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Research Article

Effect of bleaching treatment on element content of enamel

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ABSTRACT

The aim of study was to evaluate the mineral changes caused by enamel bleaching treatments at different application times by scanning electron microscope- energy dispersive X-Ray (SEM-EDX). In this study, 36 teeth were divided into 3 groups after mineral measurements (Flour, Silicon, Aluminum, Strontium) with SEM-EDX (n = 12). 40% hydrogen peroxide gel was used for bleaching in all groups. Bleaching was performed 20 min for Group 1, 40 min for Group 2, 60 min for Group 3. Mineral measurements were performed with SEM-EDX after treatment. In the intra-group comparison, although there was no significant difference between Flour (F) and Silicon (Si) values ($p > 0.05$), there was statistically significant difference in Aluminum (Al) and Strontium (Sr) values ($p < 0.05$). After the bleaching process for different application times, mineral change was observed in time-depending comparison. The best results were obtained for Group 2, appling according to manufacturer's instructions.

Keywords: Bleaching, mineral content, SEM-EDX.

Beyazlatma uygulamasının minenin mineral içeriği üzerine etkisi

ÖZ

Çalışmanın amacı, taramalı elektron mikroskobu-enerji dağıtıcılı X-Ray (SEM-EDX) cihazı ile farklı uygulama zamanlarında minedeki beyazlatma işlemleri sonrası meydana gelen mineral değişiklikleri değerlendirmektir. Bu çalışmada, 36 adet diş SEM-EDX (n = 12) ile mineral (Flor, Silisyum, Alüminyum, Stronsiyum) ölçümlerinden sonra üç gruba ayrıldı. Tüm gruplarda beyazlatma işlemi için % 40 hidrojen peroksit jel kullanıldı. Beyazlatma uygulaması, Grup 1 için 20 dakika, Grup 2 için 40 dakika, Grup 3 için 60 dakika olarak uygulandı. Mineral ölçümleri, işlemden sonra SEM-EDX cihazı ile gerçekleştirildi. Grup içi karşılaştırmada Flor (F) ve Silisyum (Si) değerleri arasında anlamlı bir fark olmamasına rağmen ($p > 0.05$), Alüminyum (Al) ve Stronsiyum (Sr) değerleri arasında istatistiksel olarak anlamlı fark vardı ($p < 0.05$). Farklı uygulama süreleri için beyazlatma işleminden sonra, zamana bağlı karşılaştırmada mineral değişikliği gözlendi. En iyi sonuçlar, üretici talimatlarına göre Grup 2 için elde edildi.

Anahtar Kelimeler: Beyazlatma, mineral içeriği, SEM-EDX.

1. INTRODUCTION

In recent years, the increasing interest in aesthetic applications has made dental aesthetic applications popular. A person's smile is the most important factor that emphasizes the importance of outward appearance. Bleaching treatment is a non-invasive and easily applied method that is often preferred for tooth discoloration. Nowadays, there are three types of bleaching techniques: office bleaching (professional application), home bleaching (individual application) and a combination of both methods.[1] Although the chosen technique varies according to the expectations and treatment needs of the patients, both techniques have advantages and disadvantages. The known major disadvantage of home bleaching is that it requires a long

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preparation process and is not easily controlled by the patient during application. In office bleaching, the main advantages are that the application of the gel under the control of the clinician can be prevented from contacting the soft tissue and the desired results can be achieved in one session.[2]

Hydrogen peroxide (HP) and carbamide peroxide (CP) gels are the most commonly used bleaching agents in discoloration.[1] HP-containing gels have been used in modern dentistry for more than 70 years.[3] The free radicals produced by HP can oxidize the conjugated chain of organic compounds and chromophores. Bleaching treatment is the brightening of tooth color by chemical agents applied to teeth oxidizing organic pigments in enamel and dentin tissue.[4] In the bleaching process, free radicals destructing double carbon bonds leads to a single carbon bond, while light and low molecular weight compounds are obtained from dark and large molecular weight compounds.

Some studies in the literature have shown that bleaching treatment disrupts the surface integrity of enamel,[5] increases the roughness,[6-7] changes the inorganic composition,[8] reduces microhardness and mineral content[7,9-10] and causes morphological changes. Therefore, the widespread use of high concentrations of peroxides is controversial.

