes in the element levels of enamel and dentin. Therefore, different concentrations of CP used according to the manufacturer's directions did not influence the change of the chemical composition of dental hard tissues in the present study.

Our study has some limitations. First, the EDS method that is used to examine the chemical changes may be much less informative than the XPS or Raman spectroscopy method. Another limitation of this study included the lack of follow-up related to the mineral content of enamel and dentin for extended periods of time after the bleaching. Therefore, further studies are needed to evaluate the ultrastructural effects of different bleaching agents on dental hard tissues after extended periods of time.

CONCLUSION

With the limitations of this *in vitro* study, it may be concluded that the use of home bleaching agents can affect the chemical composition of dental hard tissues. The change in the chemical composition of enamel and dentin may not be affected by the CP concentrations of the bleaching systems that are used according to manufacturer's instructions. However, it would also be interesting to evaluate whether tooth remineralization would have any effect on bleached enamel and dentin.

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REFERENCES

1. Chaudhri MA, & Chaudhri MN (2006) *Radiation Methods for the Study of Calcified Tissues-Teeth. World Congress on Medical Physics and Biomedical Engineering* COEX, Seoul, Korea, 1655.
2. Joiner A (2007) Review of the effects of peroxide on enamel and dentine properties *Journal of Dentistry* **35(12)** 889-896.
3. Haywood VB (1997) Nightguard vital bleaching: Current concepts and research *Journal of the American Dental Association* **128(Supplement)** 19S-25S.
4. Chen HP, Chang CH, Liu JK, Chuang SF, & Yang JY (2008) Effect of fluoride containing bleaching agents on enamel surface properties *Journal of Dentistry* **36(9)** 718-725.
5. Haywood VB, Leech T, Heymann HO, Crumpler D, & Bruggers K (1990) Nightguard vital bleaching: Effects on enamel surface texture and diffusion *Quintessence International* **21(10)** 801-804.
6. Shannon H, Spencer P, Gross K, & Tira D (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents *Quintessence International* **24(1)** 39-44.
7. Wandera A, Feigal RJ, Douglas WH, & Pintado MR (1994) Home-use tooth bleaching agents: An in vitro study on quantitative effects on enamel, dentin, and cementum *Quintessence International* **25(8)** 541-546.
8. Ben-Amar A, Liberman R, Gorfil C, & Bernstein Y (1995) Effect of mouthguard bleaching on enamel surface *American Journal of Dentistry* **8(1)** 29-32.
9. Ernst CP, Marroquín BB, & Willershausen-Zönnchen B (1996) Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel *Quintessence International* **27(1)** 53-56.
10. Haywood VB, & Heymann HO (1991) Nightguard vital bleaching: How safe is it? *Quintessence International* **22(7)** 515-523.
11. Oltu U, & Gurgan S (2000) Effects of three concentrations of carbamide peroxide on the structure of enamel *Journal of Oral Rehabilitation* **27(4)** 332-340.
12. Al-Salehi SK, Wood DJ, & Hatton PV (2007) The effect of 24h non-stop hydrogen peroxide concentration on bovine enamel and dentine mineral content and microhardness *Journal of Dentistry* **35(11)** 845-850.
13. Dankner E, Neev J, Stabholz A, & Rotstein I (1997) Effect of XeCl-308nm excimer laser on the mineral content of human dentin *Endodontics and Dental Traumatology* **13(5)** 234-237.
14. Figures KH, Ellis B, & Lamb DJ (1990) Fluoride penetration into dentine abutments in vitro *Caries Research* **24(5)** 301-305.
15. Tjaderhane L, Hietala EL, & Larmas M (1995) Mineral element analysis of carious and sound rat dentin by electron probe microanalyzer combined with back-scattered electron image *Journal of Dental Research* **74(11)** 1770-1774.
16. McCracken MS, & Haywood VB (1996) Demineralization effects of 10 percent carbamide peroxide *Journal of Dentistry* **24(6)** 395-398.

17. Potocnik I, Kosec L, & Gaspersic D (2000) Effect of 10% carbamide peroxide bleaching gel on enamel microhardness *Journal of Endodontics* **26(4)** 203-206.
18. Lee K, Kim H, Kim K, & Kwon Y (2006) Mineral loss from bovine enamel by a 30% hydrogen peroxide solution *Journal of Oral Rehabilitation* **33(3)** 229-333.
19. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, & Zalkind M (1996) Histochemical analysis of dental hard tissues following bleaching *Journal of Endodontics* **22(1)** 23-25.
20. Larsen MJ, & Brunn C (1986) *Textbook of Cariology* Munksgaard 181, Copenhagen, Denmark.
21. de Oliveira R, Paes Leme AF, & Giannini M (2005) Effect of a carbamide peroxide bleaching gel containing calcium or fluoride on human enamel surface microhardness *Brazilian Dental Journal* **16(2)** 103-106.
22. Tanizawa Y (2005) Reaction characteristics of a tooth-bleaching agent containing H_2O_2 and NaF: In vitro study of crystal structure change in treated hydroxyapatite and chemical states of incorporated fluorine *Journal of Cosmetic Science* **6(2)** 121-134.
23. Souza ROA, Lombardo GHL, Pereira SMB, Zamboni SC, Valera MC, Araujo MAM, & Ozcan M (2010) Analysis of tooth enamel after excessive bleaching: A study using scanning electron microscopy and energy dispersive x-ray spectroscopy *International Journal of Prosthodontics* **23(1)** 29-32.
24. da Silva AP, de Oliveira R, Cavalli V, Arrais CA, Giannini M, & de Carvalho RM (2005) Effect of peroxide-based bleaching agents on enamel ultimate tensile strength *Operative Dentistry* **30(3)** 318-324.
25. Ten Cate JM (1990) In vitro studies on the effects on de- and re-mineralization *Journal of Dental Research* **69(Special Issue)** 614-619.
26. Justino LM, Tames DR, & Demarco FF (2004) In situ and in vitro effects of bleaching with carbamide peroxide on human enamel *Operative Dentistry* **29(2)** 219-225.
27. Weatherell JA, Robinson C, & Hallsworth AS (1974) Variations in the chemical composition of human enamel *Journal of Dental Research* **53(2)** 180-192.
28. Titley K, Torneck CD, & Smith DC (1988) Effect of concentrated hydrogen peroxide solution on the surface morphology of cut human dentin *Endodontics and Dental Traumatology* **4(1)** 32-36.
29. Cobankara FK, Unlü N, Altinöz HC, & Füsun O (2004) Effect of home bleaching agents on the roughness and surface morphology of human enamel and dentine *International Dental Journal* **54(4)** 211-218.
30. Duschner H, Götz H, White DJ, Kozak KM, & Zoladz JR (2006) Effects of hydrogen peroxide bleaching strips on tooth surface color, surface microhardness, surface and subsurface ultrastructure, and microchemical (Raman spectroscopic) composition *Journal of Clinical Dentistry* **17(3)** 72-78.
31. Covington JS, Friend GW, Lamoreaux WJ, & Perry T (1990) Carbamide peroxide tooth bleaching: Effects on enamel composition and topography *Journal of Dental Research* Abstract **530** Abstract #69 p 175.
32. Gotz H, Duschner H, White DJ, & Klukowska MA (2007) Effects of elevated hydrogen peroxide 'strip' bleaching on surface and subsurface enamel including surface histomorphology, micro-chemical composition and fluorescence changes *Journal of Dentistry* **35(6)** 457-466.