Studies on the changes caused by HP-containing products in enamel are insufficient. Some researchers have reported negative effects such as microhardness reduction,[11-12] elastic modulus change or mineral loss,[13-17] on the other hand, other investigators also stated that these negative effects are clinically insignificant due to the remineralization power of saliva.[18-21] In the study of Goßtz and co-workers,[16] Although it has been reported that there is no significant change in enamel structure, other researchers have suggested that bleaching caused microstructural changes[22-23] in enamel at different depths and longer application times.[24]

There are few studies in the literature about fluoride (F), aluminum (Al), silicon (Si) and strontium (Sr) content of dental hard tissues after bleaching treatment.[25-28] However, no previous study evaluated the correlation of application time and the level of mineral content increase/decrease. The aim of this study was to evaluate the mineral changes in enamel after bleaching treatment for different periods by using Scanning Electron Microscopy-Energy Dispersion X-Ray Analysis (SEM-EDX) method.

2. MATERIALS AND METHODS

This study was approved by Gaziantep University Faculty of Dentistry Ethics Committee with the decision no 2019 / 468 and it was conducted in accordance with the Helsinki Declaration Principles. Each patient who participated in the study read and signed the informed consent form.

2.1. Preparation of Samples

In this study, 36 maxillary incisor teeth with no cracks or caries extracted for periodontal reasons were used. The teeth were divided into three groups as 12 teeth in each group and kept in artificial saliva during treatment.

The content of artificial saliva is; 1.1 g K_2HPO_4, 0.87 g NaCl, 0.62 g KCl, 0.3g KH_2PO_4, 0.17g $CaCl_2$ per 1000 ml and pH was 7. This chemical composition was obtained from Gaziantep University Biochemistry Department. Artificial saliva was maintained until from beginning to end of treatment and the solution was changed regularly every day. Mineral measurements with SEM-EDX were performed both before treatment and on the 14th day of treatment. The teeth were embedded in the *Si* impression material with the crown parts exposed, and their enamel surfaces were smoothed with a sanding device (Mecapol P 230 Press, Grenoble, France) using 600, 800 and 1000 grain silicon carbide water sanders.

2.2. Definition of groups and bleaching treatment

Opalescence Boost PF (Ultradent Products, Inc., SJ, USA) bleaching agent containing 40% HP was used according to the manufacturer's instructions. The samples were divided into three groups (n = 12):

Group 1: Application of the bleaching agent at the minimum time recommended by the manufacturer (20 min).

Group 2: Application of the bleaching agent to the ideal time recommended by the manufacturer (40 min).

Group 3: Application of the bleaching agent within the maximum time recommended by the manufacturer (60 min).

2.3. Mineral content analysis with SEM-EDX device

The SEM-EDX (JSM-6390LV, Jeol Inc., MA, USA) device provides information about the chemical composition of the material by allowing X-rays to be emitted from the ions and atoms on the sample surface.[29] Prepared samples were stored in an oven at 100˚C for 12 hours to isolate from the available moisture before SEM-EDX analysis. Each tooth was analyzed under 100 magnifications for elemental distribution from 3 standardized points on the crown. Al, F, Si and Sr minerals obtained from SEM-EDX analysis were investigated.

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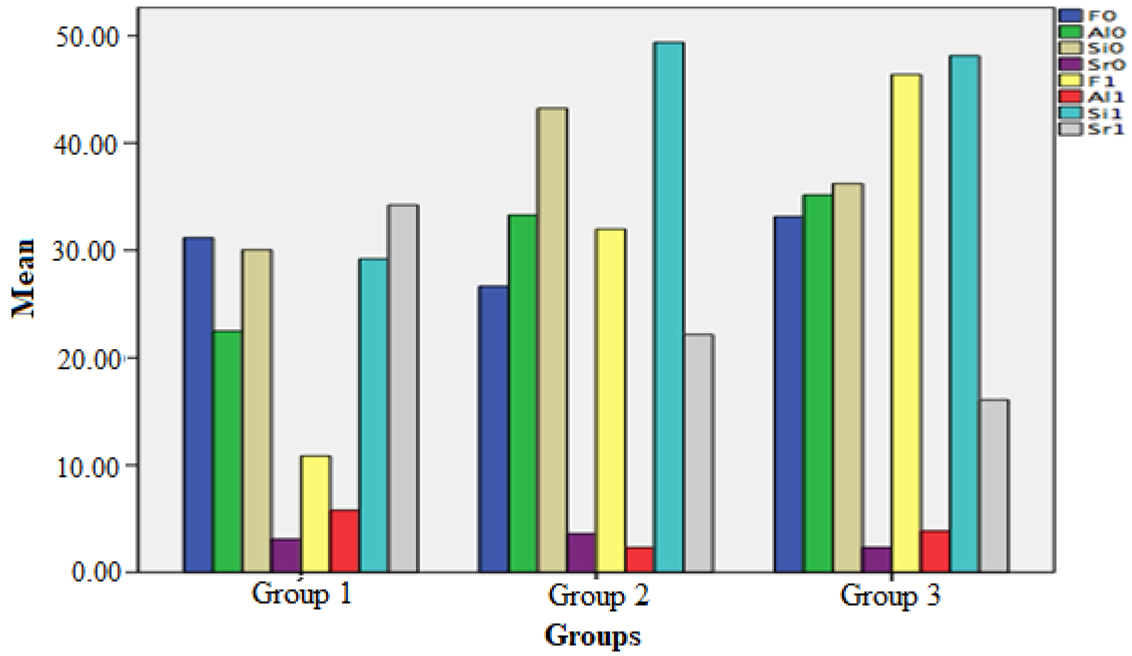

2.4. Statistical analysis

Compliance of normal distribution of numerical variables was tested by the Kolmogorov-Smirnov test. ANOVA, Bonferroni, pair *t* tests were used to compare the normally distributed variables in three independent groups; Kruskal Wallis and Wilcoxen tests were used to compare the variables that were not normally distributed in three independent groups. SPSS 22.0 Windows package program was used in the analysis and $p < 0.05$ was considered meaningful.

3. RESULTS AND DISCUSSION

3.1. Findings of fluorine content

Table 1 shows the results of F obtained from SEM-EDX analysis. Both intergroup and intragroup F value differences were evaluated in percent by weight. Although there was no statistically significant difference between the groups in terms of mineral changes before bleaching treatment (F_0) ($p > 0.05$), a significant difference was found in measurements on the 14th day of bleaching treatment (F_1) ($p = 0.002$). This significant difference is present only between Groups 1-3. No significant difference was found in F values of each group before and after treatment ($p > 0.05$).

3.2. Findings of aluminum content

Findings of Al values are shown in Table 2. Both intergroup and intragroup Al was evaluated in percent by weight. No significant difference was found between the groups in the measurements performed before (Al_0) and on the 14th day of bleaching treatment (Al_1) ($p > 0.05$). A significant difference was found in Al values of each group before and after treatment ($p < 0.05$).

3.3. Findings of silicon content

The values of Si obtained by SEM-EDX analysis are shown in Table 3. Both intergroup and intragroup Si values were evaluated in percent by weight. No statistically significant difference was found between the groups in the measurements performed before bleaching treatment (Si_0) ($p > 0.05$), however there was a significant difference in the values obtained on the 14th day of bleaching treatment (Si_1) ($p = 0.026$). Post-treatment evaluation was significantly lower in Group 1 than in Groups 2 and 3. No significant difference was found in Si values of each group before and after treatment ($p > 0.05$).

3.4. Findings of strontium content

The data related to Sr are shown in Table 4. Both inter-group and intra-group data were used to evaluate Sr in percent by weight. Although there was no statistically significant difference between the groups in terms of Sr_0 changes before the bleaching treatment ($p > 0.05$), there was a significant difference in the measurements made on the 14th day following the bleaching treatment (Sr_1) ($p = 0.010$). This significant difference was found between Groups 1and 2 and Groups 1 and 3. There was a statistically significant difference between before and 14 days following the bleaching treatment ($p = 0.005$).

All the mineral changes obtained from the study before treatment and on the 14th day of treatment are shown in Figure 1.

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Table 1. Fluorine values (mean ± standard deviation)

	Group 1	Group 2	Group 3	*p*
F_0	31.16 ± 26^{aA}	26.63 ± 25,51^{aA}	33.12 ± 37,41^{aA}	0.879
F_1	10.85 ± 20.13^{aA}	31.96 ± 16,44^{bA}	46.38 ± 15,51^{bA}	0.002*
p	0.093	0.445	0.241	

*Different letters within columns and lines indicate statistically significant differences. Lowercases represent linear differences while uppercases represent columnar differences.

Table 2. Aluminum values (mean ± standard deviation)

	Group 1	Group 2	Group 3	***p***
Al_0	22.51 ± 13.21^{aA}	33.26 ± 33.74^{aA}	35.14 ± 22.41^{aA}	0.547
Al_1	5.78 ± 5.51^{aB}	2.30 ± 2.02^{aB}	3.85 ± 2.91^{aB}	0.154
p	0.005*	0.007*	0.005*	

*The same is as in Table 1.

Table 3. Silicon values (mean ± standard deviation)

	Group 1	Group 2	Group 3	*p*
Si_0	30.03 ± 30.06^{aA}	43.24 ± 24.40^{aA}	36.21 ± 21.90^{aA}	0.348
Si_1	29.18 ± 17.39^{aA}	49.37 ± 16.21^{bA}	48.14 ± 19.85^{aA}	0.026*
p	0.959	0.333	0.203	

*The same is as in Table 1.

Table 4. Strontium values (mean ± standard deviation)

	Group 1	Group 2	Group 3	*p*
Sr_0	3.09 ± 4.69^{aA}	3.59 ± 1.96^{aA}	2.32 ± 4.38^{aA}	0.052
Sr_1	34.21 ± 17.95^{aB}	22.19 ± 8.21^{bB}	16.09 ± 8.84^{bB}	0.010*
p	0.005*	0.005*	0.005*	

*The same is as in Table 1.

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Bleaching treatment is a widely preferred method for discoloration of teeth, however the results are still controversial. It has been reported that it causes morphological changes such as erosion and porosity in enamel structure.[30-31] In this study, mineral changes in the enamel structure were evaluated with SEM-EDX device.

Oxidation-reduction reactions caused by bleaching treatment cause degradation of organic and inorganic matrix structure.[32] However, the remineralizing effect of saliva can reverse the structural changes caused by bleaching agents. In conclusion, the clinical significance of mineral loss caused by bleaching agents may be limited.[33] Therefore, in order to utilize from the remineralization effect of saliva, samples were stored in artificial saliva solution.

Hydroxyapatite crystals, whose main inorganic component is calcium (Ca) and phosphorus (P), constitute the majority of the enamel structure.[34] There are various anions and cations in enamel structure in different ratios. The most important known cations are: sodium (Na), Sr, magnesium (Mg); anions are F, Si and *Al.*[35] These changes in mineral ratios results in differences in the organic / inorganic structure of the enamel and subsequently lead to morphological changes such as surface roughness and microhardness.[36-38]

SEM-EDX is a device that determines the mineral content in dental hard tissues in a percentage by weight and provides accurate and non-destructive analysis of samples.[39-40] This method was used to evaluate changes in enamel mineral content in our study. In the study of Kutuk and co-workers,[25] it has been shown that F containing desensitizing agents used after bleaching or in addition to bleaching gels increase the F levels in enamel. The possible explanation for this increase may be the use of F-containing bleaching agent to prevent sensitivity or demineralization during the bleaching process. However, there are controversial conclusions that there is no evidence to support the effect of F-containing bleaching gels on demineralization.

In the study of Saffarpour and co-workers,[26] a Sr-based agent was applied to all samples and bleaching treatment was applied to only one group. According to the obtained data, it was reported that the amount of Sr increased significantly in the group treated with bleaching treatment. In our study, Sr values were significantly increased. We assume that the reason for this increase is the use of artificial saliva in the study.

There was an increase in *Si* measurements before and on 14th day of the bleaching treatment. The increase of Si in percentage by weight can be explained by the basic mapping of Si and its consistent distribution with beehive-like structures. In accordance with these findings, it is known that *Si* precipitates on the uneven enamel surface by reacting with free oxygen radicals produced by HP contained by bleaching agents. These values are consistent with the results obtained in a study done by Tsujimoto and co-workers.[27]

Although Olcay and co-workers[28] have showed an increase in mineral changes in Al, our results are contradictory. There are no other studies regarding the change in the percentage of Al due to bleaching treatment in the literature. Since there is no study about Al mineral in the literature, its mechanism has not been clarified, therefore more studies are needed.

One of the limitations of our study is that in-vitro conditions do not fully mimic the in-vivo environment. We assume that the morphological and chemical changes in dental hard tissues are the most important side effect of the bleaching agents. These undesired effects may be reduced with the aid of both the buffering capacity of saliva and the administration of remineralization agents when applied in vivo. Assessing the long-term impacts of bleaching agents on dental tissues should be further investigated.

4. CONCLUSIONS

When the Opalescence Boost PF 40% HP used in the study was applied in three different periods, mineral change was observed in each group. However; the best results were obtained in Group 2, which was applied in the ideal time according to the manufacturer's instructions. Although there are few studies in the relevant literature, different studies are needed to better understand the effects of bleaching.

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Conflict of interests

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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